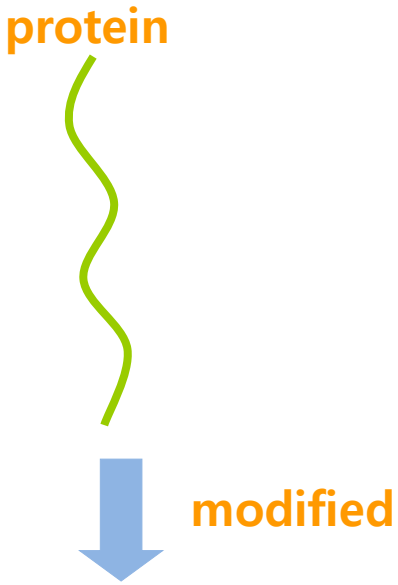
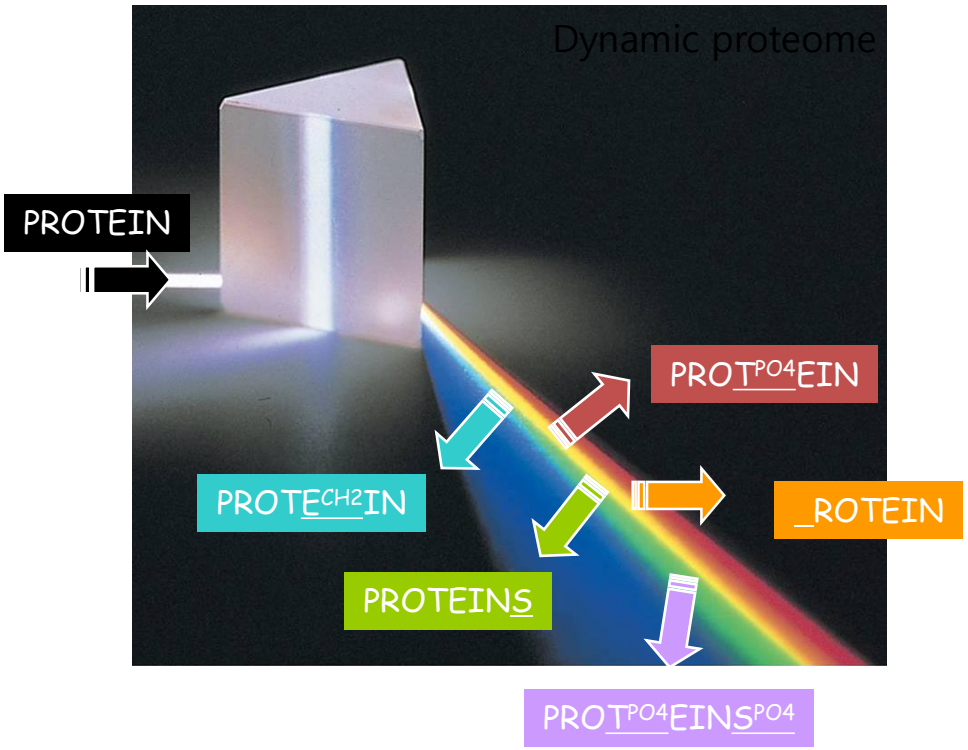
PTM analysis

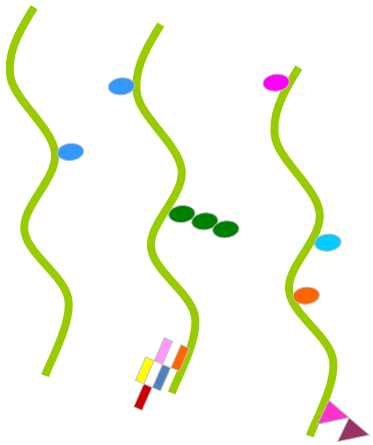
2020.06

김현우

Post-translational modification (PTM)





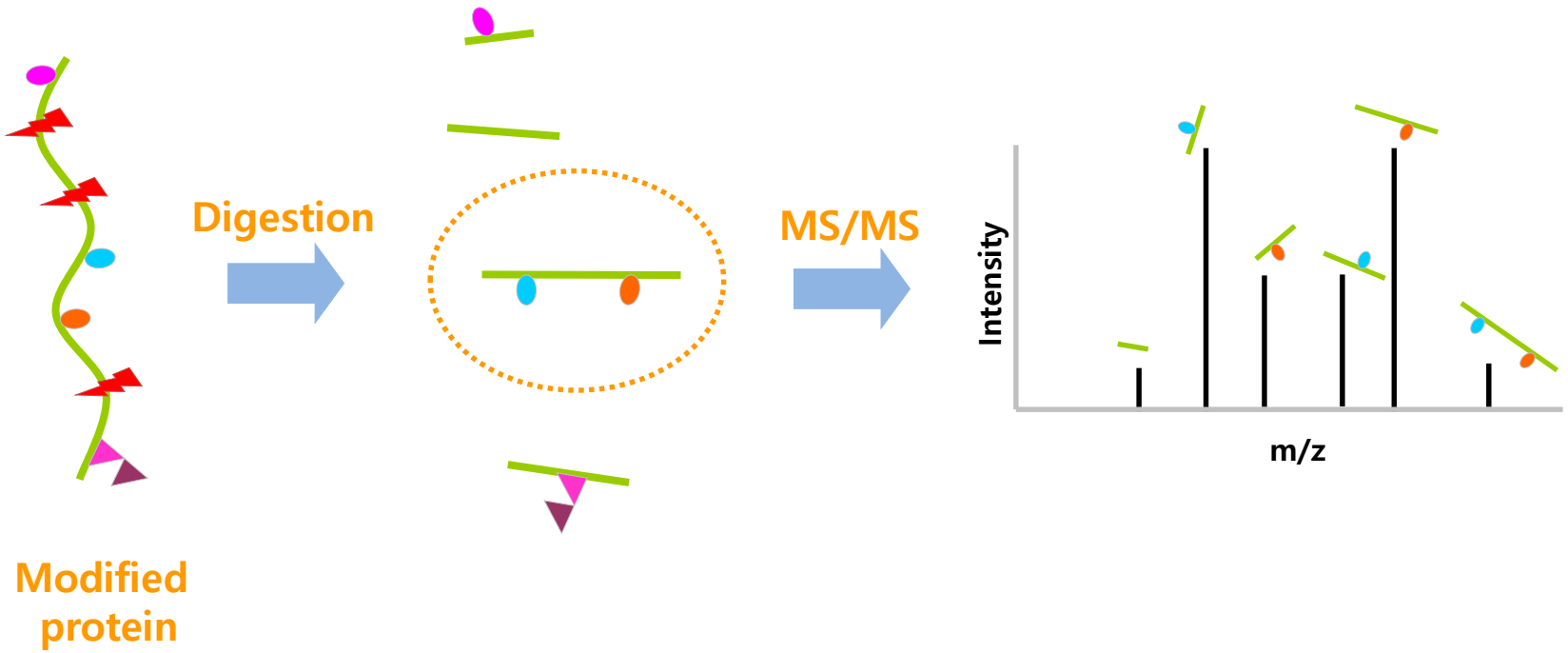


Addition of chemical groups

Structural changes

Various Cellular Functions

MS/MS of modified peptides

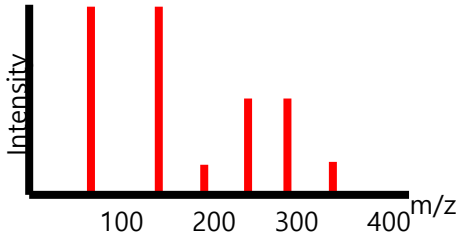


Peptide Assignment by MS/MS

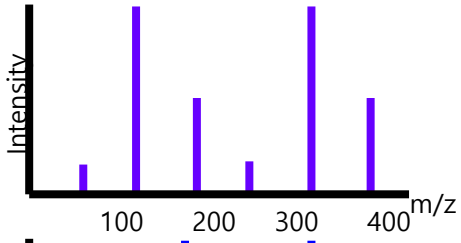
Candidate peptides

Theoretical MS/MS spectrum

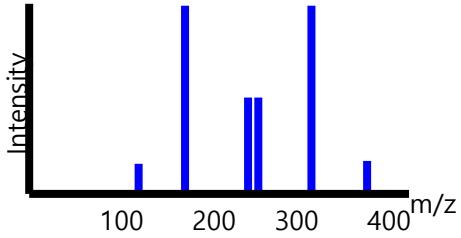
READ



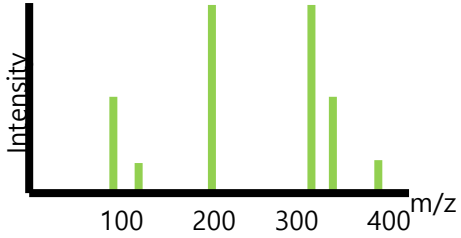
DVGAE



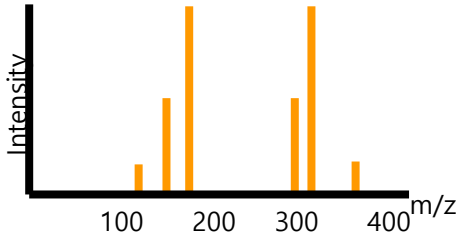
DEAR



GAGVDA

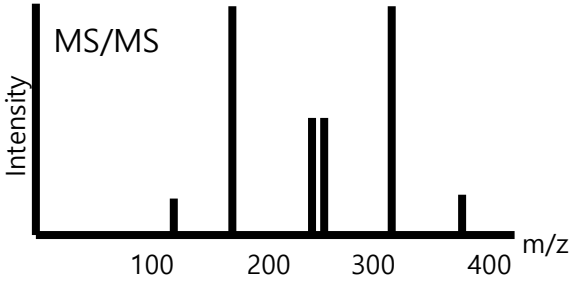


EGDVA



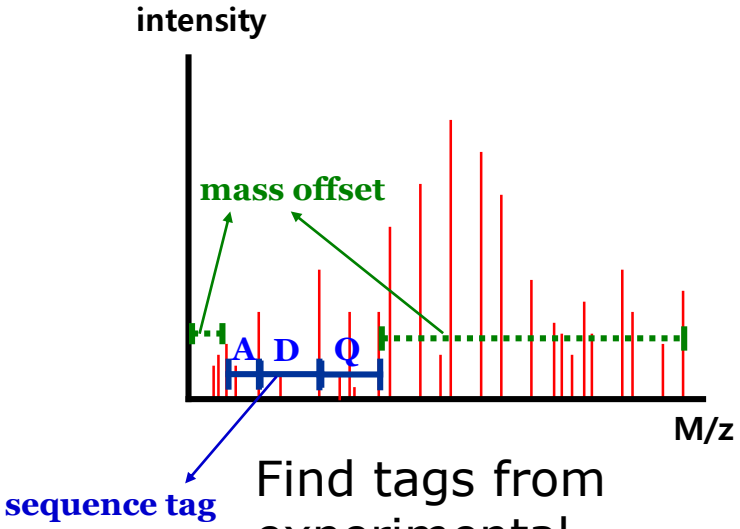
Experimental MS/MS spectrum

Parent mass = 471



Tag-based approach (Database searching + de novo)

