ⱳ ⱑ ⱘ

ⱓ ⱔ

Ⱄⱚⱳ ⱐ ⱒ ⱑ ⱓ ⱔ ⱗ ⱘ ⱙ ⱐ

2020.6.12

Ψ й ъ

2000-2004

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2004-2006

2006-2010 ⅵ

2011-2013

↑

2013-2018

2019-

№ Ţ

ѭ ï σ ɖ ḱ ̀ ▒ ~ Ẹ ɨ ڭ ڑ Ẇ ɨ ڭ

ㄩ ק ה ᴇ ᴺ ᵊ ̣ ̈ Ḥ ̈ Ḫ ɨ ڭ

a Ѧ ṵ ᴇ ᴺ ᵊ ~ ɔ̀ ! ɲ ɨ ڭ

¾ ͡ و ⅃ e̯ ḧ ǫ́ ˥ ε MS2 Æ ⅃ Æ ͡ ζ

@ ǐ̀ ǂ ٹ ḧ ͡ ˊ ڑ Ӣ ة ɥ ᴾ ٹ ḧ ...

¾ ḧ ڀ ͡ و ⅃ e̯ ε LFQ TMT/iTRAQ SWATH ... ζ

@ ʌ ℵ Ṯ ȁ ͡ ʾ Ӣ, Ă ɨ ⅃ PTM ...

¾ ᵇ ͡ و ⅃ e̯ ε MRM^{HR}/PRM ζ

@ و ؤ ³ ⌧WB

Ʊ (Discovery proteomics)

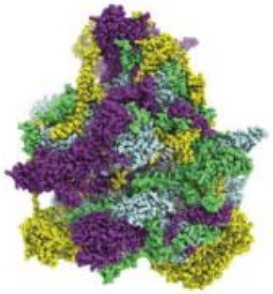
͡ و ㇳ e̯ χ ʁ ḧ ó̞ ḧ ˮ

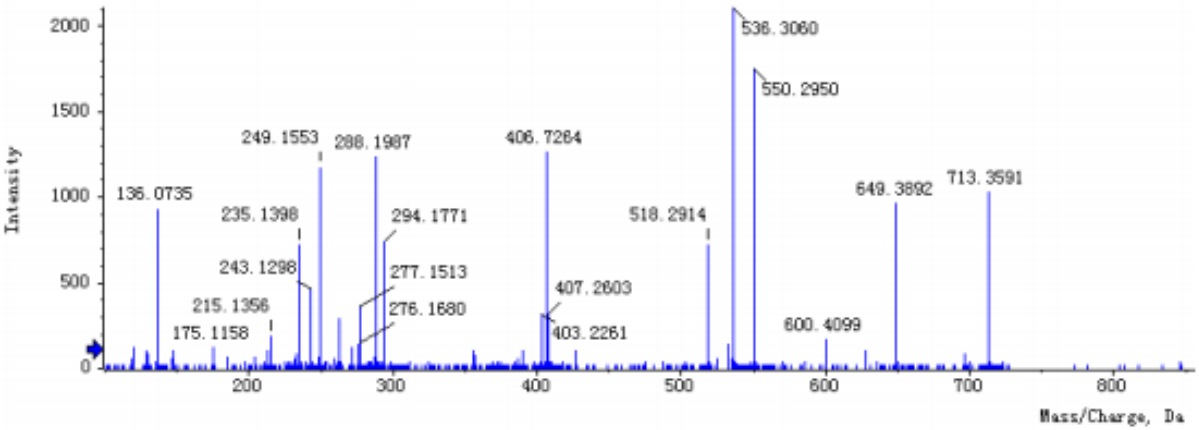
- ḧ ó̞ χ ͡ و ȶ ḧ ʜ ᵀ ɥ ȶ ḧ ㇳ ɲ ͡ و ㇳ ǻ PTM...
- ḧ ˮ χ ⅝ ɗ j ᵃ ↑ õ ï ͡ ↨ Ẇ⃞ Ψ ק Й Ă ㇳ e̯

--- ˚ ‰ L ḧ ˮ χ LFQ/SWATH

--- ‰ L ḧ ˮ χ SILAC/ ⚲ ᵍ ק /TMT/iTRAQ...

μ Ƕ





MW	ion			ion	MW
88	b_1	S	GFLEEDELK	y_9	1080
145	b_2	SG	FLEEDELK	y_8	1022
292	b_3	SGF	LEEDELK	y_7	875
405	b_4	SGFL	EEDELK	y_6	762
534	b_5	SGFLE	EDELK	y_5	633
663	b_6	SGFLEE	DELK	y_4	504
778	b_7	SGFLEED	ELK	y_3	389
907	b_8	SGFLEEDE	LK	y_2	260
1020	b_9	SGFLEEDEL	K	y_1	147

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á
á

W

Protein database → 模拟酶切 → …… NQWFFSK ……

模拟碎裂：

b_1	N——QWFFSK	y_1
b_2	NQ——WFFSK	y_2
b_3	NQW——FFSK	y_3
b_4	NQWF——FSK	y_4
b_5	NQWFF——SK	y_5
b_6	NQWFFS——K	y_6

