

Effective design of multiplexed quantitative SRM assays with Skyline

Joseph Brown, Ph.D.

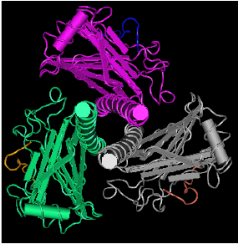
Pacific Northwest National Laboratories

Minneapolis, MN; Skyline Workshop; 61st ASMS

SRM pipeline flowchart

Target Selection

Protein Selection

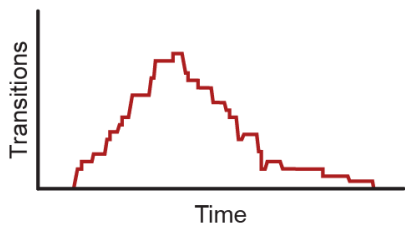


Peptide/
Transition Selection

M A S S S P E C T R



Transition Scheduling



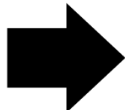
Assay Development

Collision Energy
Optimization

Assess Transition
Quality
(e.g. Matrix Interference)

Calibration Curves

Retention Time
Reproducibility

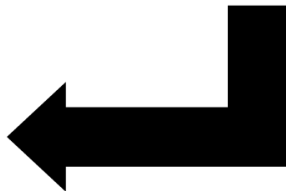


Data
Acquisition

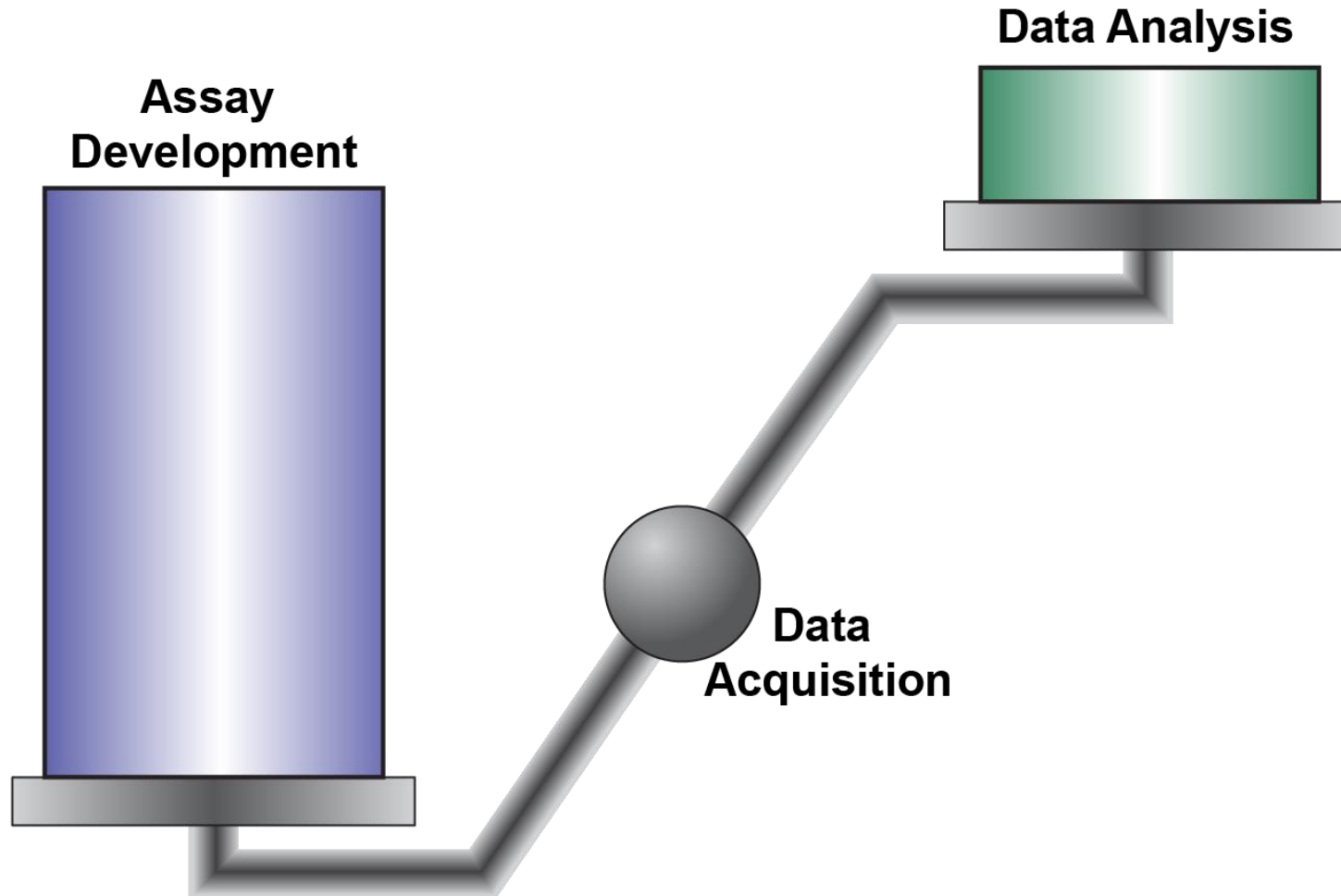


Data Analysis

Abundance Calc.
Statistical Tests
Sensitivity / Selectivity



Investing on assay development simplifies data analysis



How do you choose the “best” transition, or is an “OK” transition sufficient?