- PeptideSearch, InspecT, MODi



Find tags from experimental spectrum

DB search *using sequence tag + mass offset*

Peptide DB

VTAEDKGTGNK
RALSSQHQAR
VYADQRPLTK
ETMEKAVEEK
EFFNGKEPSR
DAGTIAGLNVMR
VEIINQDAGNR
FLPFKVVEKK
LIPRNTVVPTK
MKETAEAYLGK
NQIGDKEKLGGK
EKLGGKLSSEDK
LSSEDKETMEK

MS/MS spectrum of modified peptides

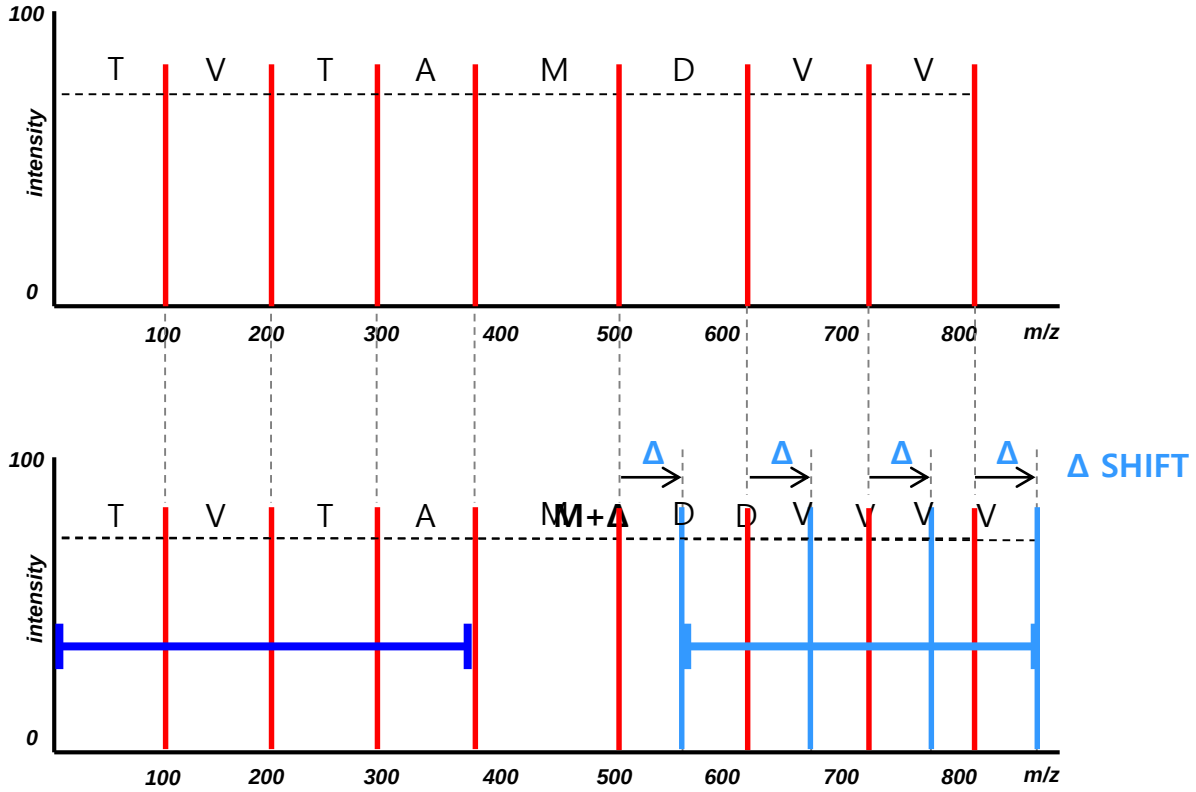
TVTAMDVVY *vs.* TVTAM$^{\Delta}$DVVY

'TVTAMDVVY'

T VTAMDVVY
TV TAMDVVY
TVT AMDVVY
TVTA MDVVY
TVTAM DVVY
TVTAMD VVY
TVTAMDV VY
TVTAMDVV Y

'TVTAM$^{\Delta}$DVVY'

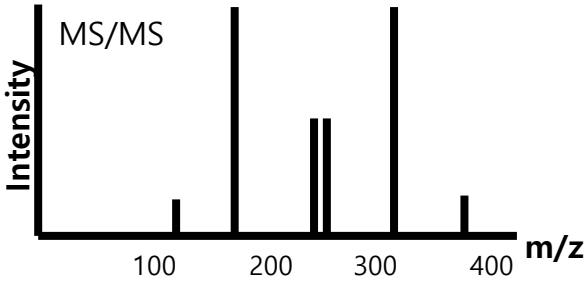
T
TV
TVT
TVTA
TVTAM$^{\Delta}$
TVTAM$^{\Delta}$D
TVTAM$^{\Delta}$DV
TVTAM$^{\Delta}$DVV



MS/MS spectrum of peptide 'TVTAM$^{\Delta}$DVVY' with a modification of +Δ mass

Database search - modification analysis

Parent mass = 471



>Protein A
MEMEKEFEQIDKSGSWAAIYQDI**DVGAE**DFPCRVAKLPK
NKNRNRYRDVSPFDHSRK**READ**DNDYINASLIKMEEAQR
SYILTQQIDKSGSWAAIYQDIRHEASDFHEASDFPCRVA
KLPKNK**DEAR**YMEKEFEQIDK**GAGVDA**DIRHEMEKEFEQ
IDKSGSWAAIYQDIRHE
>Protein B
…

Candidate peptides

DVGAE
READ
DEAR
GAGVDA
…

471

Modification analysis

Candidate peptides

Every substring

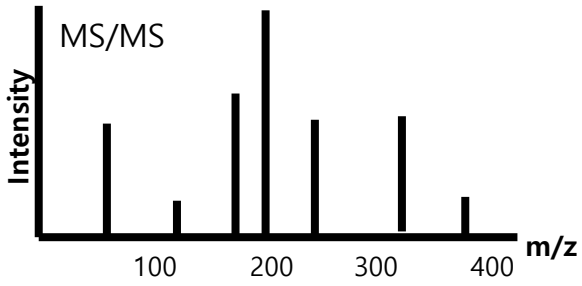
Explosion of the no. of candidate peptides

46

Complexity for analyzing modified peptides

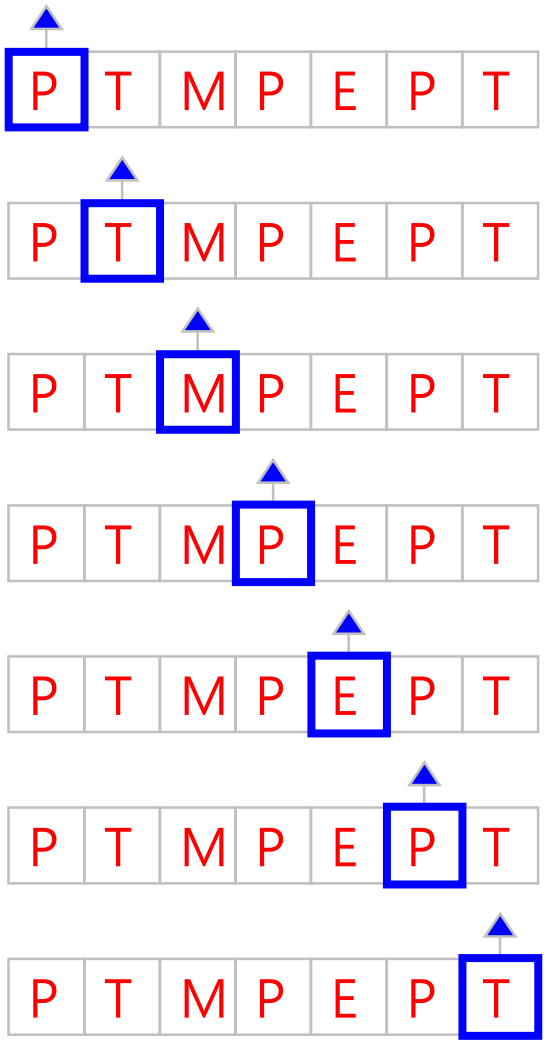
Considering one modification per peptide

Parent mass = 769



PTMPEPT 753

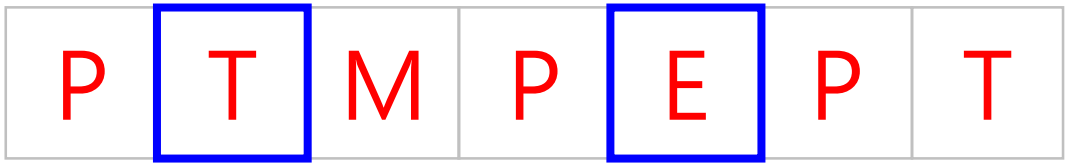
16



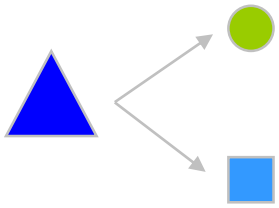
$O(N)$

Complexity for analyzing modified peptides

Considering two modifications per peptide



$N(N\text{-}1)$



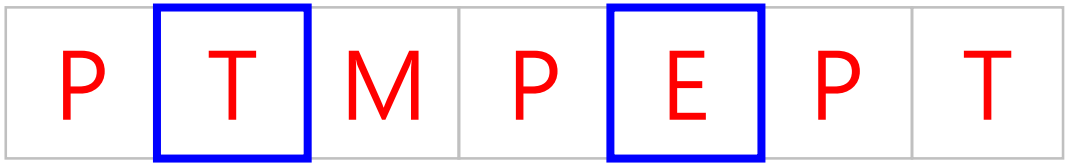
100 = 1 + 99
= 2 + 98
= 3 + 97
…
…
= 101 + -1
= 102 + -2
…
…
= 300 + -200

d (-200 ~ +200)

$O(dN^2)$

Complexity for analyzing modified peptides

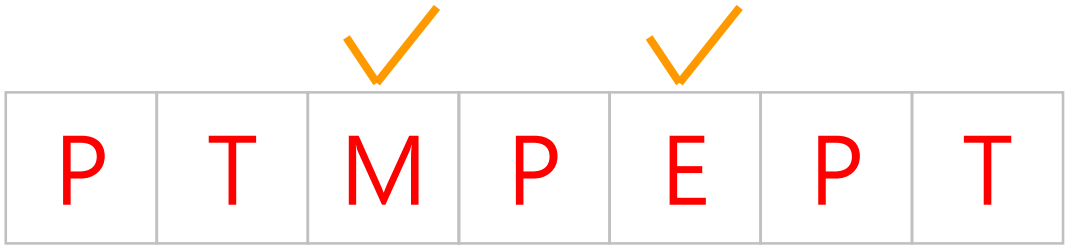
Considering two modifications per peptide

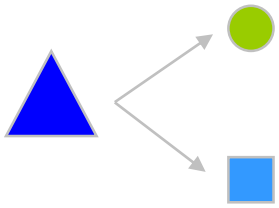


$N(N\text{-}1)$

mass	# tryptic peptides	phos STY tryptic	factor	unconstr phos STY
1000 Da	1,430	5,093	3.5x	1,167,740
2000 Da	466	7,283	15.6x	4,538,383
3000 Da	249	16,761	67.3x	15,641,722