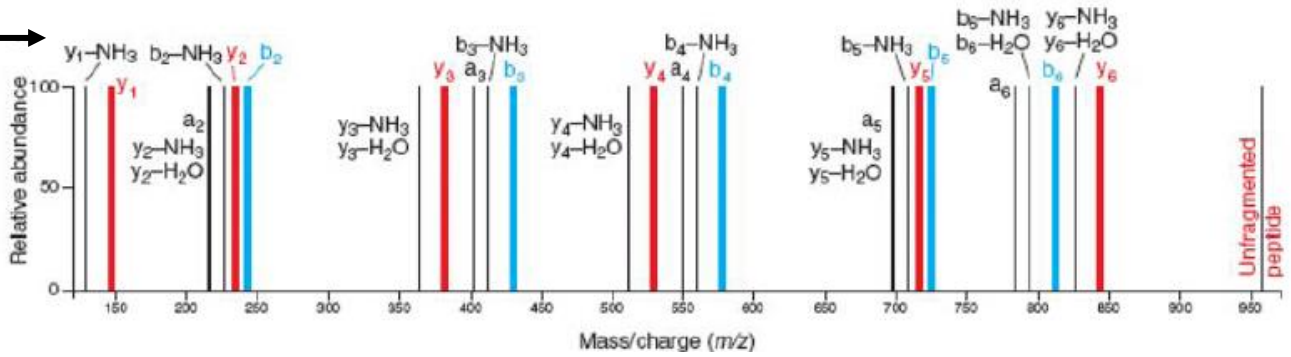
推测图谱

数据库检索比对

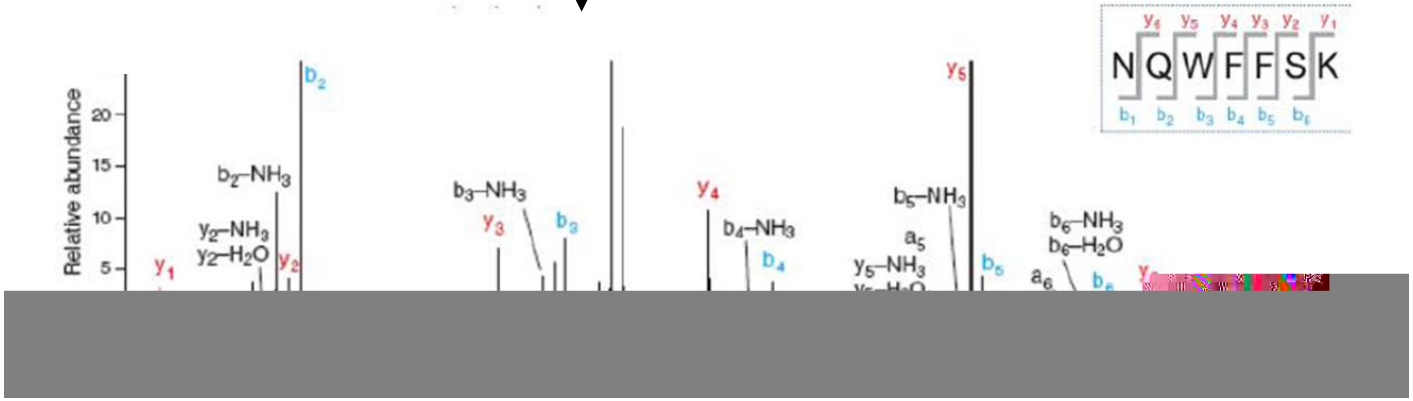
肽段鉴定

蛋白鉴定

相对定量



比对匹配



ئز

¾ ѣ ⅃ ḧ ⸗ ẻ Ã̃

z ⅄ Ḿ ḧ ⸗ : ת ỳ Ά Ã̃ ⁀ ⃞ ⅄ Ḿ ᵍ ⸗ ι Ẃ ƿ Ѫ Ẑ ₅ ⅃⅃

ѧ ε ⅝ ḅ χ ng/mL ⊢ ζ

z ʾ Ḿ ḧ ⸗ : ̈ ̃ ̿ ἄ ⁀ ƿ j ᵃ ̸ ʈ ï ⃞ Ḿ ᵍ ⸗ ⅝

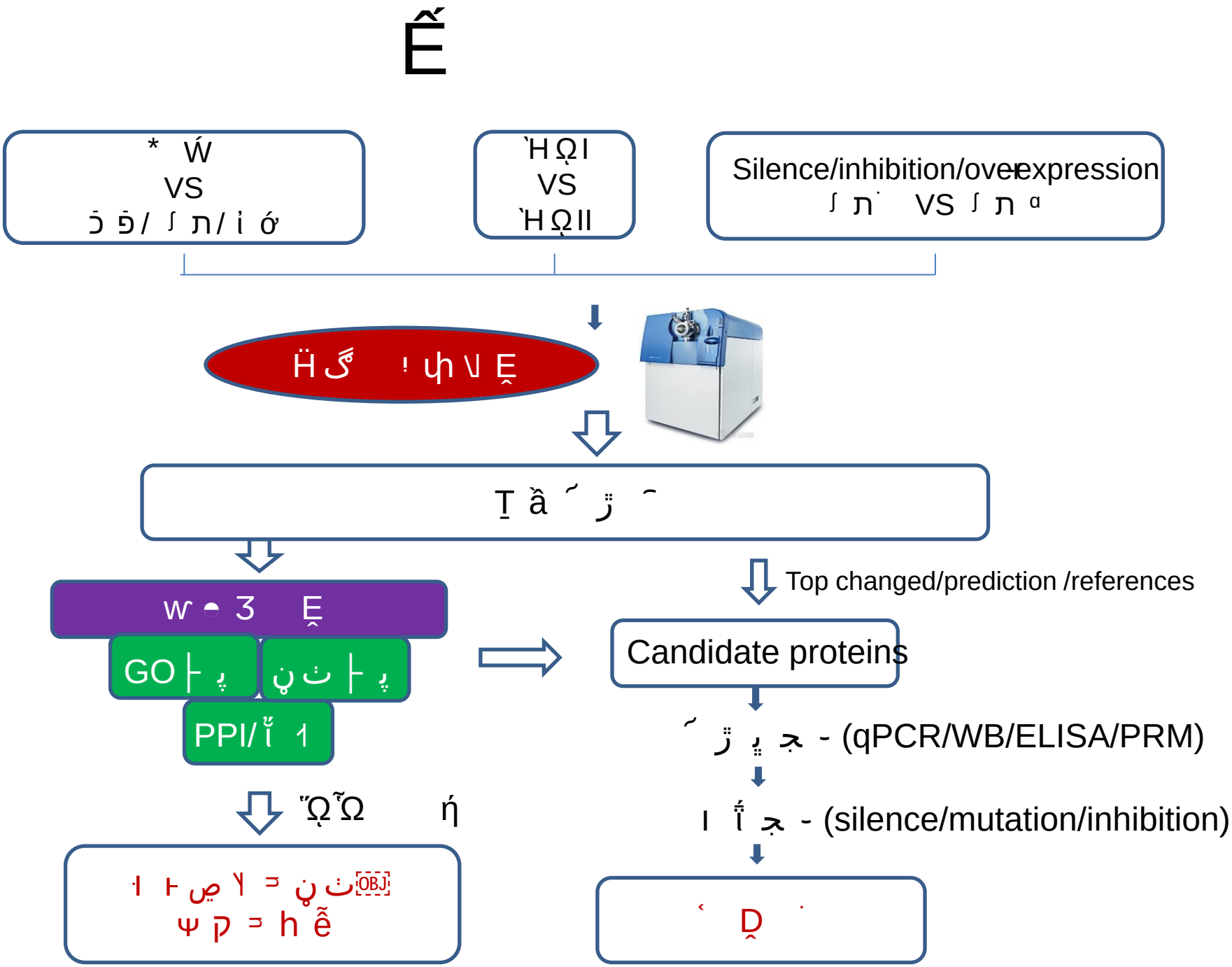
ᴗ ι Ẃ ƿ ᵍ ̃ ̣ ⅄ ε ⅝ ḅ χ ⊢ ̣ ☼/Ḿ Ł ζ

¾ ⁀ و ⅃ ḛ ḧ ⸗ ʿH

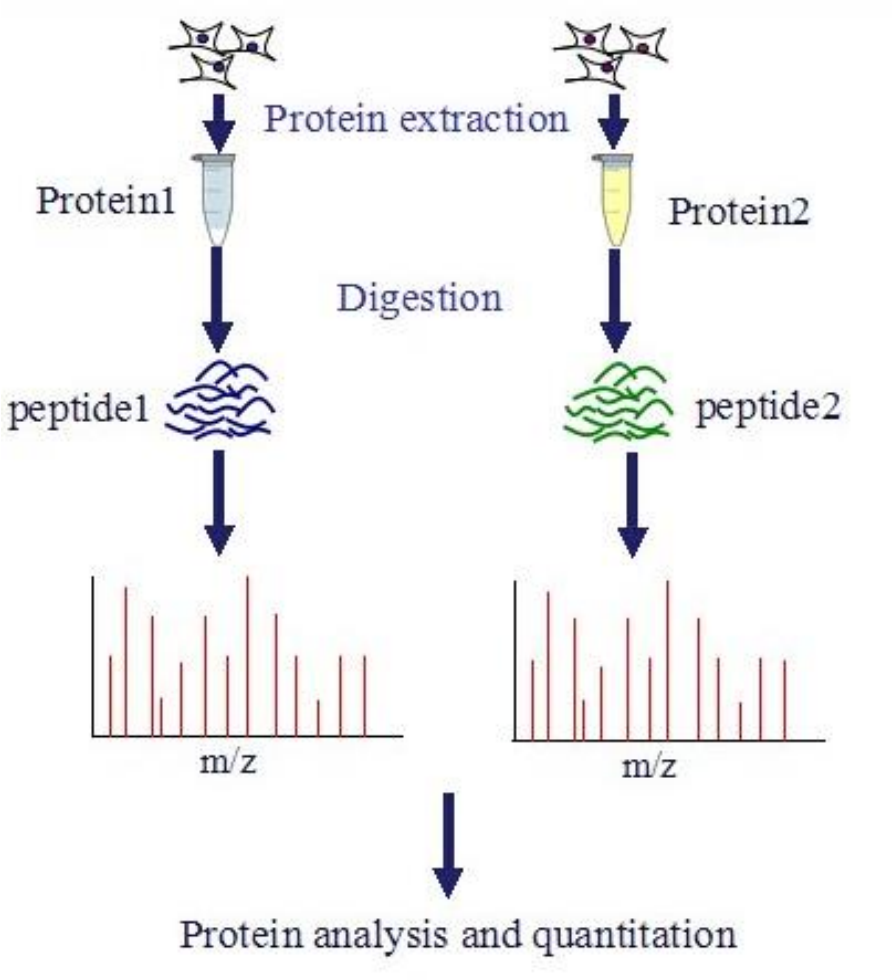
z ʾ ‰ L ḧ ⸗