Peptides

Skyline

File Edit View Settings Tools Help

Targets

ref|NP_940978.2

- R.ESLLDGGNK.V [82, 90]
- K.VVISGFGDPLICDNQVSTGDTR.I [91, 112]
- R.IFRVNPAPPYLPWAHKN [113, 128]
- K.NELMLNSSLMR.I [129, 139]
- R.GMLCGFGAVCEPNAEGPSR.A [173, 191]
- R.DPGSNVTCSFGSTCAR.S [245, 260]
- R.SADGLTASGLCPATCAR.G [251, 276]
- R.GAPEGTVCGSDGADYFGEQLLR.R [277, 299]
- K.FDGFCDPCGGALPDPFSR.S [312, 328]
- R.RPEMLLRPESCPAR.Q [338, 351]
- R.QAPVCGDDGVTYENDVMGR.S [352, 371]
- R.DQCPEPCR.F [392, 399]
- R.FNAVCLSR.R [400, 407]
- R.VTGDGAYRPVCAQDGR.T [418, 433]
- R.TYDSDCWR.Q [434, 441]
- R.FGALCEAETGR.C [541, 551]
- R.CEHPHPPGPVCGSDGVTYGSACELR.E [622, 645]
- R.EAACLQQTQIEEAR.A [646, 659]
- R.GLYVAAGACR.G [740, 750]
- R.CEFGFWNFR.G [825, 833]
- R.SGCTPCSDPQGA VR.D [841, 855]
- R.DDCEQM TGLCSKPGVAGPK.C [856, 875]
- K.GQC PDGR.A [876, 883]
- R.ALGPAGCEADASAPATCAEMR.C [884, 904]
- R.CVVEESGAHCVCFMLTCEANATK.V [911, 934]
- K.VCGSDGVTYGNEQLK.T [935, 950]
- R.ASCYNALGCCSDGK.T [1102, 1116]
- K.TPSLDAEGSNCPATK.V [1117, 1131]
- K.SELFGETAR.S [1158, 1166]
- R.SIESTLDDLFR.N [1167, 1177]
- R.AIVDHFDPPTAFR.A [1201, 1214]
- R.RPLQEHVR.F [1238, 1245]
- R.LPSSAVTPR.A [1278, 1296]
- R.APHPSHTSOPVAK.T [1287, 1299]
- K.TTAAPTTR.R [1300, 1307]
- R.RPPTAPSR.V [1308, 1316]
- K.VLGAPVFAFEGR.S [1368, 1379]
- R.SFLAFTLR.A [1380, 1388]
- R.ALEPQGLLYGNAR.G [1401, 1415]
- K.DFLALALLDGR.V [1418, 1428]
- R.FDTGSGPAVLTSVAVPFGQWHR.L [1433, 1455]
- R.TFVGAGLR.G [1509, 1516]
- R.LLDVNNQR.L [1521, 1528]
- R.LELGGPGAATR.G [1529, 1540]
- R.FHQCPGPR.V [1571, 1579]
- R.VGPTCADEK.S [1580, 1588]
- K.SPQPNPCHGAAPCR.V [1589, 1603]
- R.VLPEGGAQCEPLGR.E [1604, 1618]
- K.MALEVFLAR.G [1661, 1670]
- R.GPSGLLYNGOK.T [1671, 1682]
- K.GDFVSLAR.D [1687, 1695]
- R.EPVTLGAWTR.V [1716, 1725]
- R.VLGESEPVHTVNLK.E [1745, 1759]
- K.EPLYGGAPDFSKL [1760, 1772]
- R.AAAVSSGFDGAIQLVSLGGR.Q [1776, 1795]
- R.QLLTPEHLR.Q [1796, 1805]
- R.QVDVTSFAGHPCR.A [1806, 1819]
- R.ASGHPCLNGASCVPR.E [1820, 1834]
- R.EAAYVCLCPGGFSGPHCEK.G [1835, 1853]
- K.SAGDVDTLAFDGR.T [1859, 1871]
- R.TFVEYLNVAETSEK.A [1872, 1885]
- K.ALQSNHFELSLR.T [1886, 1897]
- R.TEATQGLVLSGK.A [1898, 1910]
- R.STVPVNTNR.W [1942, 1950]
- K.AYGTGFVGLR.D [2007, 2017]
- R.HPLHLEDAVTKPELRCP.TP. [2024, 2044]



Transitions

ref|NP_940978.2

R.ESLLDGGNK.V [82, 90]

K.VVISGFGDPLICDNQVSTGDTR.I [91, 112]

1175.5708++

- L [y13] - 1478.6904+
- I [y12] - 1365.6063+
- C [y11] - 1252.5223+
- D [y10] - 1092.4916+
- N [y9] - 977.4647+
- Q [y8] - 863.4217+
- V [y7] - 735.3632+
- S [y6] - 636.2947+
- T [y5] - 549.2627+
- G [y4] - 448.2150+
- D [y3] - 391.1936+
- T [y2] - 276.1666+
- R [y1] - 175.1190+
- V [y21] - 1126.0366++
- I [y20] - 1076.5024++
- S [y19] - 1019.9604++
- G [y18] - 976.4444++
- F [y17] - 947.9336++
- G [y16] - 874.3994++
- D [y15] - 845.8887++
- P [y14] - 788.3752++
- L [y13] - 739.8488++
- I [y12] - 683.3068++
- C [y11] - 626.7648++
- D [y10] - 546.7494++
- N [y9] - 489.2360++
- Q [y8] - 432.2145++
- V [y7] - 368.1852++
- S [y6] - 318.6510++
- T [y5] - 275.1350++
- G [y4] - 224.6112++
- D [y3] - 196.1004++
- T [y2] - 138.5870++
- R [y1] - 88.0631++
- 784.0496+++
- 588.2890++++
- 470.8327, +5
- 392.5285, +6



- Prior Knowledge
 - Previous Experiments
 - Public Databases
 - NIST
 - PeptideAtlas
 - GPM
- Prediction Software
- SRM Collider

SRM Collider to predict potential matrix interferences

```

TEMTASSSSDYGQTSK/2, TEMTASSSSDYGQTSK, 2, 840.356881, 1217.528051, #N/A, 1, y12
TEMTASSSSDYGQTSK/2, TEMTASSSSDYGQTSK, 2, 840.356881, 1146.490937, #N/A, 1, y11
TEMTASSSSDYGQTSK/2, TEMTASSSSDYGQTSK, 2, 840.356881, 1059.458909, #N/A, 1, y10
TEMTASSSSDYGQTSK/2, TEMTASSSSDYGQTSK, 2, 840.356881, 520.272552, #N/A, 1, y5
TEMTASSSSDYGQTSK[136]/2, TEMTASSSSDYGQTSK[136], 2, 844.36398, 1225.54225, #N/A, 1, y12
TEMTASSSSDYGQTSK[136]/2, TEMTASSSSDYGQTSK[136], 2, 844.36398, 1154.505136, #N/A, 1, y11
TEMTASSSSDYGQTSK[136]/2, TEMTASSSSDYGQTSK[136], 2, 844.36398, 1067.473108, #N/A, 1, y10
TEMTASSSSDYGQTSK[136]/2, TEMTASSSSDYGQTSK[136], 2, 844.36398, 528.286751, #N/A, 1, y5
MEC[160]NPSQVNGSR/2, MEC[160]NPSQVNGSR, 2, 689.795542, 958.470083, #N/A, 1, y9
MEC[160]NPSQVNGSR/2, MEC[160]NPSQVNGSR, 2, 689.795542, 844.427155, #N/A, 1, y8
MEC[160]NPSQVNGSR/2, MEC[160]NPSQVNGSR, 2, 689.795542, 532.283785, #N/A, 1, y5
MEC[160]NPSQVNGSR/2, MEC[160]NPSQVNGSR, 2, 689.795542, 433.215371, #N/A, 1, y4
    
```

SSRCalc window: arbitrary units

Q1 mass window: Th

Q3 mass window: Th

Low mass threshold for transitions: Th

High mass threshold for transitions: Th

Genome:

Consider isotopes up to: amu

Missed Cleavages:

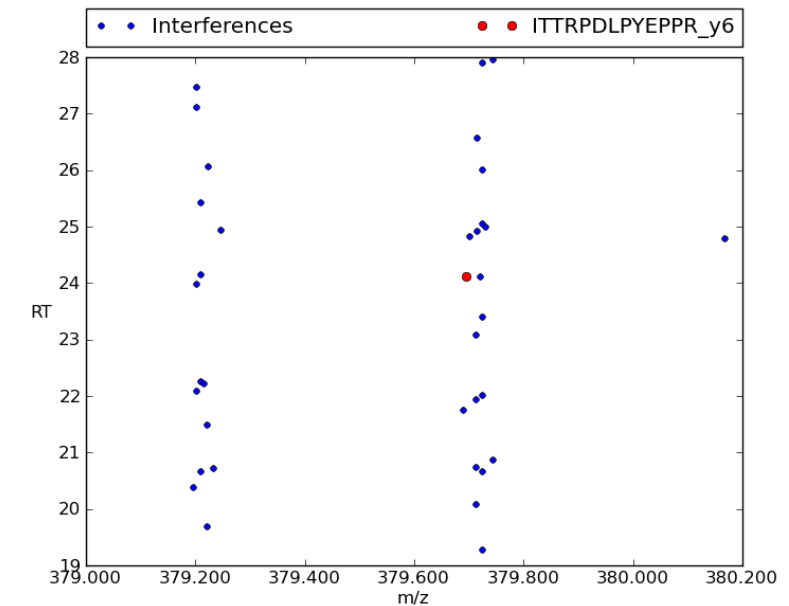
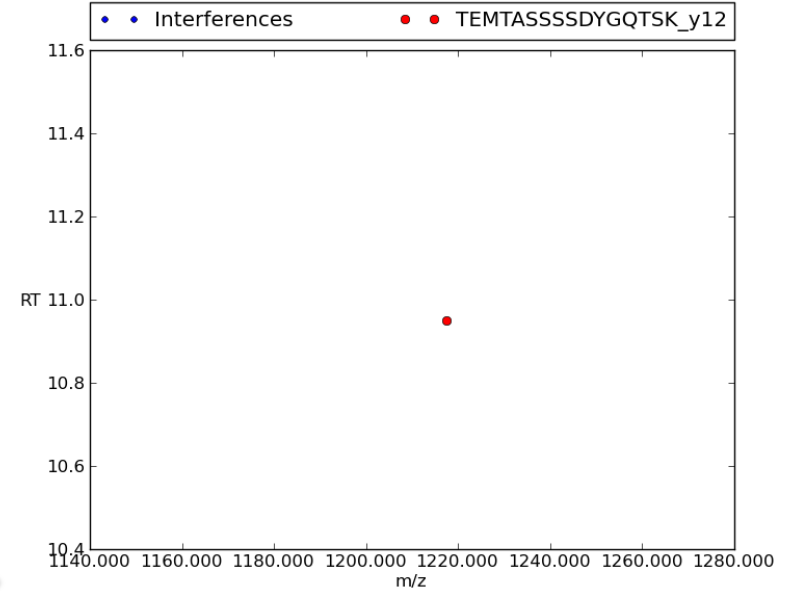
Find UIS up to order*:

Charge check: Check that interfering signal can actually hold charge (e.g. 2+ charge)

Modifications: oxidized Methionines deamidated Asparagines

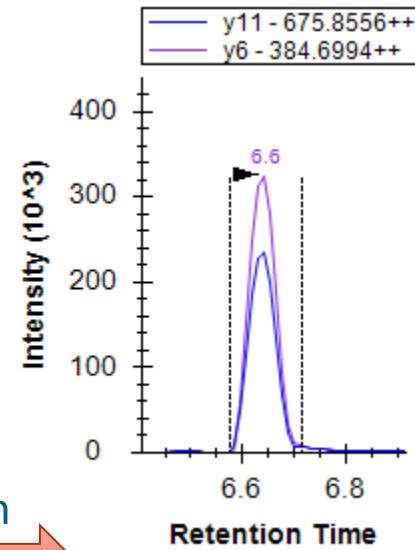
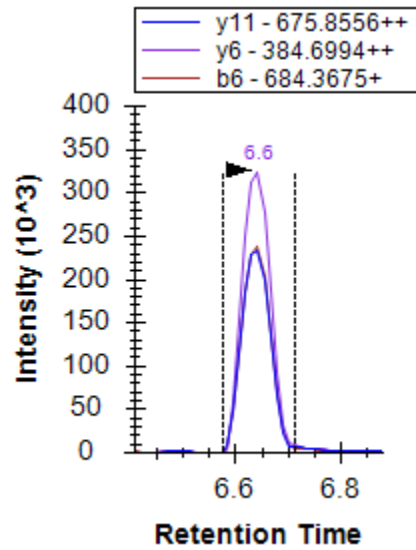
[Background Ion Series](#)

- a ions
- aMinusNH3
- b ions
- bMinusH2O
- bMinusNH3
- bPlusH2O
- c ions
- x ions
- y ions
- yMinusH2O
- yMinusNH3
- z ions
- MMinusH2O
- MMinusNH3