Standard method for modification search





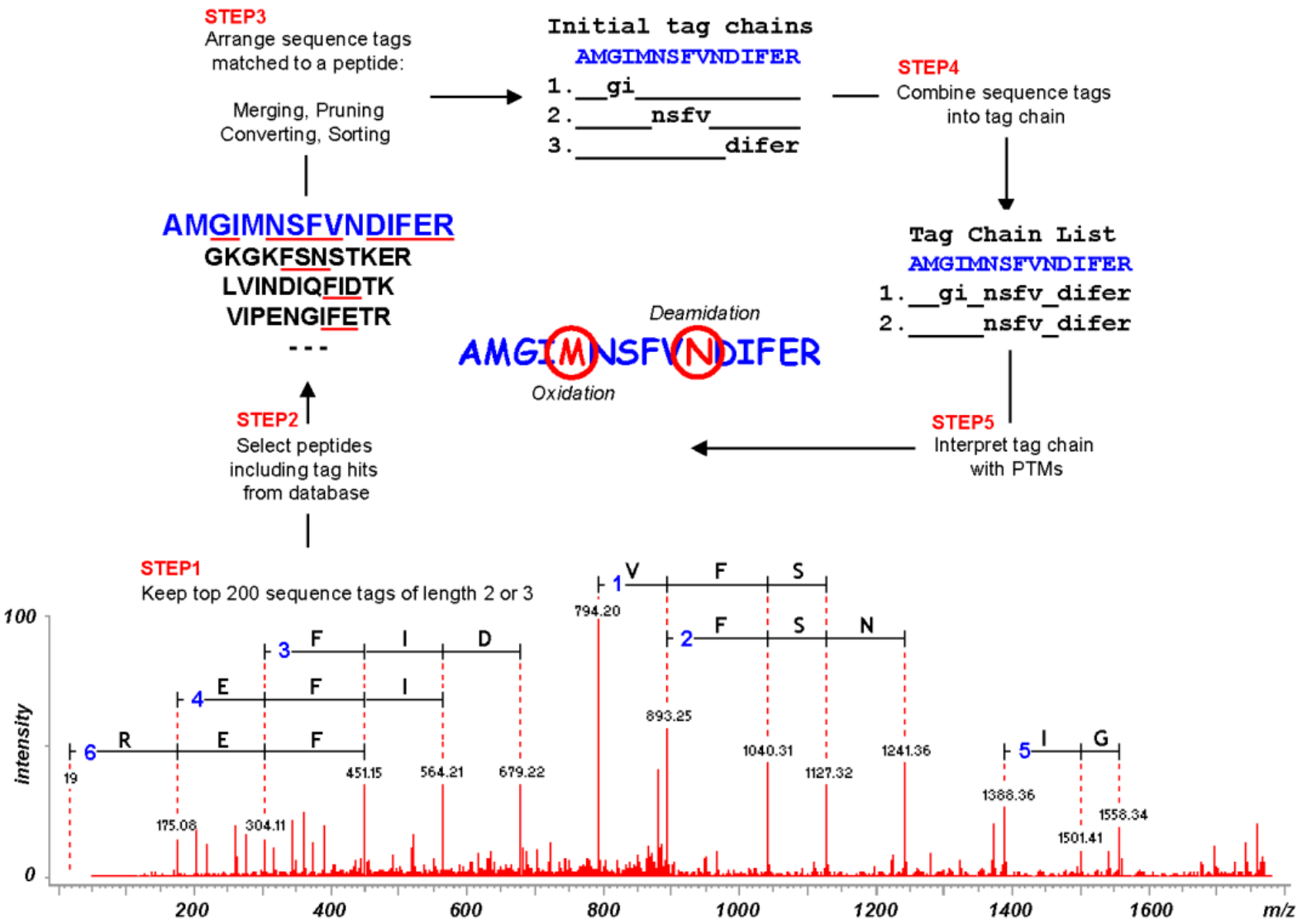
100 = 1 + 99
= 2 + 98
= 3 + 97
...
...
= 101 + -1
= 102 + -2
...
...
= 300 + -200

Input modifications

+1 on N
+3 on M
+97 on E
+102 on T

Restrictive search

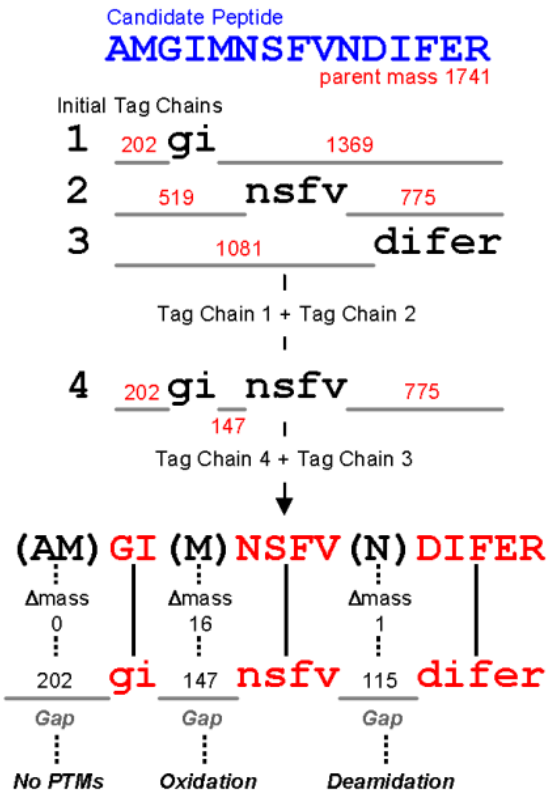
Overview of MODi algorithm



Na, Seungjin, et al. "Unrestrictive identification of multiple post-translational modifications from tandem mass spectrometry using an error-tolerant algorithm based on an extended sequence tag approach." *Molecular & Cellular Proteomics* 7.12 (2008): 2452-2463.

Overview of MODi algorithm

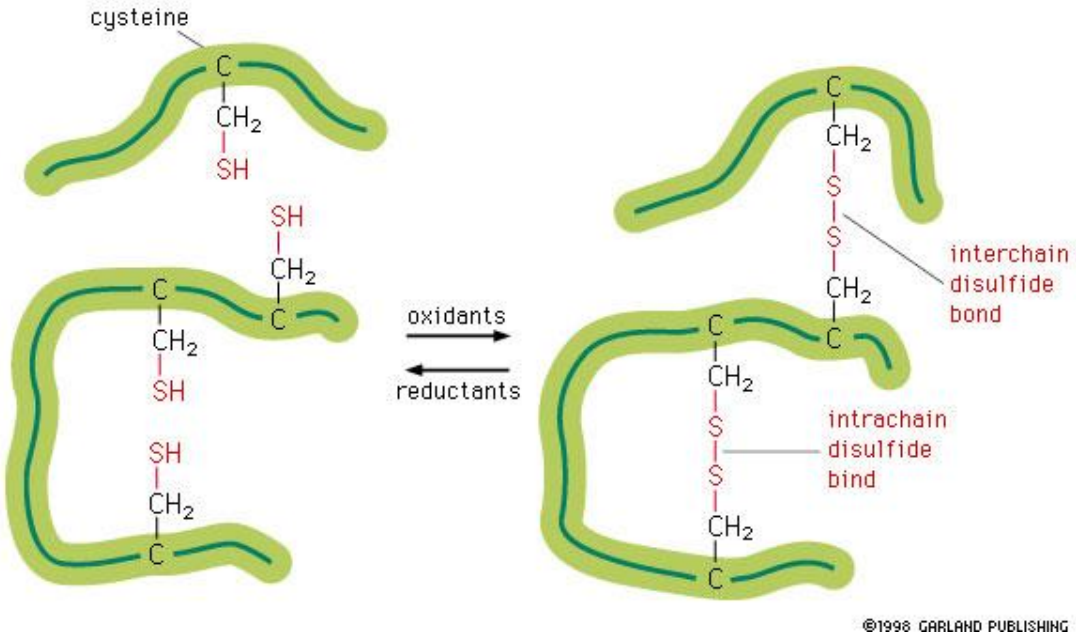
Interpretation of tag chains in terms of modifications

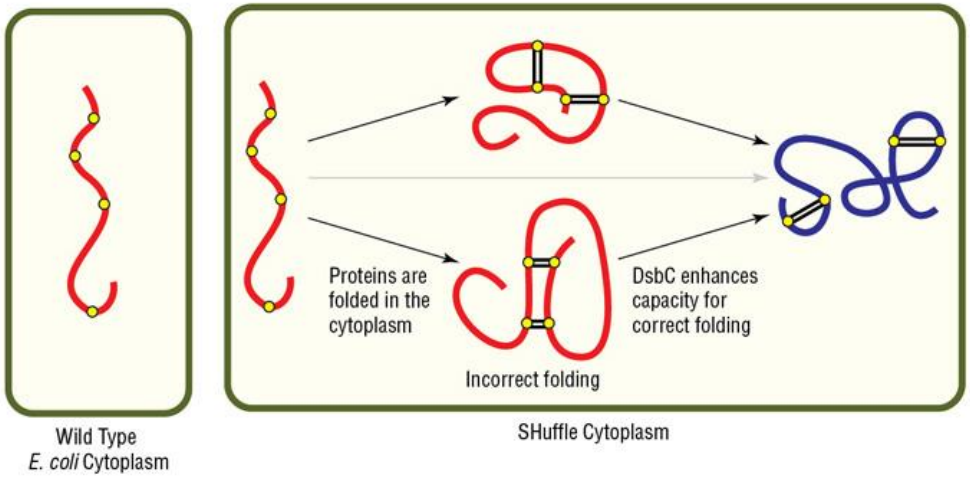


Search Parameters

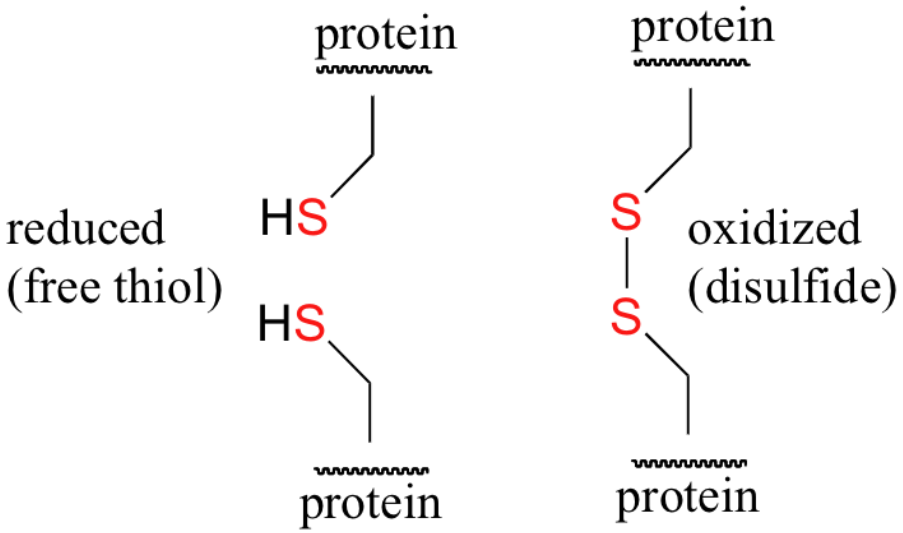
- Variable modification
  - endogenous modifications (e.g. phosphorylation)
  - artefacts from sample handling (e.g. oxidation)
- Fixed modification: replace amino acid mass with a different value (example: cysteine carbamidomethylation => 160 Da for all occurrences of cysteine)
- www.unimod.org
  http://www.abrf.org/index.cfm/dm.home
  http://home.earthlink.net/~jsgaravelli/RESIDInfo.HTML
- modification mass range
- number of modified sites per peptide