z ‰ L ḧ ⸗ ε SILAC/ ☠ ᵍ ק /TMT/iTRAQ ζ

z SWATH

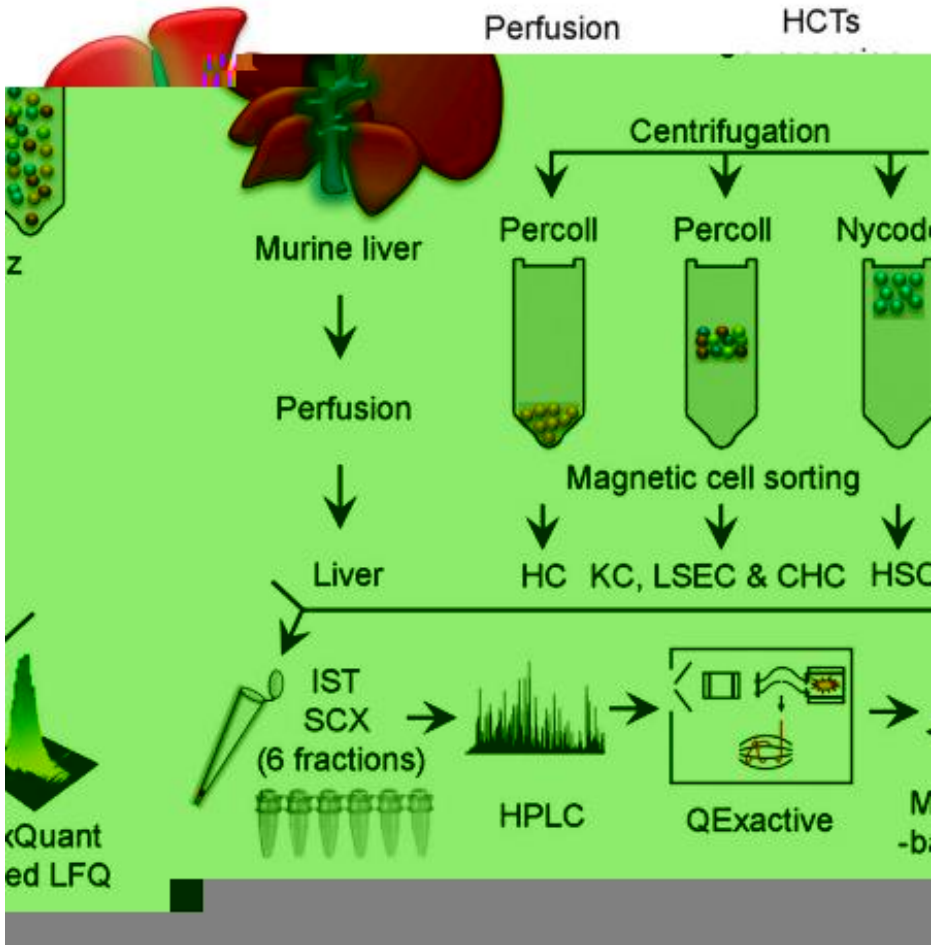

Ḗ
* Ẃ
VS
ɔ̄ ɔ̄ / ʃ ʈ / ɪ ɔ́
ˋH Ω̣ I
VS
ˋH Ω̣ II
Silence/inhibition/overexpression
ʃ ʈ ˙ VS ʃ ʈ ɑ
Ḧ گ ! ɥ ∨ Ḕ
T̲ ȁ ˊ ʒ ˆ
Top changed/prediction /references
w ◒ ʒ Ḕ
GO ⊢ پ
پ ⊢ ث ڹ
PPI/ ǐ ɹ
Candidate proteins
ˊ ʒ ڀ ج - (qPCR/WB/ELISA/PRM)
ɪ ǐ ج - (silence/mutation/inhibition)
Ω̣̃ Ω̣̃ ή
ɪ ⊢ ص ∨ ⊃ ڹ ث OBJ
ψ ק ⊃ h ẽ
ˋ D̲ ˙