Assessing matrix interference

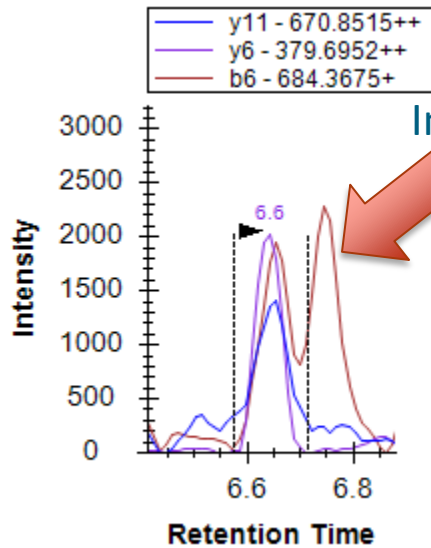
Heavy



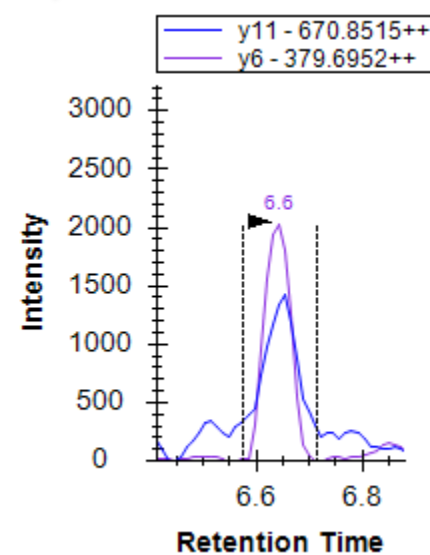
Remove b6-ion



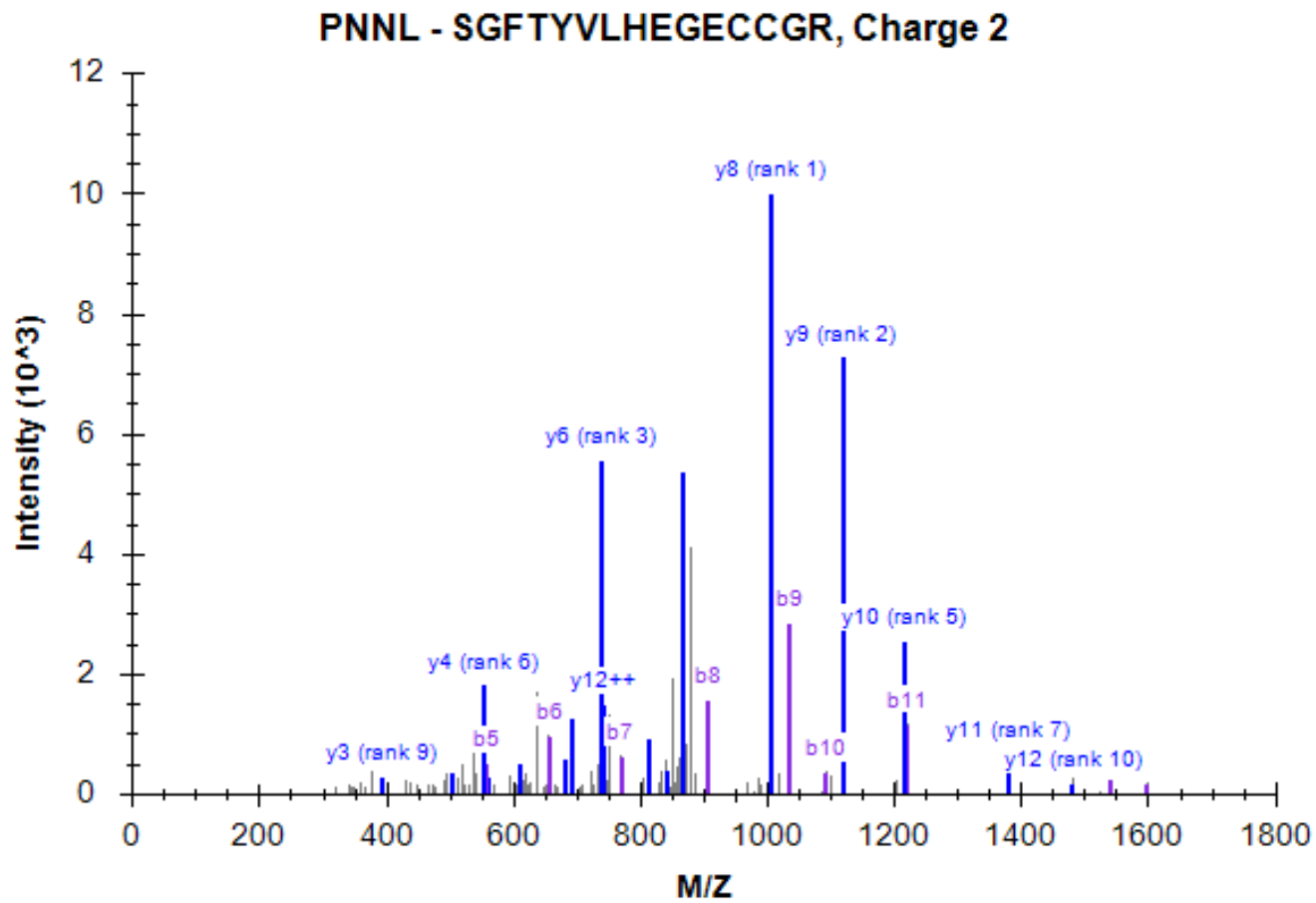
Light



Interfering Peak

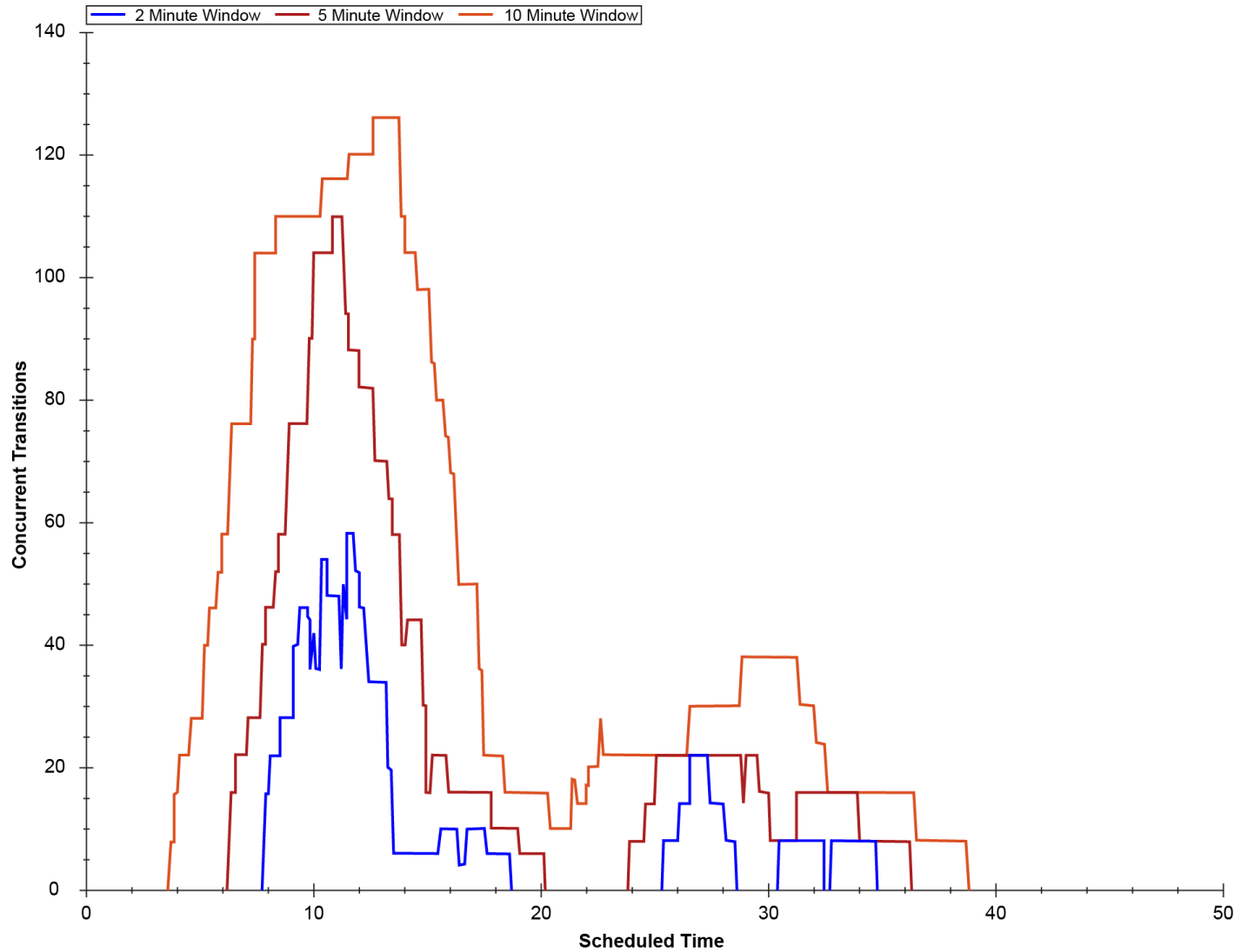


Spectral libraries visualization through Skyline