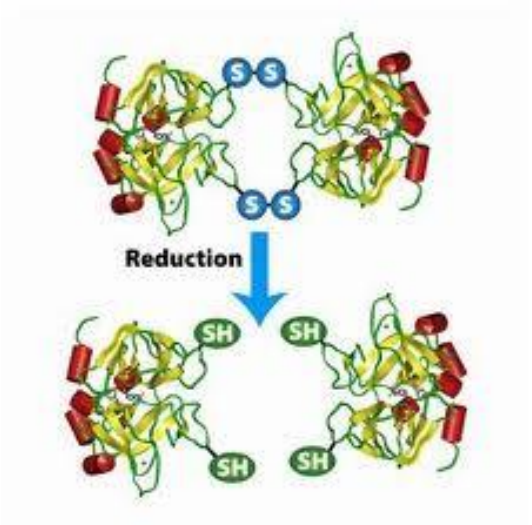
Disulfide Bond between Cysteines

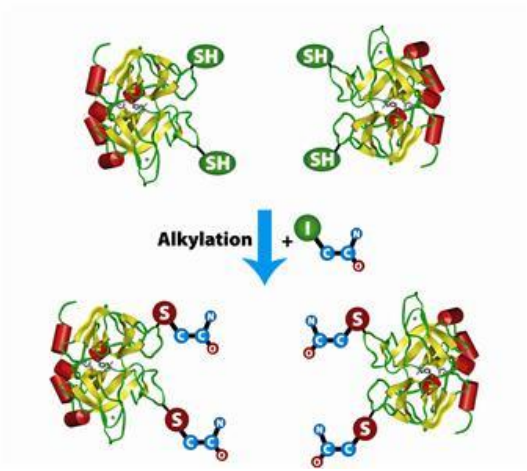




Alkylation Reduction => Fixed Modification

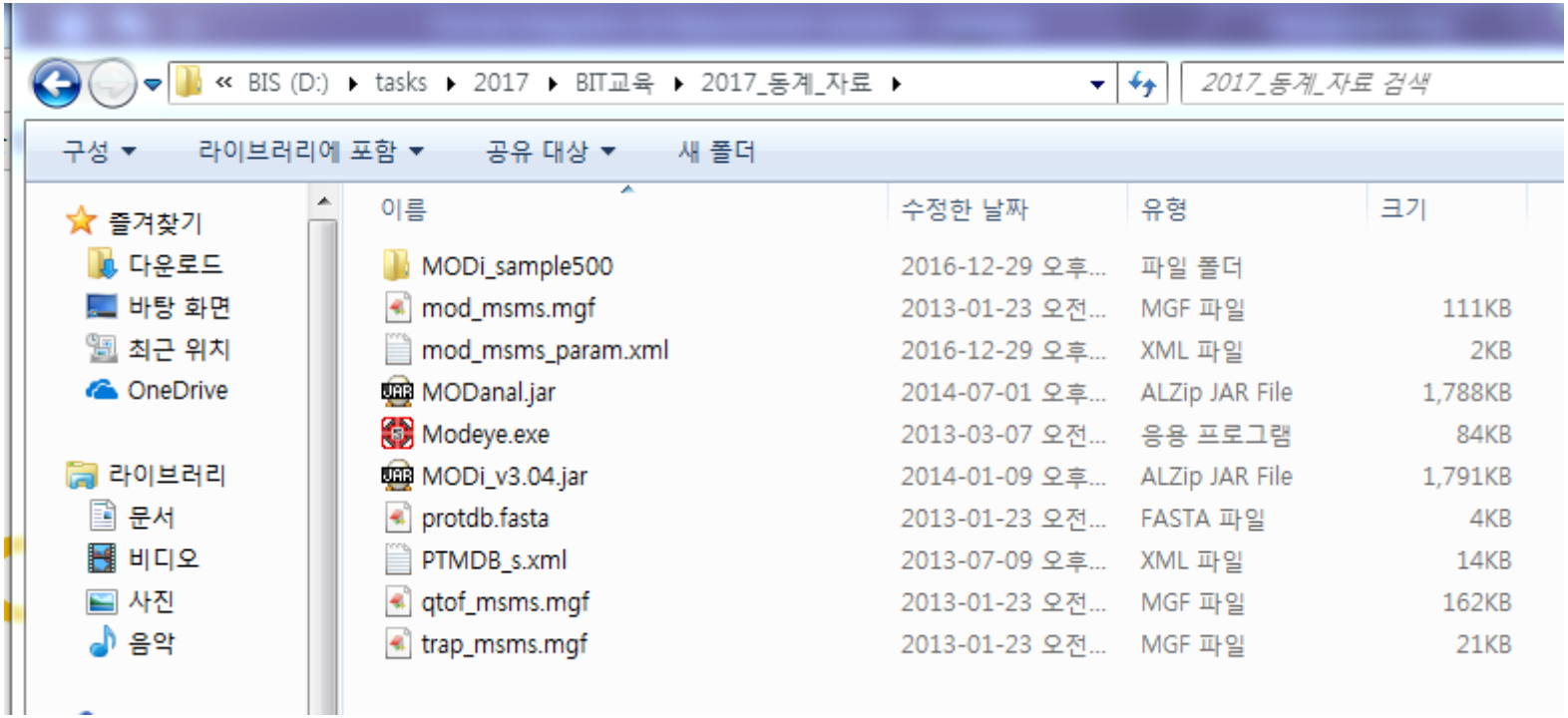






Lab: MODi

- 파일 확인



  - Data file(*.mgf)
  - Database(protdb.fasta)

Parameter setting

- mod_msms_param.xml 실행(메모장)
- Parameter setting

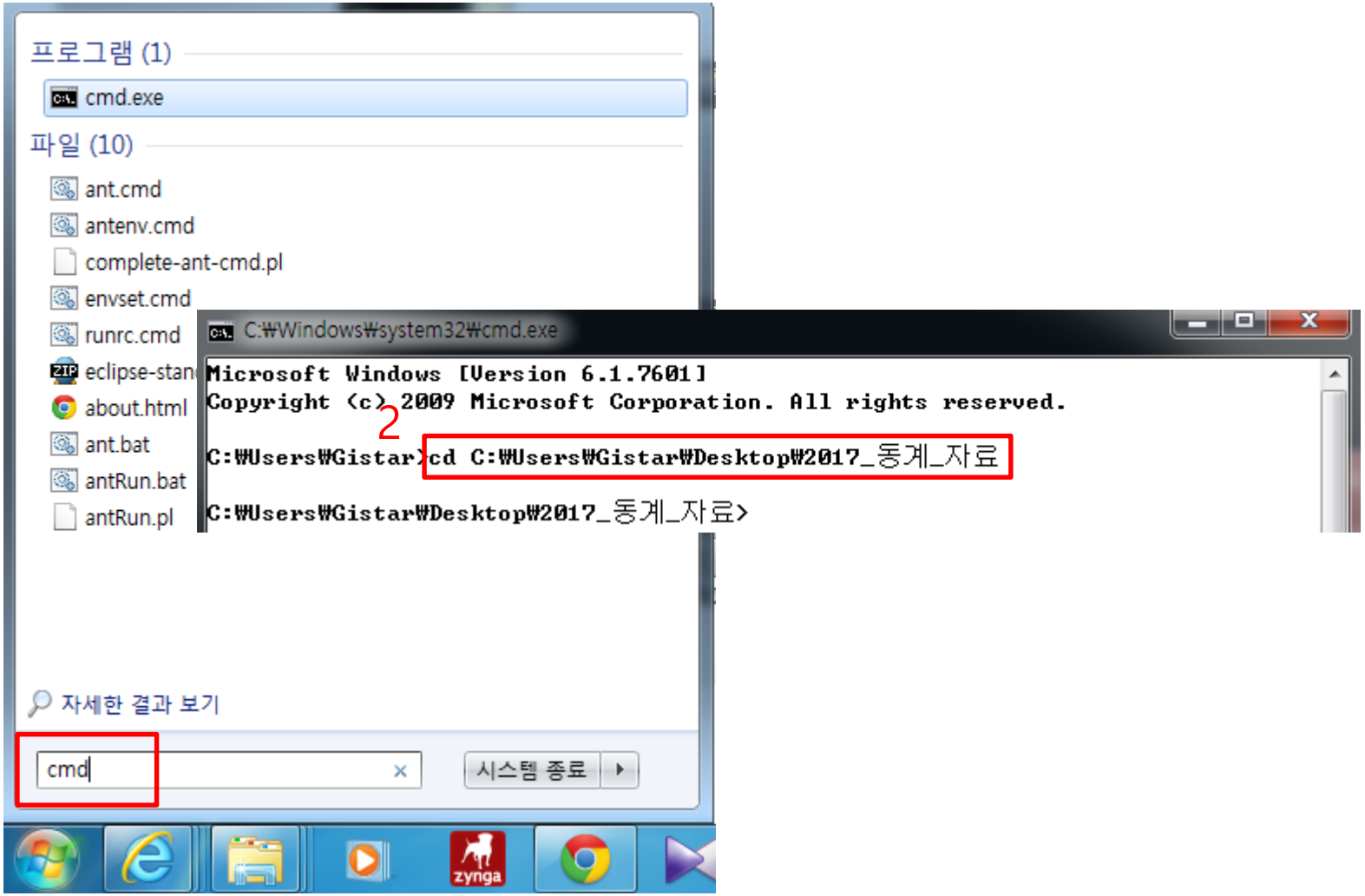
Parameter setting

- mod_msms_param.xml

```
<?xml version="1.0" encoding="UTF-8"?>
<search  "Na" title="Sample" >

    <data  local_path="mod_msms.mgf" format="mgf" instrument="TRAP" />    <!--
    format=[mgf|pkl|dta], instrument=[QTOF|TRAP] -->
    <database local_path="protdb.fasta" />

    <combined_enzyme name="Trypsin" nterm_cleave="" cterm_cleave="KR" />

    <instrument_resolution ms="high" msms="low" /> <!-- ms / msms =[high|low] -->

    <parameters>
        <enzyme_constraint max_miss_cleavages="2" min_number_termini="2" />
        <isotope_error C13_number="0" />
        <peptide_mass_tol value="15" unit="ppm" /> <!-- unit=[Da|ppm] -->
        <fragment_ion_tol value="0.6" unit="Da" /> <!-- unit=[Da] (NOT ALLOWED PPM) -->

        <modified_mass_range min_value="-150" max_value="250" />
    </parameters>

    <decoy_search checked="0" /> <!-- with reverse sequences -->
    <mod_map checked="0" />

    <modifications>
    <!-- for mod, site=[AA|N-term|C-term],
    position=[ANYWHERE|ANY_N(C)_TERM|PROTEIN_N(C)_TERM] -->
        <fixed>
            <mod name="Carbamidomethyl" site="C" position="ANYWHERE" massdiff="57.02150"
            />
            <!-- for fix mod, NOT ALLOWED a combination of site="AA" and
            position="ANY_N_TERM" -->
        </fixed>
        <variable local_path="PTMDB_s.xml" canBeModifiedOnFixedAA="1"> <!--
        local_path=external ptm file path (attached), canBeModifiedOnFixedAA=[0|1] -->
            <!--mod name="NT+12" site="N-term" position="ANY_N_TERM" massdiff="12.00"
            /--> <!-- specify additional mods -->
        </variable>
    </modifications>

</search>
```