Label-free quantification



- j a ¯ β ᴅ˜ ʃ ɲ ˂ ˛ 5 ⅃⅃
- ʃ ɲ ℓ ح ٩ ^ ı Ậ ʒ OBJ Ṯ ầ ٩ ʑ ı ẽ φ ḧ ˛ ´ . ớ
- Ḿ ɑ ʜ OBJ ↑ õ ⊃ ⅄ ḧ ớ ⌊ ∂ ɔ̤ ح
- ⅄ ᴅ j ɹ ‰ L ɔ̥ ˛ ح

Label-free quantification example: Murine Liver



- HC/KC/LSEC/CHC/HSC
- MaxLFQ
- Perseus, R 2

Cell Metabolism: 20, 1076-1087, 2014

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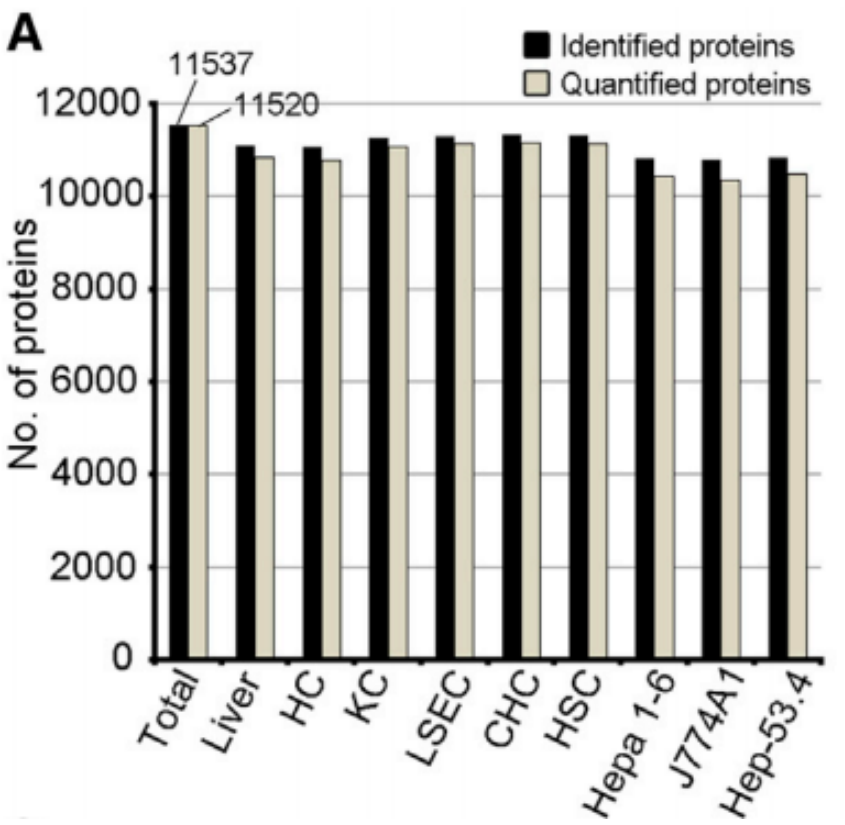
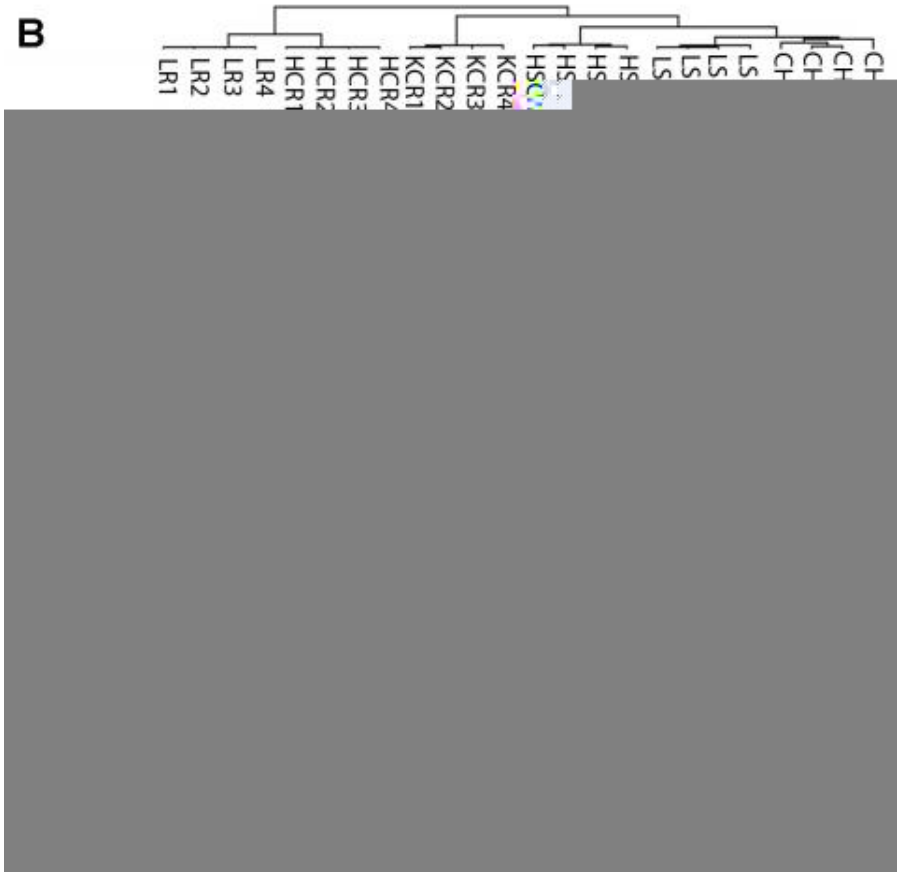
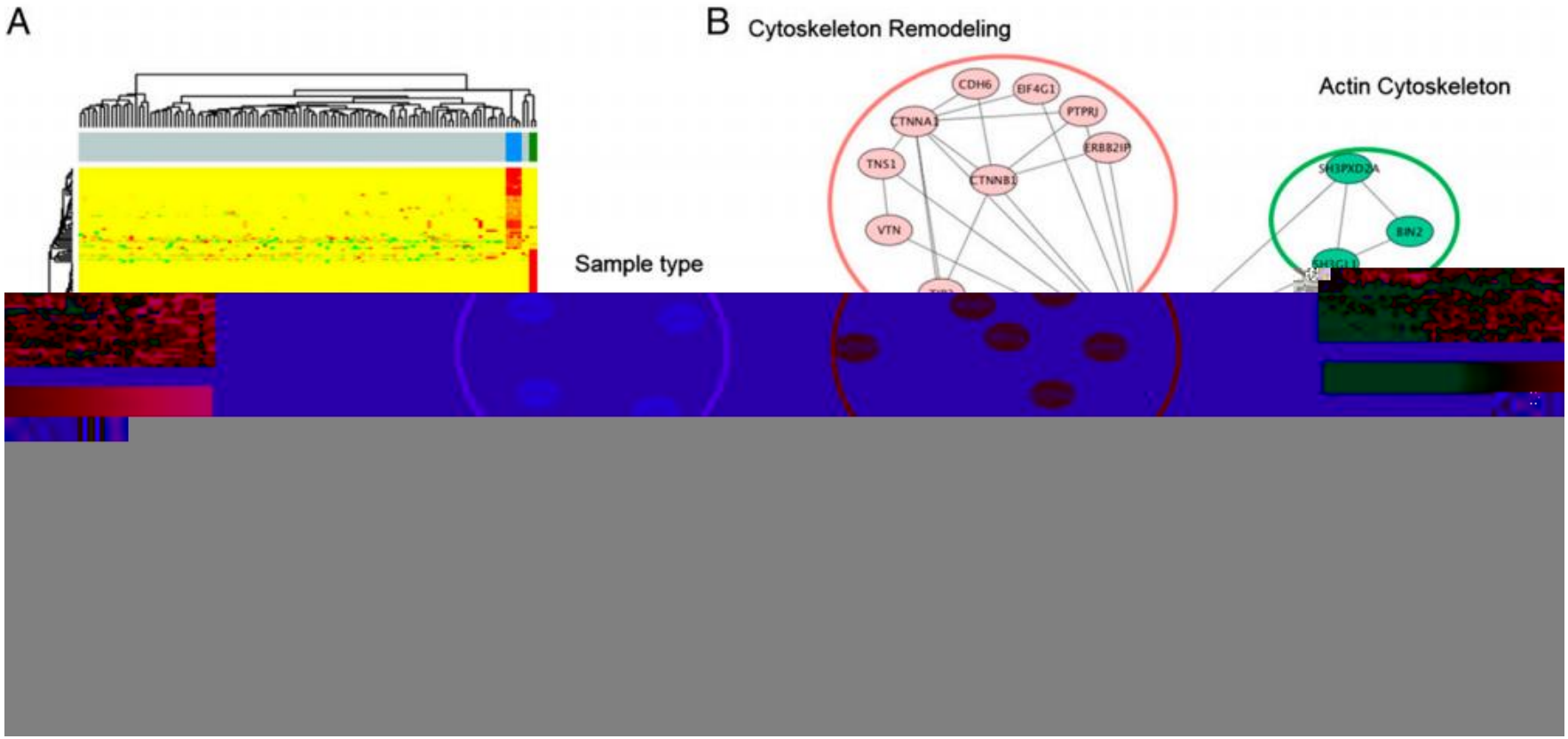
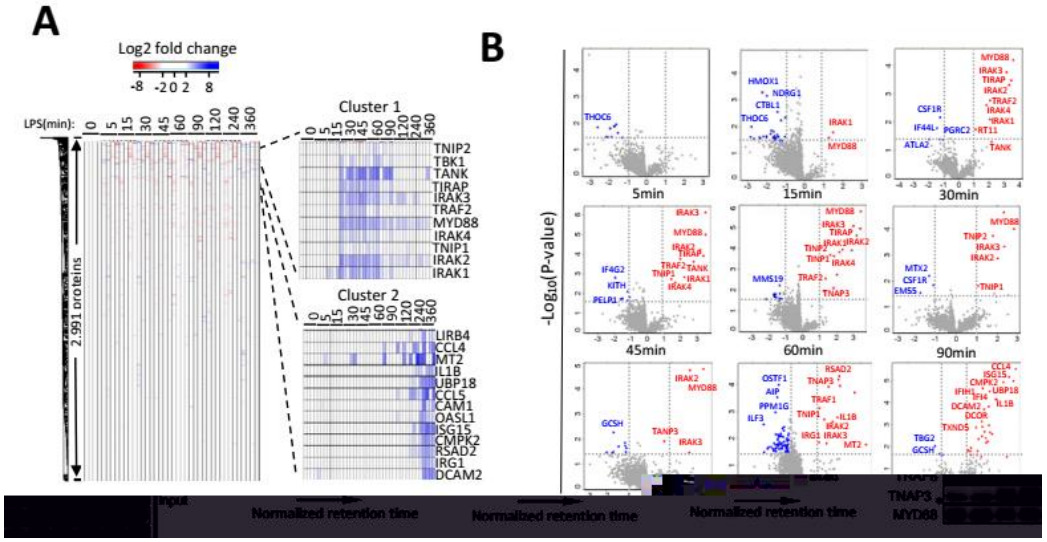