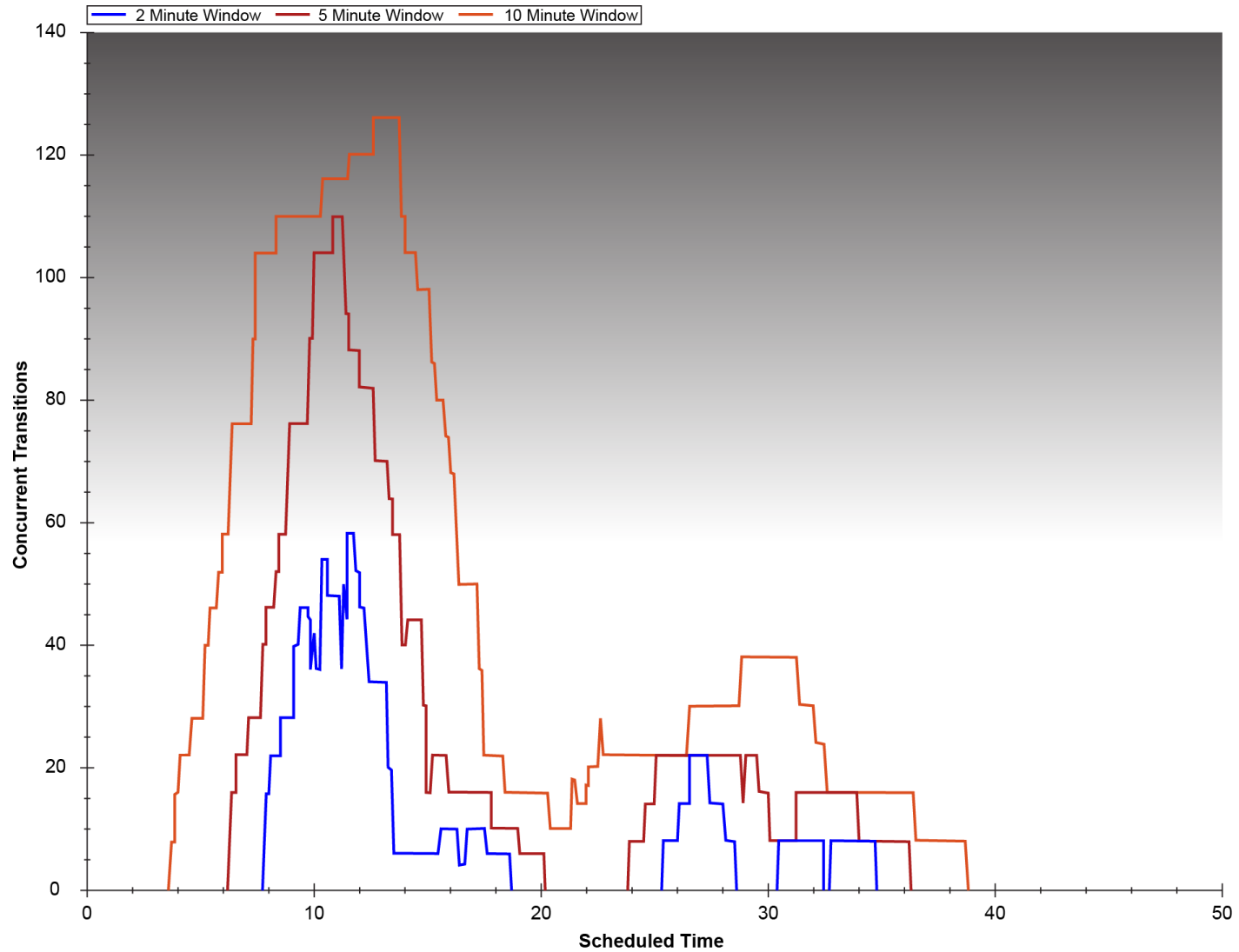


A
B
C
X
Y
Z
1
2
[Icons: Document, Folder, Monitor, Printer]

Transition scheduling



Transition scheduling



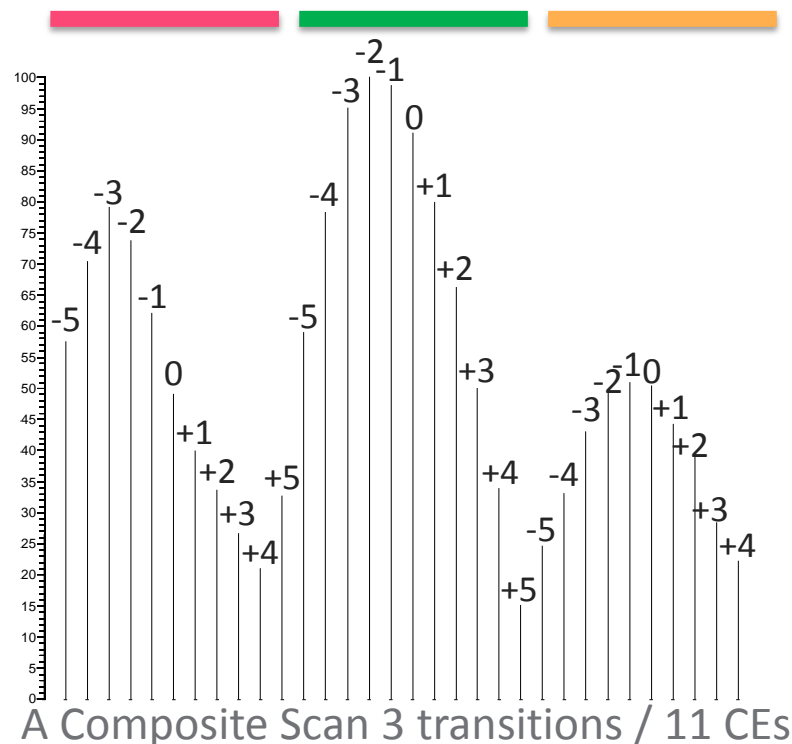
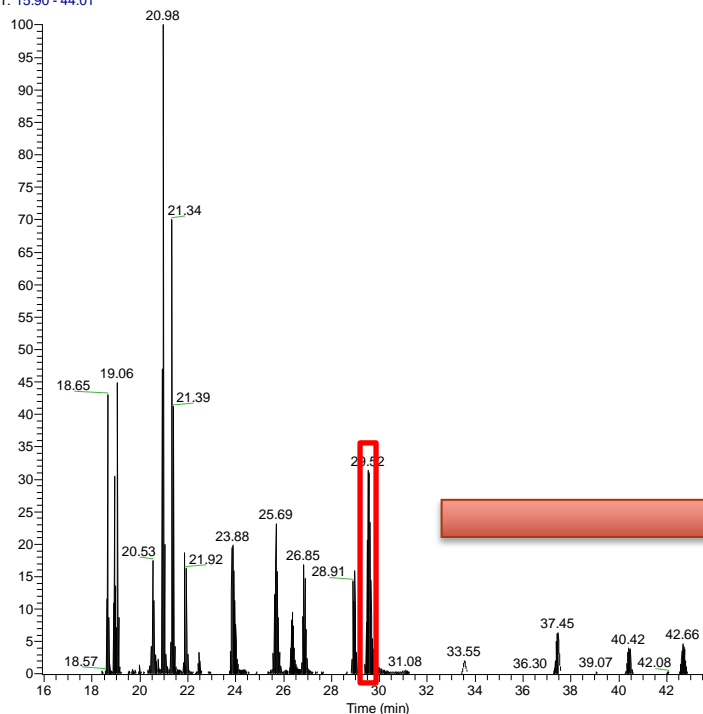
Collision energy optimization