1 2 3 4 5

- CMD창 실행 후 실습 폴더 이동



Execute MODi

- Execute MODi
  - 명령어 : java –jar MODi.jar [파라미터 파일]

ex)java –jar MODi_v3.04.jar mod_msms_param.xml

```
Microsoft Windows [Version 6.1.7601]
Copyright (c) 2009 Microsoft Corporation. All rights reserved.

C:₩Users₩Gistar>cd C:₩Users₩Gistar₩Desktop₩2017_동계_자료

C:₩Users₩Gistar₩Desktop₩2017_동계_자료>java -jar MODi_v3.04.jar mod_msms_param.x
ml
**************************************************************************
Modeye v3.04 - Identification of post-translational modifications
Release Date: JULY 01, 2013
Hanyang University, Seoul, Korea
**************************************************************************

Reading parameter.....
Input datasest : mod_msms.mgf / Specified type : MGF
Input database : protdb.fasta
High resolution MS!!
Fixed modifications : 1 selected
Variable modifications : 43 selected

Reading MS/MS spectra.....  8 scans
Reading protein database.....  13 proteins / 2563 residues

Processing 1/8 (title:1)
Processing 2/8 (title:2)
Processing 3/8 (title:3)
Processing 4/8 (title:4)
Processing 5/8 (title:5)
Processing 6/8 (title:6)
Processing 7/8 (title:7)
Processing 8/8 (title:8)
Interpreted : 8 / 8
Elapsed Time : 2 Sec

C:₩Users₩Gistar₩Desktop₩2017_동계_자료>
```

Result

- [dataname].modi.txt 확인

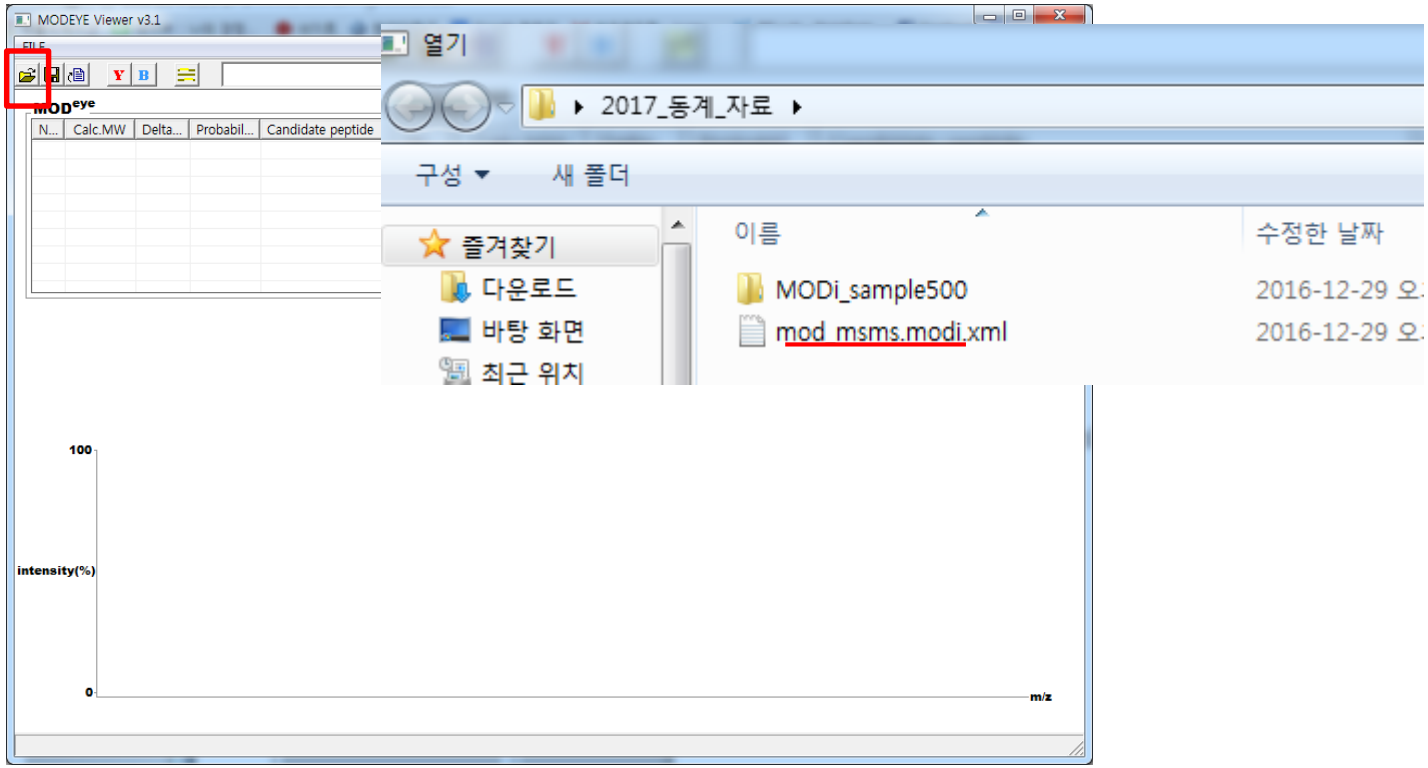
mod_msms.modi.txt - 메모장

파일(F) 편집(E) 서식(O) 보기(V) 도움말(H)

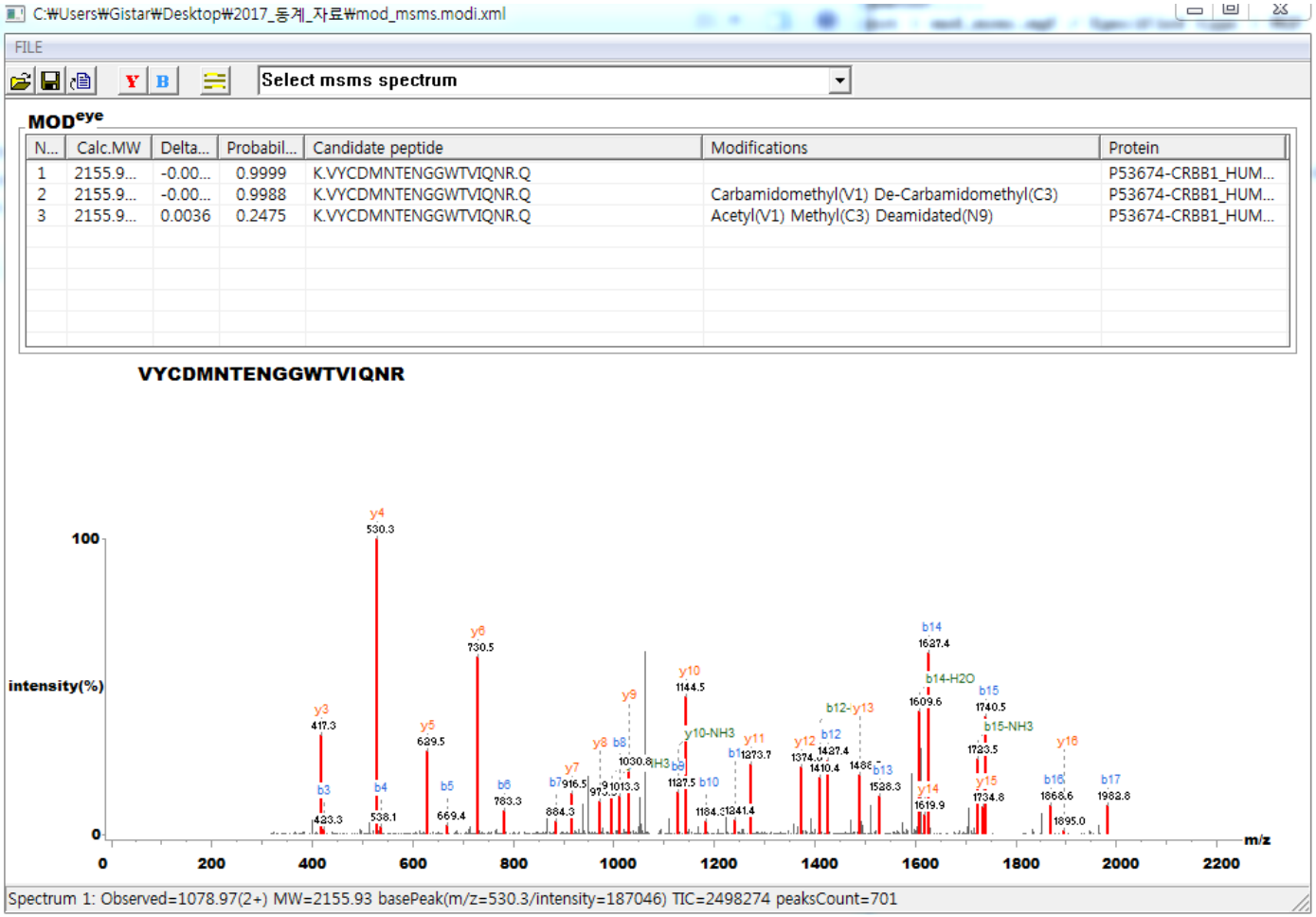
```
MODi v3.04

>>mod_msms.mgf  1       2155.9339       2
2155.9416       -0.0076 91      0.9999  K.VYCDMNTENGGWTVIQNR.Q  P53674-CRBB1_HUMAN              253~270
2155.9415       -0.0076 79      0.9988  K.VYCDMNTENGGWTVIQNR.Q  P53674-CRBB1_HUMAN      Carbamidomethyl(V1) De-Carbamidomethyl(C3)      253~270
2155.9303       0.0036  43      0.2475  K.VYCDMNTENGGWTVIQNR.Q  P53674-CRBB1_HUMAN      Acetyl(V1) Methyl(C3) Deamidated(N9)    253~270

>>mod_msms.mgf  2       2171.9455       2
2171.9365       0.0091  93      0.9999  K.VYCDMNTENGGWTVIQNR.Q  P53674-CRBB1_HUMAN      Oxidation(M5)   253~270

>>mod_msms.mgf  3       2203.9196       2
2203.9263       -0.0067 92      0.9994  K.VYCDMNTENGGWTVIQNR.Q  P53674-CRBB1_HUMAN      Oxidation(M5) Dioxidation(W12)  253~270

>>mod_msms.mgf  4       2331.1378       2
2331.1342       0.0036  94      0.9994  K.TGKEYIPGQPPLSQSSDSSPTR.N      P53672-CRBA2_HUMAN              199~220

>>mod_msms.mgf  5       2411.1054       2
2411.1006       0.0049  80      0.9925  K.TGKEYIPGQPPLSQSSDSSPTR.N      P53672-CRBA2_HUMAN      Phospho(S19)    199~220
2411.1006       0.0049  79      0.9915  K.TGKEYIPGQPPLSQSSDSSPTR.N      P53672-CRBA2_HUMAN      Phospho(S18)    199~220
2411.1006       0.0049  76      0.9877  K.TGKEYIPGQPPLSQSSDSSPTR.N      P53672-CRBA2_HUMAN      Phospho(T21)    199~220
2411.1006       0.0049  72      0.9609  K.TGKEYIPGQPPLSQSSDSSPTR.N      P53672-CRBA2_HUMAN      Phospho(S16)    199~220
2411.1006       0.0049  70      0.9335  K.TGKEYIPGQPPLSQSSDSSPTR.N      P53672-CRBA2_HUMAN      Phospho(S15)    199~220
2411.1006       0.0049  65      0.7697  K.TGKEYIPGQPPLSQSSDSSPTR.N      P53672-CRBA2_HUMAN      Phospho(S13)    199~220
2411.1241       -0.0186 42      0.2662  K.TGKEYIPGQPPLSQSSDSSPTRN.-     P53672-CRBA2_HUMAN      Deamidated(Q9) Dehydrated(S19) Ammonia-loss(N23)        199~221
2411.1241       -0.0186 41      0.2426  K.TGKEYIPGQPPLSQSSDSSPTRN.-     P53672-CRBA2_HUMAN      Deamidated(Q9) Dehydrated(S18) Ammonia-loss(N23)        199~221
2411.1241       -0.0186 36      0.1021  K.TGKEYIPGQPPLSQSSDSSPTRN.-     P53672-CRBA2_HUMAN      Deamidated(Q9) Dehydrated(T21) Ammonia-loss(N23)        199~221

>>mod_msms.mgf  6       2331.1378       3
2331.1342       0.0035  78      0.9999  K.TGKEYIPGQPPLSQSSDSSPTR.N      P53672-CRBA2_HUMAN              199~220

>>mod_msms.mgf  7       2108.1908       3
2108.1881       0.0027  84      1.0000  K.APLKPVPVSPSDKVLIQEK.T P53673-CRBA4_HUMAN              198~216

>>mod_msms.mgf  8       2188.1623       3
2188.1544       0.0078  50      0.9691  K.APLKPVPVSPSDKVLIQEK.T P53673-CRBA4_HUMAN      Phospho(S9)     198~216
2188.1544       0.0078  41      0.8485  K.APLKPVPVSPSDKVLIQEK.T P53673-CRBA4_HUMAN      Phospho(S11)    198~216
```