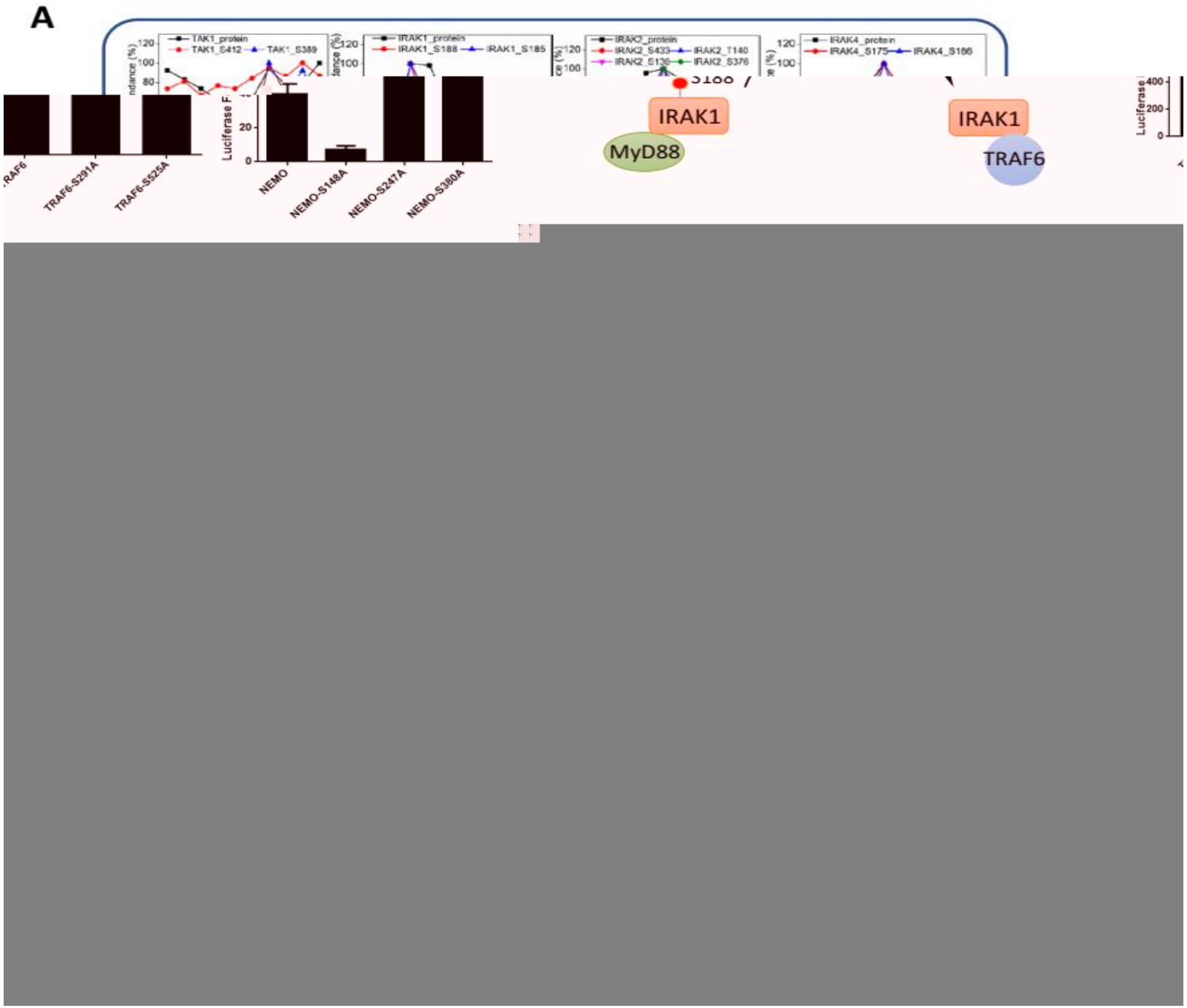
ʹ ˙ ω ʑ OBJ H ḥ ˜ و ˧ ̈Ω̣ ̃Ω

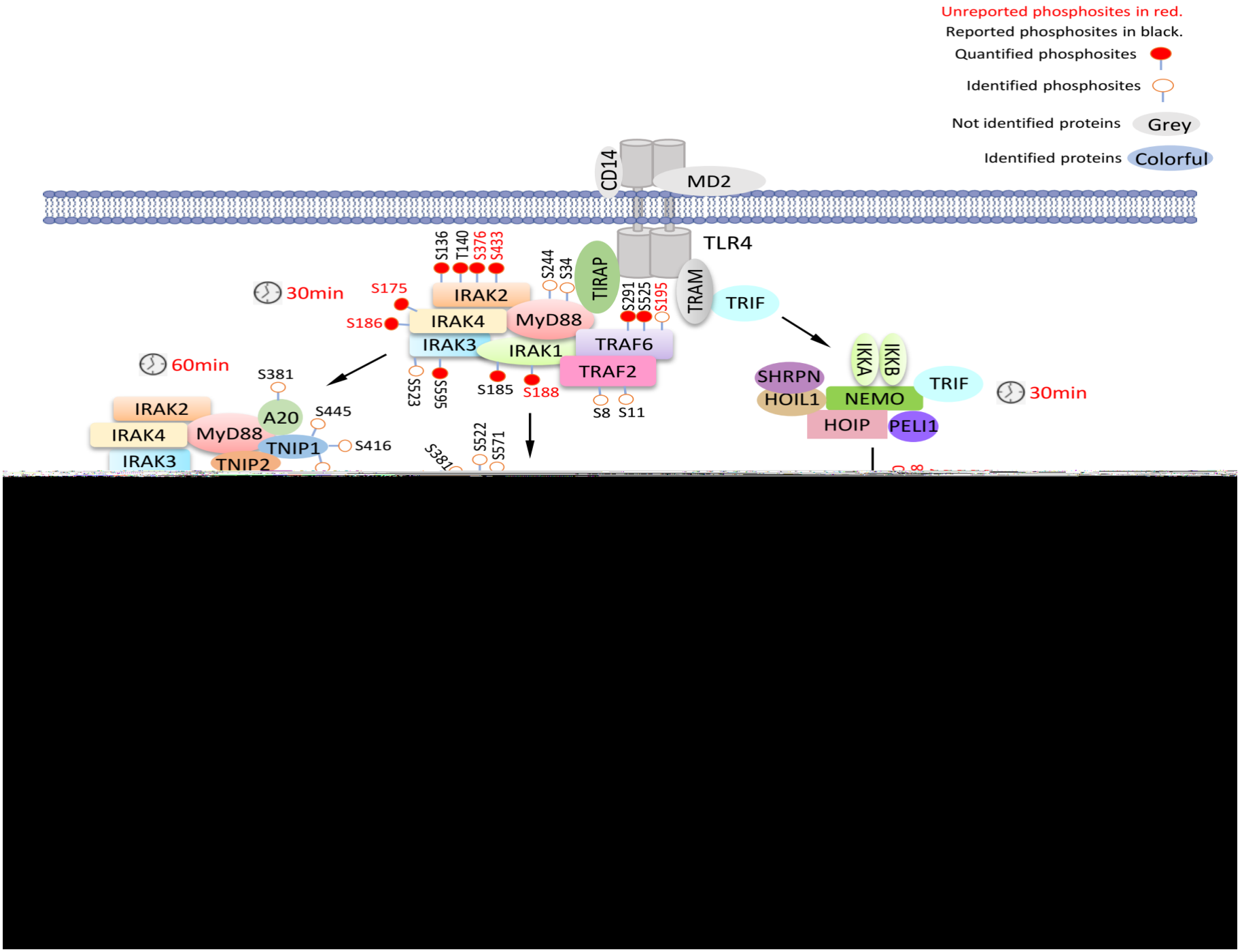

A
B
Cytoskeleton Remodeling
Actin Cytoskeleton
CDH6
EIF4G1
CTNNA1
PTPRJ
ERBB2IP
TNS1
CTNNB1
VTN
SH3PXD2A
BIN2
Sample type


A
Log2 fold change
-8 -2 0 2 8
LPS(min):
0 5 15 30 45 60 90 120 240 360
2,991 proteins
Cluster 1
TNIP2
TBK1
TANK
TIRAP
IRAK3
TRAF2
MYD88
IRAK4
TNIP1
IRAK2
IRAK1
Cluster 2
LIRB4
CCL4
MT2
IL1B
UBP18
CCL5
CAM1
OASL1
ISG15
CMPK2
RSAD2
IRG1
DCAM2
B
-Log10(P-value)
5min
15min
30min
45min
60min
90min
Normalized retention time
Normalized retention time
Normalized retention time
TNAP3
MYD88