Precursor	Product ion	Ramping CE
504.2363	613.3043	11.3
504.2363	613.3043	12.3
504.2363	613.3043	13.3
504.2363	613.3043	14.3
504.2363	613.3043	15.3
504.2363	613.3043	16.3
504.2363	613.3043	17.3
504.2363	613.3043	18.3
504.2363	613.3043	19.3
504.2363	613.3043	20.3
504.2363	613.3043	21.3

Precursor	Product ion	Ramping CE
504.2363	243.8715	11.3
504.2363	243.8715	12.3
504.2363	243.8715	13.3
504.2363	243.8715	14.3
504.2363	243.8715	15.3
504.2363	243.8715	16.3
504.2363	243.8715	17.3
504.2363	243.8715	18.3
504.2363	243.8715	19.3
504.2363	243.8715	20.3
504.2363	243.8715	21.3

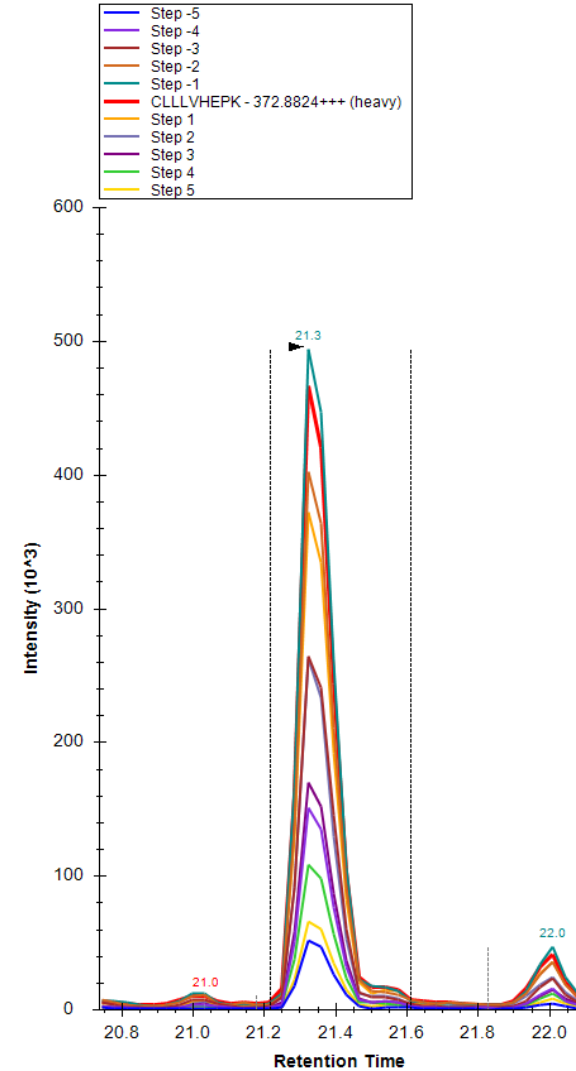
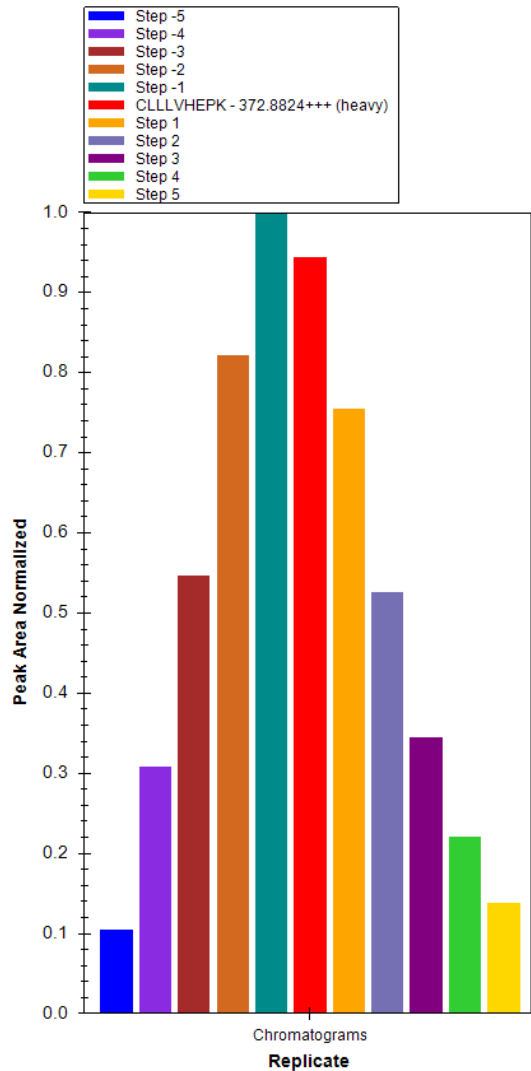
Precursor	Product ion	Ramping CE
504.2363	874.3346	11.3
504.2363	874.3346	12.3
504.2363	874.3346	13.3
504.2363	874.3346	14.3
504.2363	874.3346	15.3
504.2363	874.3346	16.3
504.2363	874.3346	17.3
504.2363	874.3346	18.3
504.2363	874.3346	19.3
504.2363	874.3346	20.3
504.2363	874.3346	21.3