Result

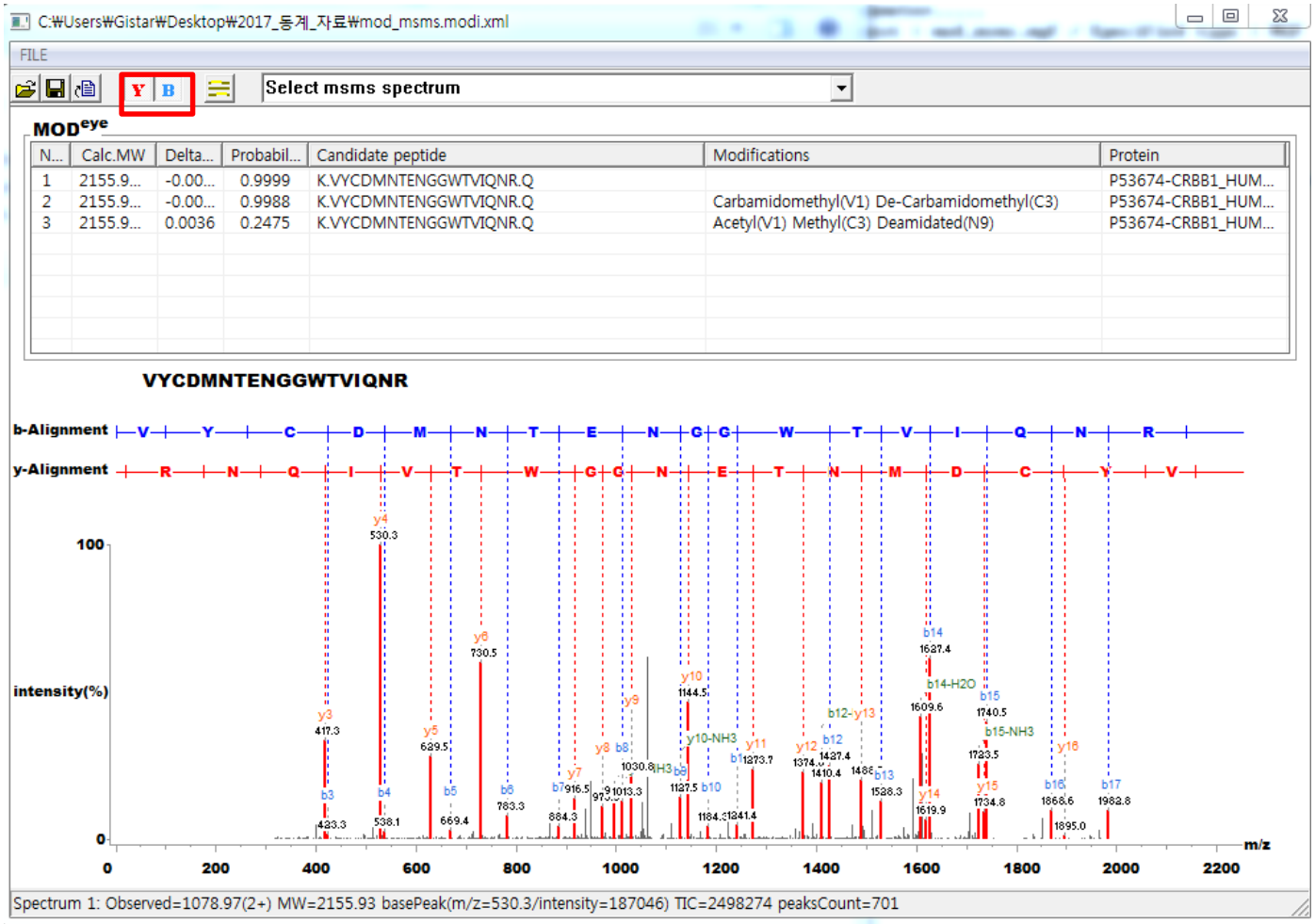
- [dataname].modi.xml 확인
  - MODeyeViewer.exe 실행
  - [dataname].modi.xml 열기



- [dataname].modi.xml 확인
  - 결과 확인 (spectrum 확인)

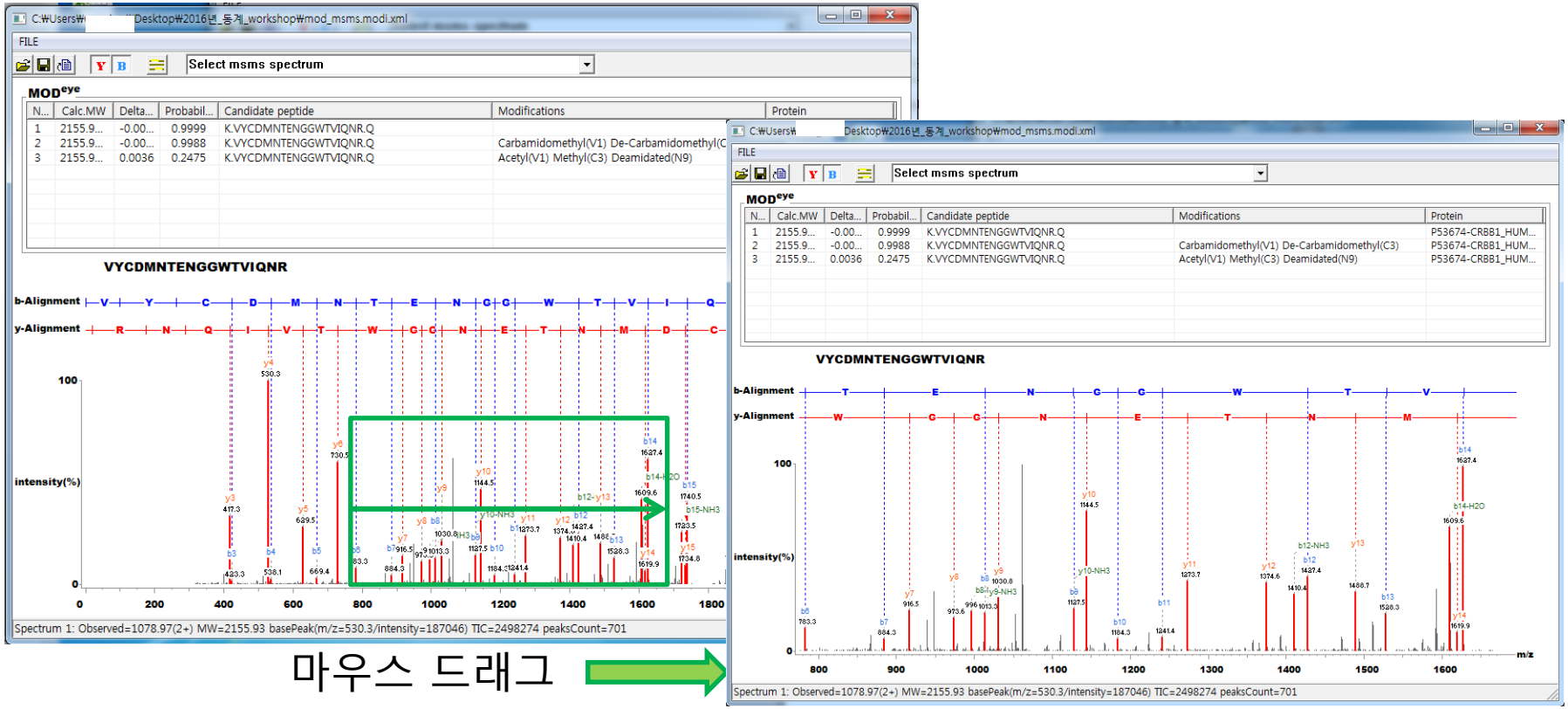


- [dataname].modi.xml 확인
  - 결과 확인 (Y, B-ion alignment)

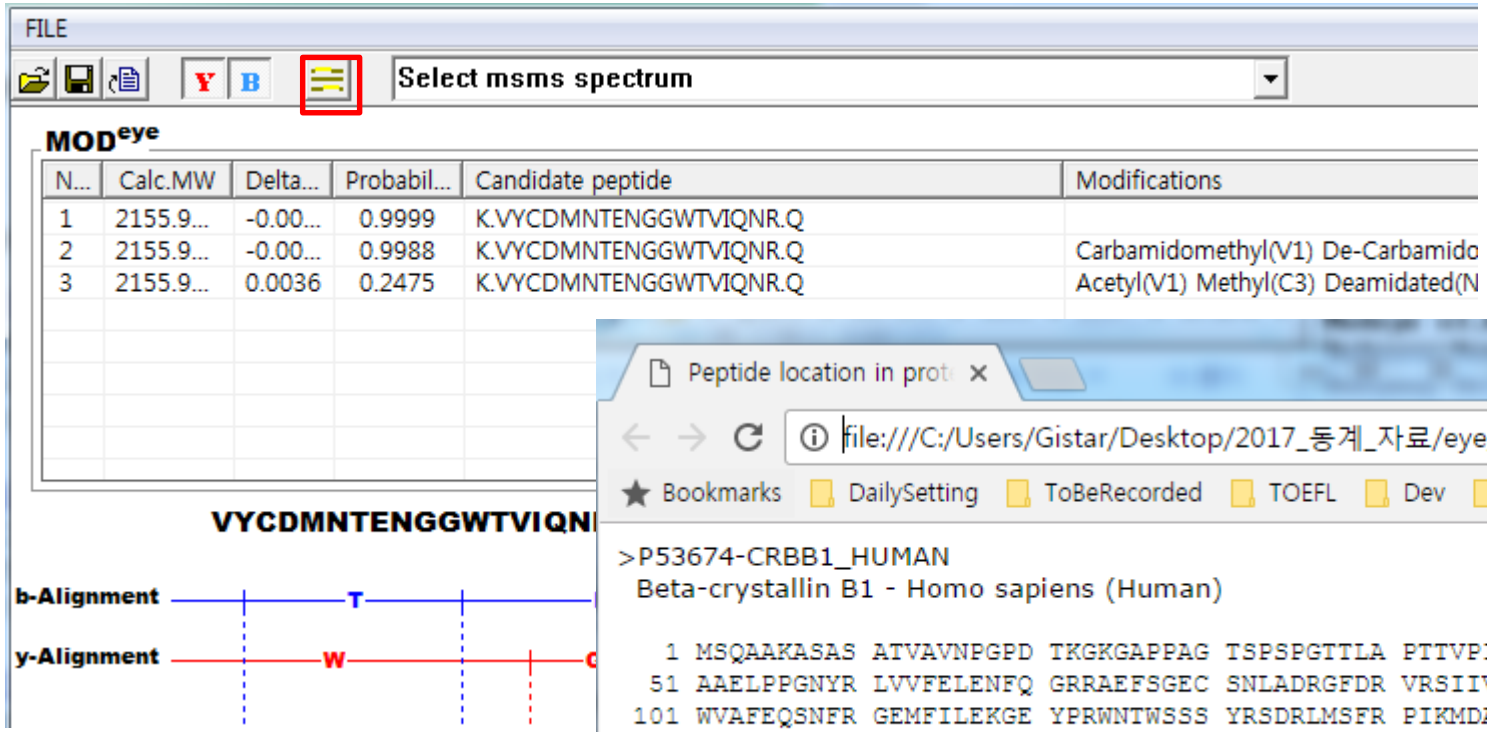


Result

- [dataname].modi.xml 확인
  - 결과 확인 (spectrum 확대)