F

15min


A
TAK1_protein
TAK1_S412
TAK1_S389
IRAK1_protein
IRAK1_S188
IRAK1_S185
IRAK2_protein
IRAK2_S423
IRAK2_T140
IRAK2_S376
IRAK4_protein
IRAK4_S175
IRAK4_S186
TRAF6-S291A
TRAF6-S525A
NEMO
NEMO-S148A
NEMO-S247A
NEMO-S380A
IRAK1
MyD88
IRAK1
TRAF6


Unreported phosphosites in red.
Reported phosphosites in black.
Quantified phosphosites
Identified phosphosites
Not identified proteins
Grey
Identified proteins
Colorful
CD14
MD2
TLR4
TIRAP
TRAM
TRIF
S136
T140
S376
S433
S244
S34
S291
S525
S195
30min
S175
S186
IRAK2
IRAK4
IRAK3
IRAK1
MyD88
TRAF6
TRAF2
S523
S595
S185
S188
S8
S11
60min
S381
A20
S445
TNIP1
S416
TNIP2
S522
S571
IKKA
IKKB
SHRPN
HOIL1
NEMO
HOIP
PELI1
TRIF
30min

. (Targeted proteomics)

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discovery-based investigation

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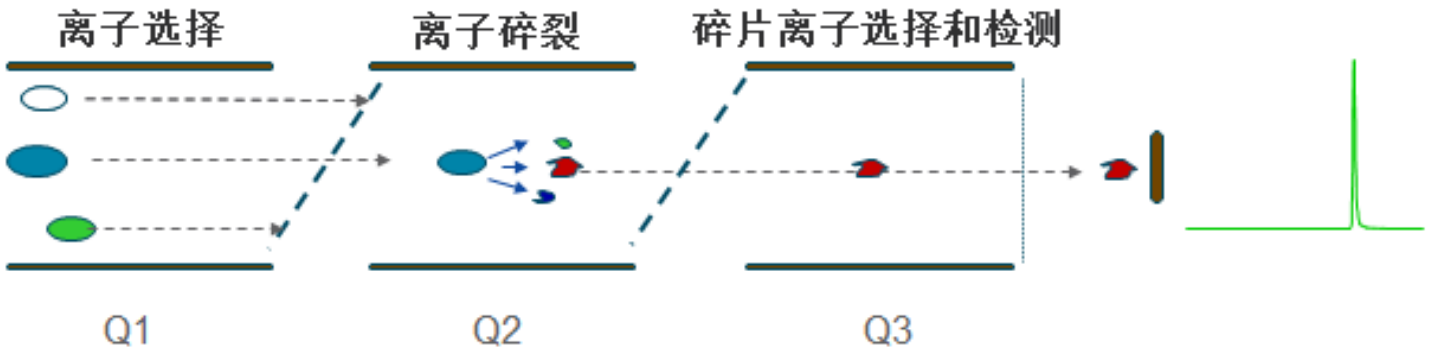
hypothesis-driven investigation