RT: 15.90 - 44.01



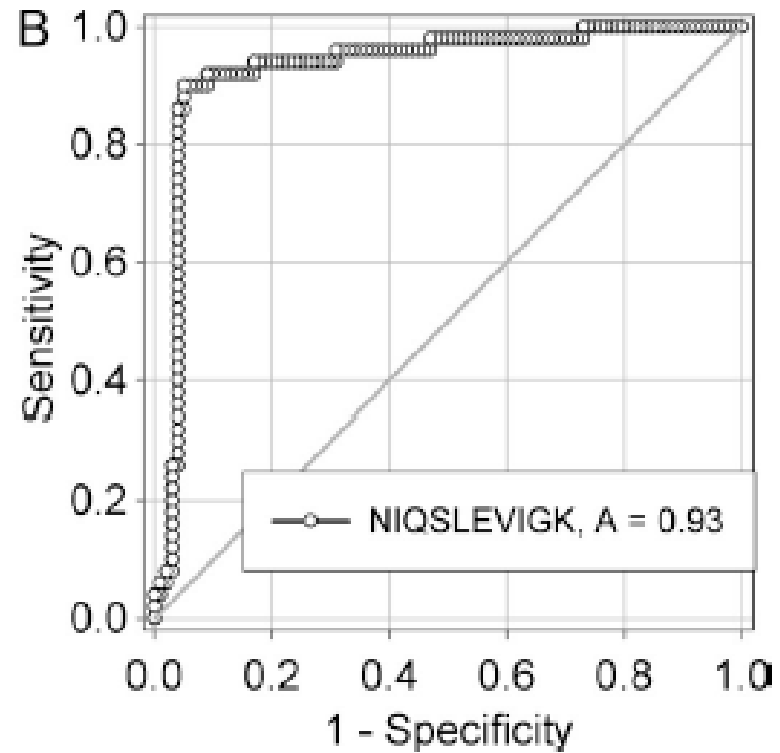
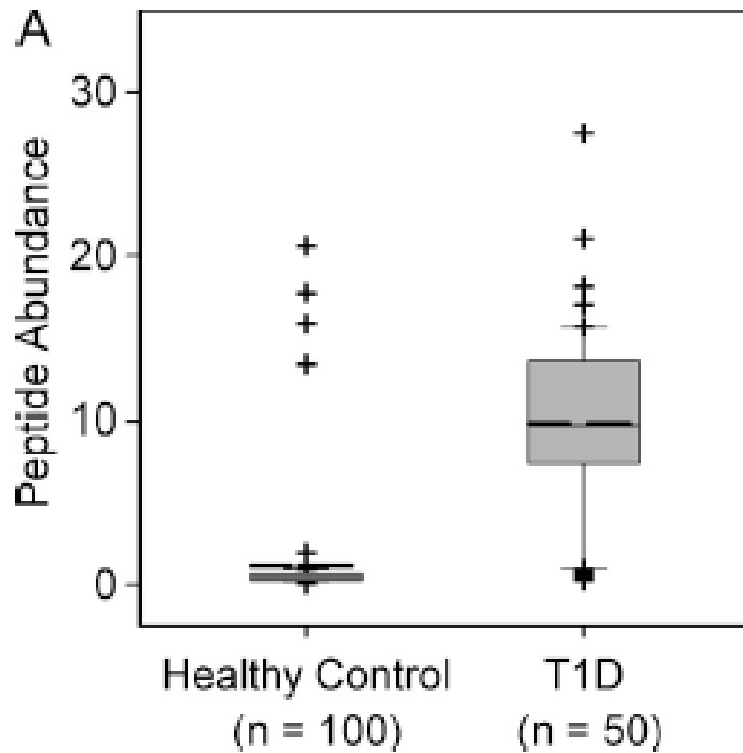
A Composite Scan 3 transitions / 11 CEs

Skyline automates collision energy optimization



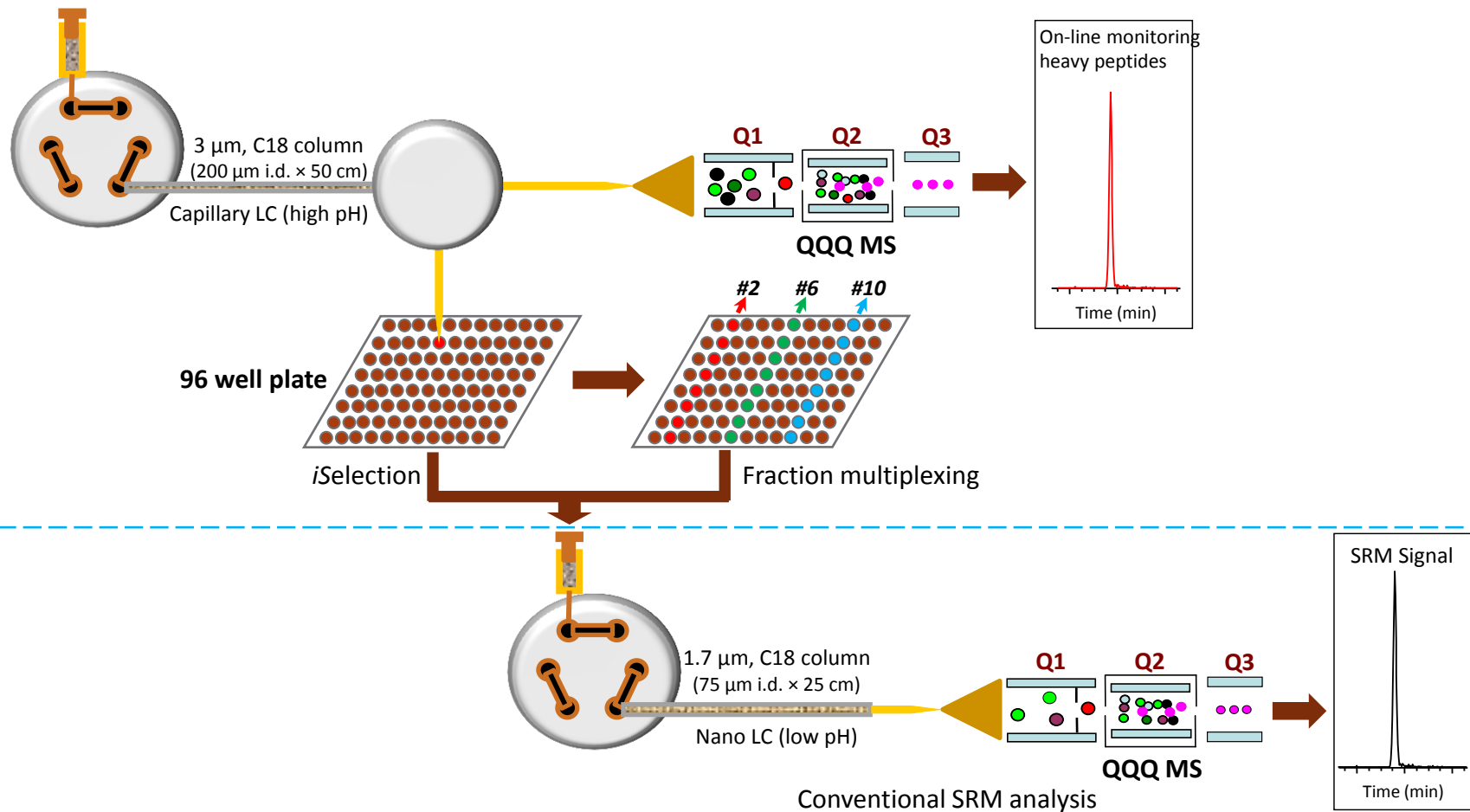
Peptide distinguishes T1D from healthy control

Platelet basic protein abundance in human serum



Increased fractionation with PRISM-SRM facilitates deep-dive approach

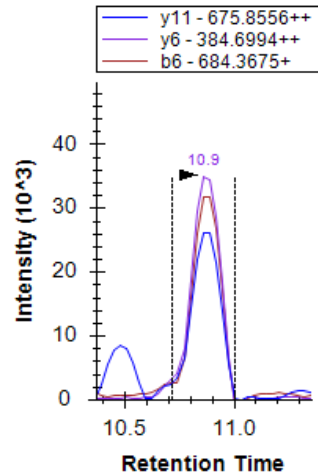
High-pressure, high-resolution separations coupled with intelligent selection & multiplexing