- [dataname].modi.xml 확인
  - 결과 확인 (match to protein sequence)

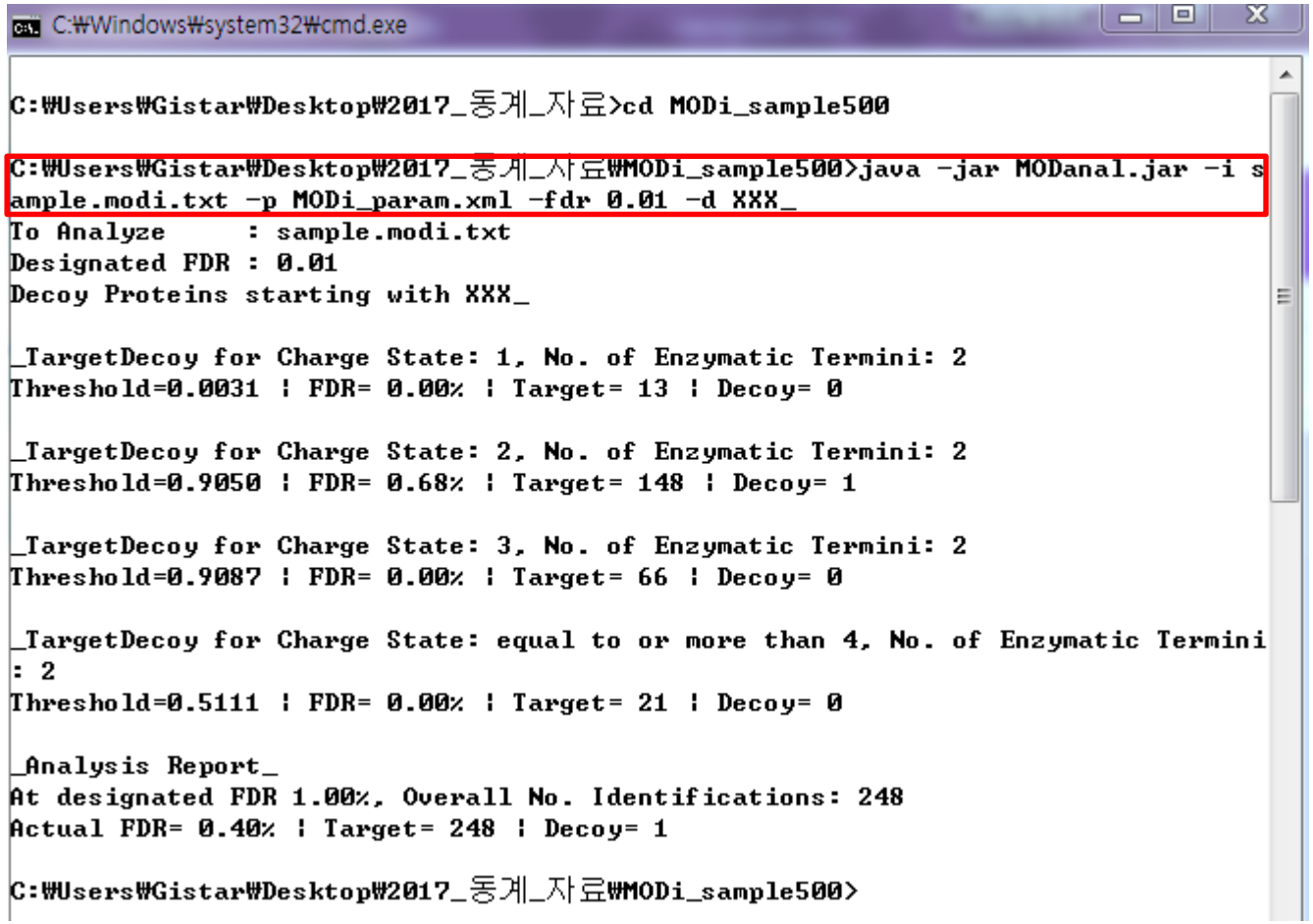


MODi result analysis 실습

- MODanal 실행
  - MODi 결과에 대한 analysis
    - MODanal 실행
      - Java –jar MODanal.jar – i [결과파일.txt] –p [파라미터파일.xml] –fdr [FDR value] –d [Decoy tag]
        - » [FDR value] : false discovery rate.
          ex) fdr 1% ➡ 0.01
        - » [Decoy tag] : Decoy protein을 구분하기 위한 Protein DB에 추가한 Tag

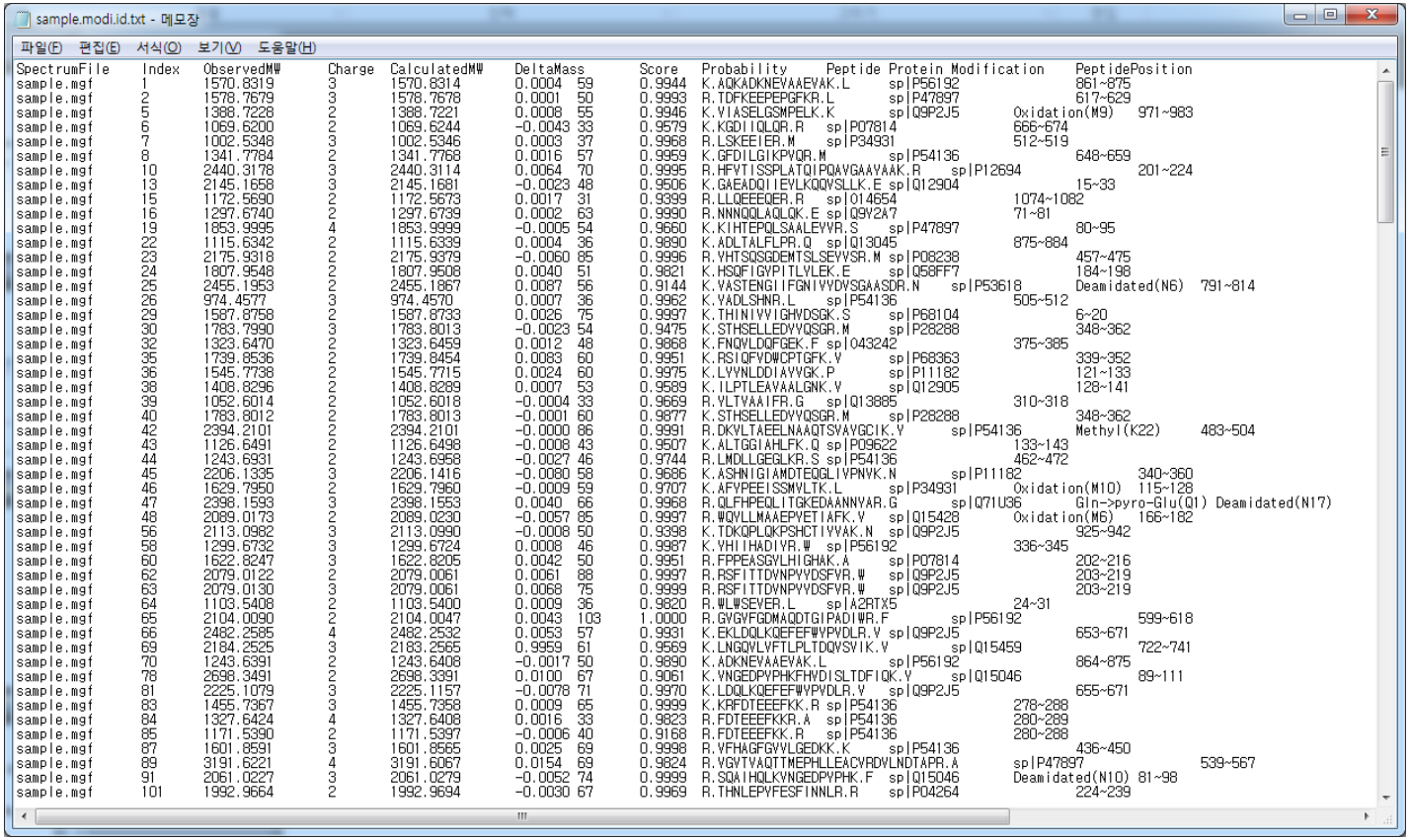
ex) java –jar MODanal.jar – i sample.modi.txt –p sample_param.xml –fdr 0.01 –d XXX_

- MODanal 실행
  - MODi 결과에 대한 analysis
    - 실행 화면



MODi result analysis 실습

- MODanal 실행
  - MODi 결과에 대한 analysis
    - 실행 결과 [dataname].modi.id.txt



sample.modi.id.txt - 메모장

파일(F) 편집(E) 서식(O) 보기(V) 도움말(H)