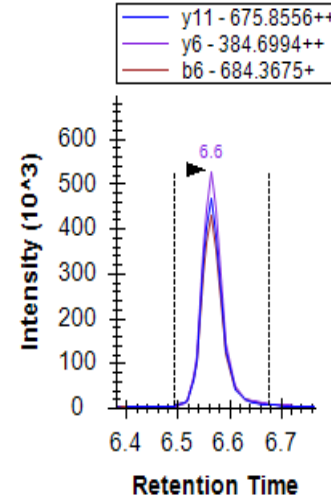
Use Skyline to visualize and inspect fractions

ITTRPDLPYEPPR

Conventional SRM

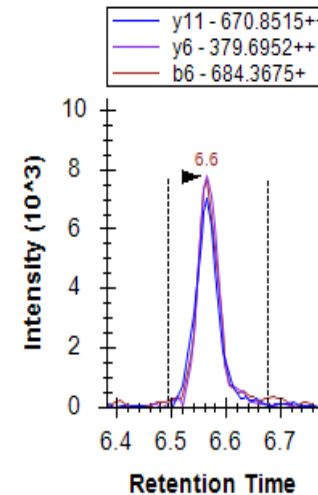
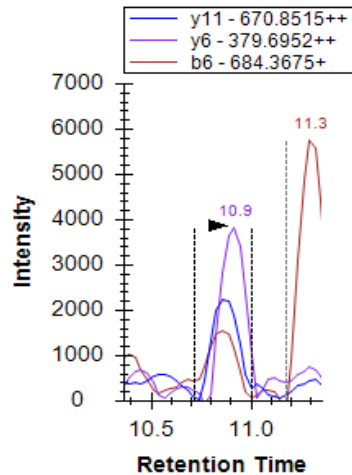


PRISM-SRM



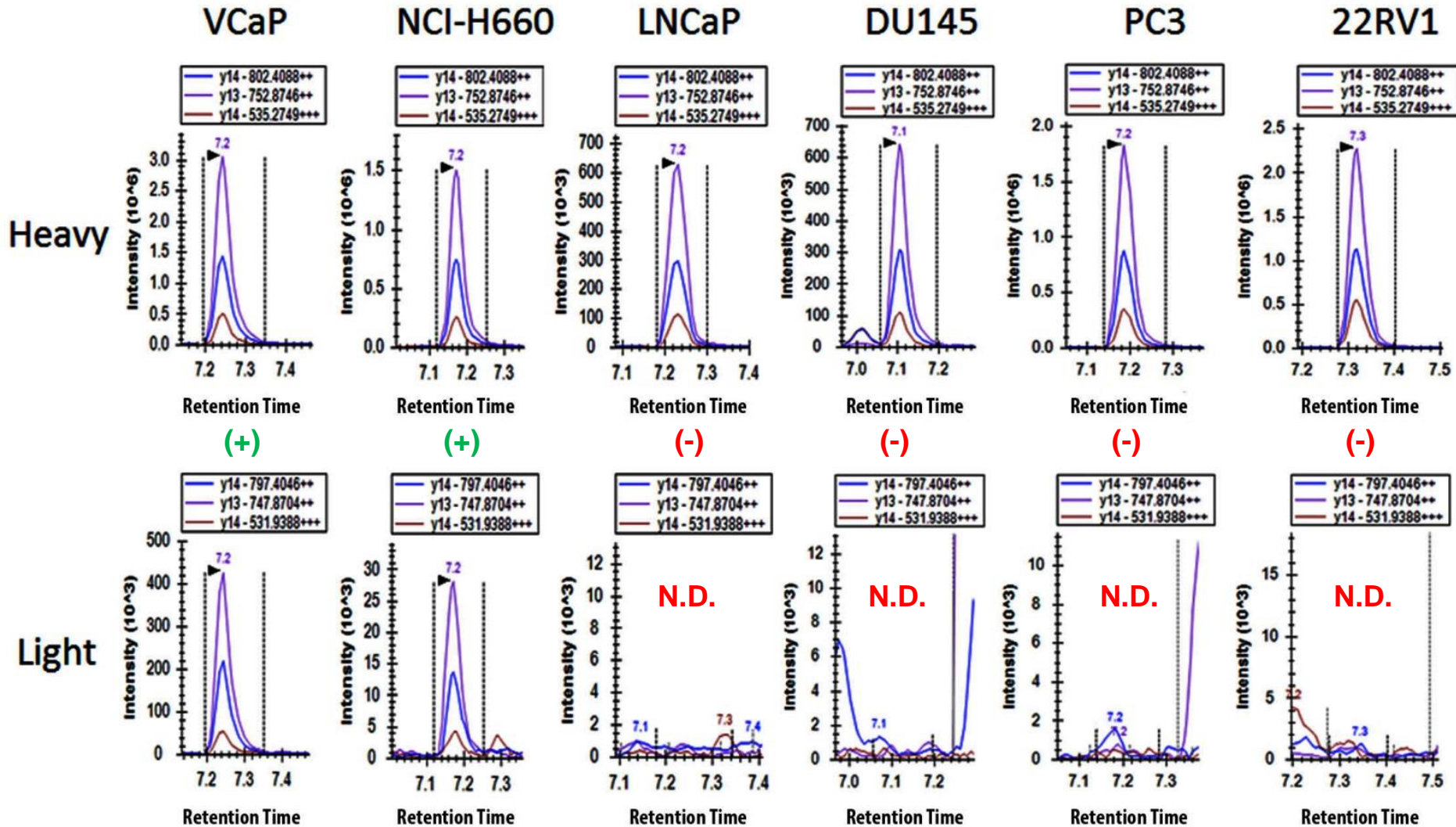
Increased S/N

Reduced Interference



Detection of an ERG peptide in TMPRSS2-ERG positive vs negative prostate cancer cell lines

VIVPADPTLWSTDHVR



Acknowledgments

Tao Liu

Weijun Qian

Tujin Shi

Tom Fillmore

Qibin Zhang

Chaochao Wu

Jintang He

Yuqian Gao

Tom Metz

Sam Payne

Gordon Anderson

David Camp

Karin Rodland

Dick Smith

T1D-specific peptide

Pro-platelet basic protein

Protein	Peptide	100 HC, 50 T1D			10 HC, 10 T1D			50 T2D				
		DASP Verification Cohort			DASP Blind Validation Cohort			T2D Specificity Cohort				
		FC ^a	P-value ^a	AUC	Specificity	Sensitivity	FC ^a	P-value ^a	FC ^b	P-value ^b	FC ^c	P-value ^c
PPBP	EESLDSLYAELR	1.6	8.41E-08	0.77	0.3	1	1.6	4.93E-02	-1.7	1.71E-04	11.0	4.44E-12
PPBP	NIQSLEVIGK	8.5	6.62E-18	0.93	1	1	30.4	1.08E-05	-1.2	7.44E-02	32.6	4.44E-12