SpectrumFile	Index	ObservedMW	Charge	CalculatedMW	DeltaMass	Score	Probability	Peptide	Protein	Modification	PeptidePosition
sample.mgf	1	1570.8319	3	1570.8314	0.0004 59	0.9944	K.AQKADKNEVAAEVAK.L		sp|P56192		861~875
sample.mgf	2	1578.7679	3	1578.7678	0.0001 50	0.9993	R.TDFKEEPEPGFKR.L		sp|P47897		617~629
sample.mgf	5	1388.7228	2	1388.7221	0.0008 55	0.9946	K.VIASELGSMPELK.K		sp|Q9P2J5	Oxidation(M9)	971~983
sample.mgf	6	1069.6200	2	1069.6244	-0.0043 33	0.9579	K.KGDIIQLQR.R	sp|P07814			666~674
sample.mgf	7	1002.5348	3	1002.5346	0.0003 37	0.9968	R.LSKEEIER.M	sp|P34931			512~519
sample.mgf	8	1341.7784	2	1341.7768	0.0016 57	0.9959	K.GFDILGIKPVQR.M		sp|P54136		648~659
sample.mgf	10	2440.3178	3	2440.3114	0.0064 70	0.9995	R.HFVTISSPLATQIPQAVGAAVAAK.R		sp|P12694		201~224
sample.mgf	13	2145.1658	3	2145.1681	-0.0023 48	0.9506	K.GAEADQIIEVLKQQVSLLK.E	sp|Q12904			15~33
sample.mgf	15	1172.5690	2	1172.5673	0.0017 31	0.9399	R.LLQEEEQER.R	sp|O14654			1074~1082
sample.mgf	16	1297.6740	2	1297.6739	0.0002 63	0.9990	R.NNNQQLAQLQK.E	sp|Q9Y2A7			71~81
sample.mgf	19	1853.9995	4	1853.9999	-0.0005 54	0.9660	K.KIHTEPQLSAALEVVR.S		sp|P47897		80~95
sample.mgf	22	1115.6342	2	1115.6339	0.0004 36	0.9890	K.ADLTALFLPR.Q	sp|Q13045			875~884
sample.mgf	23	2175.9318	2	2175.9379	-0.0060 85	0.9996	R.VHTSQSGDEMTSLSEVVSR.M	sp|P08238			457~475
sample.mgf	24	1807.9548	2	1807.9508	0.0040 51	0.9821	K.HSQFIGVPITLVLEK.E	sp|Q58FF7			184~198
sample.mgf	25	2455.1953	2	2455.1867	0.0087 56	0.9144	K.VASTENGIIFGNIVVDVSGAASDR.N		sp|P53618	Deamidated(N6)	791~814
sample.mgf	26	974.4577	3	974.4570	0.0007 36	0.9962	K.VADLSHNR.L	sp|P54136			505~512
sample.mgf	29	1587.8758	2	1587.8733	0.0026 75	0.9997	K.THINIVVIGHVDSGK.S		sp|P68104		6~20
sample.mgf	30	1783.7990	3	1783.8013	-0.0023 54	0.9475	K.STHSELLEDVYQSGR.M		sp|P28288		348~362
sample.mgf	32	1323.6470	2	1323.6459	0.0012 48	0.9868	K.FNQVLDQFGEK.F	sp|O43242			375~385
sample.mgf	35	1739.8536	2	1739.8454	0.0083 60	0.9951	K.RSIQFVDWCPTGFK.V		sp|P68363		339~352
sample.mgf	36	1545.7738	2	1545.7715	0.0024 60	0.9975	K.LYYNLDDIAVVGK.P		sp|P11182		121~133
sample.mgf	38	1408.8296	2	1408.8289	0.0007 53	0.9589	K.ILPTLEAVAALGNK.V		sp|Q12905		128~141
sample.mgf	39	1052.6014	2	1052.6018	-0.0004 33	0.9669	R.YLTVAAIFR.G	sp|Q13885			310~318
sample.mgf	40	1783.8012	2	1783.8013	-0.0001 60	0.9877	K.STHSELLEDVYQSGR.M		sp|P28288		348~362
sample.mgf	42	2394.2101	2	2394.2101	-0.0000 86	0.9991	R.DKVLTAEELNAAQTSVAVGCIK.V		sp|P54136	Methyl(K22)	483~504
sample.mgf	43	1126.6491	2	1126.6498	-0.0008 43	0.9507	K.ALTGGIAHLFK.Q	sp|PO9622			133~143
sample.mgf	44	1243.6931	2	1243.6958	-0.0027 46	0.9744	R.LMDLLGEGLKR.S		sp|P54136		462~472
sample.mgf	45	2206.1335	3	2206.1416	-0.0080 58	0.9686	K.ASHNIGIAMDTEQGLIVPNVK.N		sp|P11182		340~360
sample.mgf	46	1629.7950	2	1629.7960	-0.0009 59	0.9707	K.AFVPEEISSMVLTK.L		sp|P34931	Oxidation(M10)	115~128
sample.mgf	47	2398.1593	3	2398.1553	0.0040 66	0.9968	R.QLFHPEQLITGKEDAANNVAR.G		sp|Q71U36	Gln->pyro-Glu(Q1) Deamidated(N17)	
sample.mgf	48	2089.0173	2	2089.0230	-0.0057 85	0.9997	R.WQVLLMAAEPVETIAFK.V	sp|Q15428		Oxidation(M6)	166~182
sample.mgf	56	2113.0982	3	2113.0990	-0.0008 50	0.9398	K.TDKQPLQKPSHCTIVVAK.N	sp|Q9P2J5			925~942
sample.mgf	58	1299.6732	3	1299.6724	0.0008 45	0.9987	K.YHIIHADIVR.W	sp|P56192			336~345
sample.mgf	60	1622.8247	3	1622.8205	0.0042 50	0.9951	R.FPPEASGVLHIGHAK.A		sp|P07814		202~216
sample.mgf	62	2079.0122	2	2079.0061	0.0061 88	0.9997	R.RSFITTDVNPVYVDSFVR.W		sp|Q9P2J5		203~219
sample.mgf	63	2079.0130	3	2079.0061	0.0068 75	0.9999	R.RSFITTDVNPVYVDSFVR.W		sp|Q9P2J5		203~219
sample.mgf	64	1103.5408	2	1103.5400	0.0009 36	0.9820	R.WLWSEVER.L	sp|A2RTX5			24~31
sample.mgf	65	2104.0090	2	2104.0047	0.0043 103	1.0000	R.GVGVFGDMAQDTGIPADIWR.F		sp|P56192		599~618
sample.mgf	66	2482.2585	4	2482.2532	0.0053 57	0.9931	K.EKLDQLKQEFEFWYPVDLR.V	sp|Q9P2J5			653~671
sample.mgf	69	2184.2525	3	2183.2565	0.9959 61	0.9569	K.LNGQVLVFTLPLTDQVSVIK.V		sp|Q15459		722~741
sample.mgf	70	1243.6391	2	1243.6408	-0.0017 50	0.9890	K.ADKNEVAAEVAK.L		sp|P56192		864~875
sample.mgf	78	2698.3491	2	2698.3391	0.0100 67	0.9061	K.VNGEDPVPHKFHVDISLTDFIQK.V		sp|Q15046		89~111
sample.mgf	81	2225.1079	3	2225.1157	-0.0078 71	0.9970	K.LDQLKQEFEFWYPVDLR.V	sp|Q9P2J5			655~671
sample.mgf	83	1455.7367	3	1455.7358	0.0009 65	0.9999	K.KRFDTEEEFKK.R	sp|P54136			278~288
sample.mgf	84	1327.6424	4	1327.6408	0.0016 33	0.9823	R.FDTEEEFKKR.A	sp|P54136			280~289
sample.mgf	85	1171.5390	2	1171.5397	-0.0006 40	0.9168	R.FDTEEEFKK.R	sp|P54136			280~288
sample.mgf	87	1601.8591	3	1601.8565	0.0025 69	0.9998	R.VFHAGFGVVLGEDKK.K		sp|P54136		436~450
sample.mgf	89	3191.6221	4	3191.6067	0.0154 69	0.9824	R.VGVTVAQTTMEPHLLEACVRDVLNDTAPR.A		sp|P47897		539~567
sample.mgf	91	2061.0227	3	2061.0279	-0.0052 74	0.9999	R.SQAIHQLKVNGEDPVPHK.F	sp|Q15046		Deamidated(N10)	81~98
sample.mgf	101	1992.9664	2	1992.9694	-0.0030 67	0.9969	R.THNLEPYFESFINNLR.R	sp|P04264			224~239

Practice: Change the parameter as below and run MODi.

- qtof_msms_param.xml

```
<?xml version="1.0" encoding="UTF-8"?>
<search user="Na" title="Sample" >

    <dataset local_path="qtof_msms.mgf" format="mgf" instrument="QTOF" />   <!--
    format=[mgf|pkl|dta], instrument=[QTOF|TRAP] -->
    <database local_path="protdb.fasta" />

    <combined_enzyme name="Trypsin" nterm_cleave="" cterm_cleave="KR" />

    <instrument_resolution ms="low" msms="low" /> <!-- ms / msms =[high|low] -->

    <parameters>
        <enzyme_constraint max_miss_cleavages="2" min_number_termini="2" />
        <isotope_error C13_number="0" />
        <peptide_mass_tol value="0.5" unit="Da" /> <!-- unit=[Da|ppm] -->
        <fragment_ion_tol value="0.5" unit="Da" /> <!-- unit=[Da] (NOT ALLOWED PPM) -->

        <modified_mass_range min_value="-150" max_value="250" />
    </parameters>

    <decoy_search checked="0" /> <!-- with reverse sequences -->
    <mod_map checked="0" />

    <modifications>
    <!-- for mod, site=[AA|N-term|C-term],
    position=[ANYWHERE|ANY_N(C)_TERM|PROTEIN_N(C)_TERM] -->
        <fixed>
            <mod name="Carbamidomethyl" site="C" position="ANYWHERE" massdiff="57.02150"
            />
            <!-- for fix mod, NOT ALLOWED a combination of site="AA" and
            position="ANY_N_TERM" -->
        </fixed>
        <variable local_path="PTMDB_s.xml" canBeModifiedOnFixedAA="1"> <!--
        local_path=external ptm file path (attached), canBeModifiedOnFixedAA=[0|1] -->
            <!--mod name="NT+12" site="N-term" position="ANY_N_TERM" massdiff="12.00"
            /--> <!-- specify additional mods -->
        </variable>
    </modifications>

</search>
```

Practice: Change the parameter as below and run MODi.

- trap_msms_param.xml

```
<?xml version="1.0" encoding="UTF-8"?>
<search user="Na" title="Sample" >

    <dataset local_path="trap_msms.mgf" format="mgf" instrument="TRAP" />   <!--
    format=[mgf|pkl|dta], instrument=[QTOF|TRAP] -->
    <database local_path="protdb.fasta" />

    <combined_enzyme name="Trypsin" nterm_cleave="" cterm_cleave="KR" />

    <instrument resolution ms="low" msms="low" /> <!-- ms / msms =[high|low] -->

    <parameters>
        <enzyme_constraint max_miss_cleavages="2" min_number_termini="2" />
        <isotope_error C13_number="0" />
        <peptide_mass_tol value="3" unit="Da" /> <!-- unit=[Da|ppm] -->
        <fragment_ion_tol value="0.6" unit="Da" /> <!-- unit=[Da] (NOT ALLOWED PPM) -->

        <modified_mass_range min_value="-150" max_value="250" />
    </parameters>

    <decoy_search checked="0" /> <!-- with reverse sequences -->
    <mod_map checked="0" />

    <modifications>
    <!-- for mod, site=[AA|N-term|C-term],
    position=[ANYWHERE|ANY_N(C)_TERM|PROTEIN_N(C)_TERM] -->
        <fixed>
            <mod name="Carbamidomethyl" site="C" position="ANYWHERE" massdiff="57.02150"
            />
            <!-- for fix mod, NOT ALLOWED a combination of site="AA" and
            position="ANY_N_TERM" -->
        </fixed>
        <variable local_path="PTMDB_s.xml" canBeModifiedOnFixedAA="1"> <!--
        local_path=external ptm file path (attached), canBeModifiedOnFixedAA=[0|1] -->
            <!--mod name="NT+12" site="N-term" position="ANY_N_TERM" massdiff="12.00"
            /--> <!-- specify additional mods -->
        </variable>
    </modifications>

</search>
```