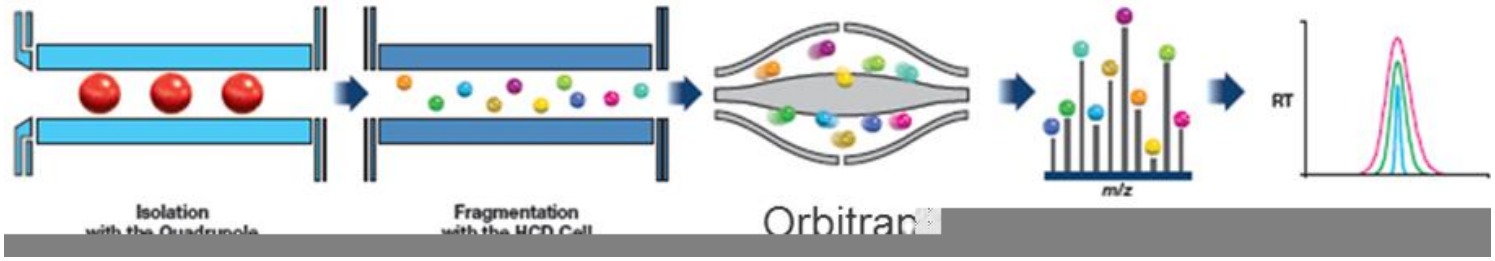
Ӭ ب Æ و ̈Ω̃Ω Æ جـ -

MS strategy: Targeted VS. Large-scale

MRM & PRM



MRM/SRM



MRM^{HR}

PRM

Molecular & Cellular Proteomics **Editorial**

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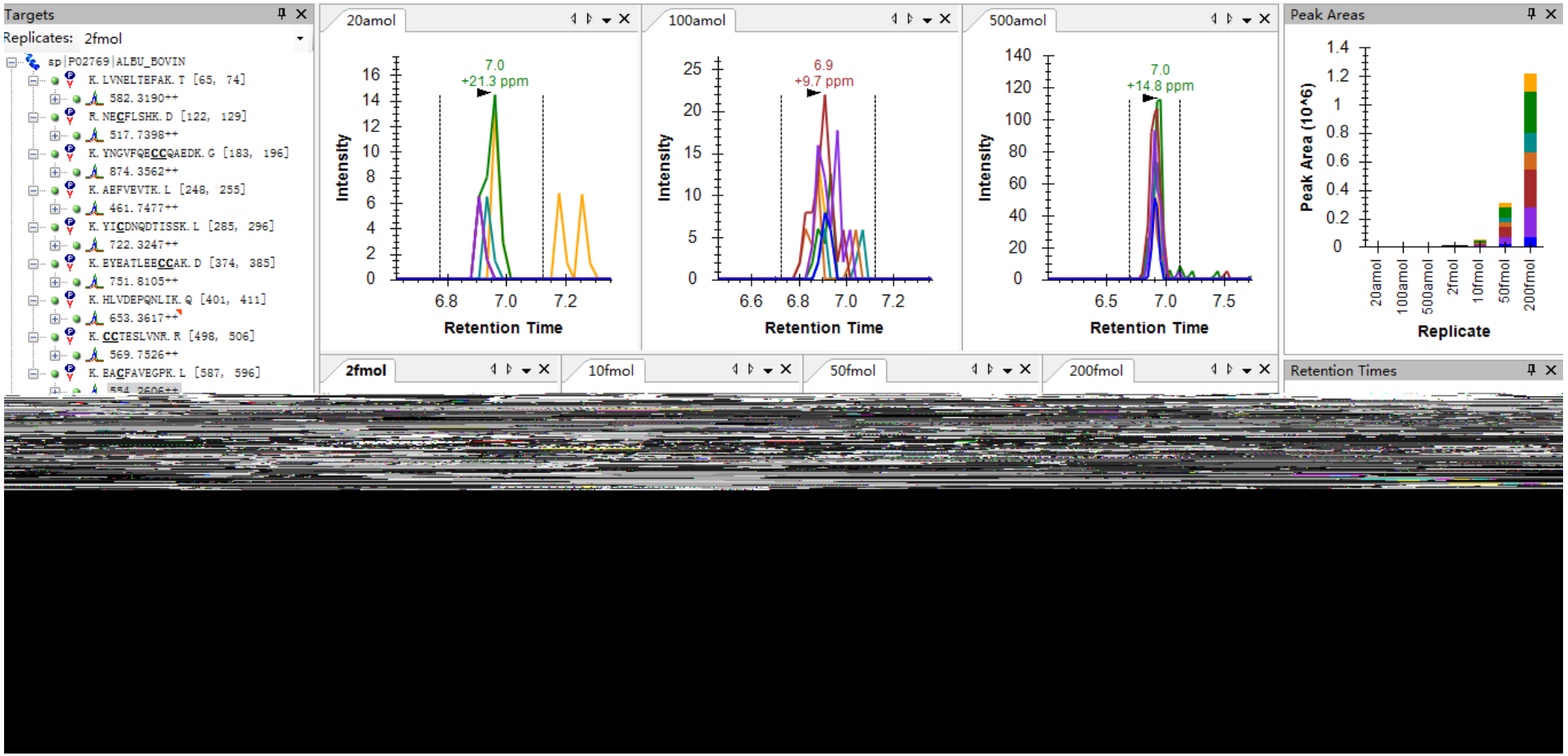
This paper is available on line at http://www.mcponline.org

Western Blots *versus* Selected Reaction Monitoring Assays: Time to Turn the Tables?

Ruedi Aebersold, Alma L. Burlingame, and Ralph A. Bradshaw

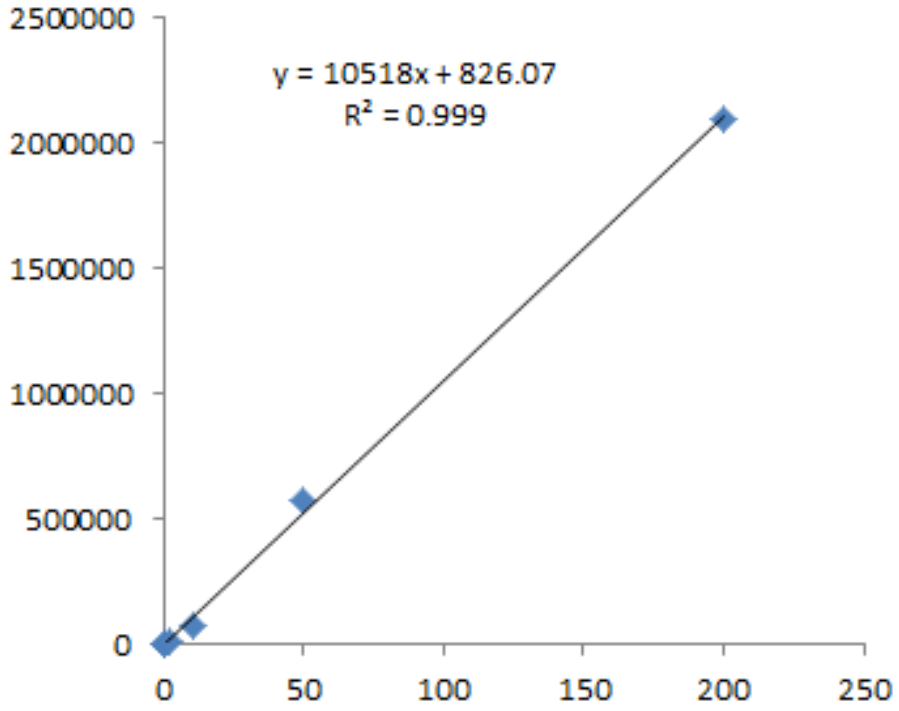
- Authors who submit papers containing quantitative protein data generated via MS are frequently asked by reviewers to validate some of the values with Western blotting.
- Unfortunately, the quality of quantitative data obtained via Western blotting is not comparable to that obtained with SRM.
- We posit that the request to validate quantitative MS data by Western blotting is no longer justified.
- In fact, it may be time to ñ t u the tn a b l and request that Western blotting results be validated by MS.

PRM


Targets
Replicates: 2fmol
sp|P02769|ALBU_BOVIN
K.LVNELTEFAK.T [65, 74]
582.3190++
R.NECFLSHK.D [122, 129]
517.7398++
K.YNGVFQECCQAEDK.G [183, 196]
874.3562++
K.AEFVEVTK.L [248, 255]
461.7477++
K.YICDNQDTISSK.L [285, 296]
722.3247++
K.EYEATLEECCAK.D [374, 385]
751.8105++
K.HLVDEPQNLIK.Q [401, 411]
653.3617++
K.CCTESLVNR.R [498, 506]
569.7526++
K.EACFAVEGPK.L [587, 596]
20amol
100amol
500amol
2fmol
10fmol
50fmol
200fmol
7.0
+21.3 ppm
6.9
+9.7 ppm
7.0
+14.8 ppm
Intensity
Retention Time
Peak Areas
Peak Area (10^6)
Replicate
20amol
100amol
500amol
2fmol
10fmol
50fmol
200fmol
Retention Times

́ 4Ψ

Con. (fmol)	Total Area
0.02	545
0.1	840
0.5	4206
2	13443
10	78509
50	579807
200	2106256



ɒ Ӈ ̈Ω \ χ 1 mol = 10^{3} mmol = 10^{6} ε mol = 10^{9} nmol = 10^{12} pmol = 10^{15} fmol = 10^{18} amol

PRM ـم

¾ b ‿ و ⊣ e̯ ʿH χ ñ و ؤ ³ We s t e r n E

¾ ‿ / ⊣ OBJ Ḿ/ ʌ Ḿ ḧ ٍ χ

z ج - ḧ ٍ ‿ و ⊣ e̯ ʿΩ̣ ʿΩ

z ⌊ Ȳ WB ι ≡ ω̃ ỉ ч OBJ ε e.g. ‿ ק ḧ ٍ ζ

z ı ṯ ʌ ⊥ ʒ ˙ ‿ و ḧ ٍ OBJ

¾PRM ├ Ẓ’ ° χ

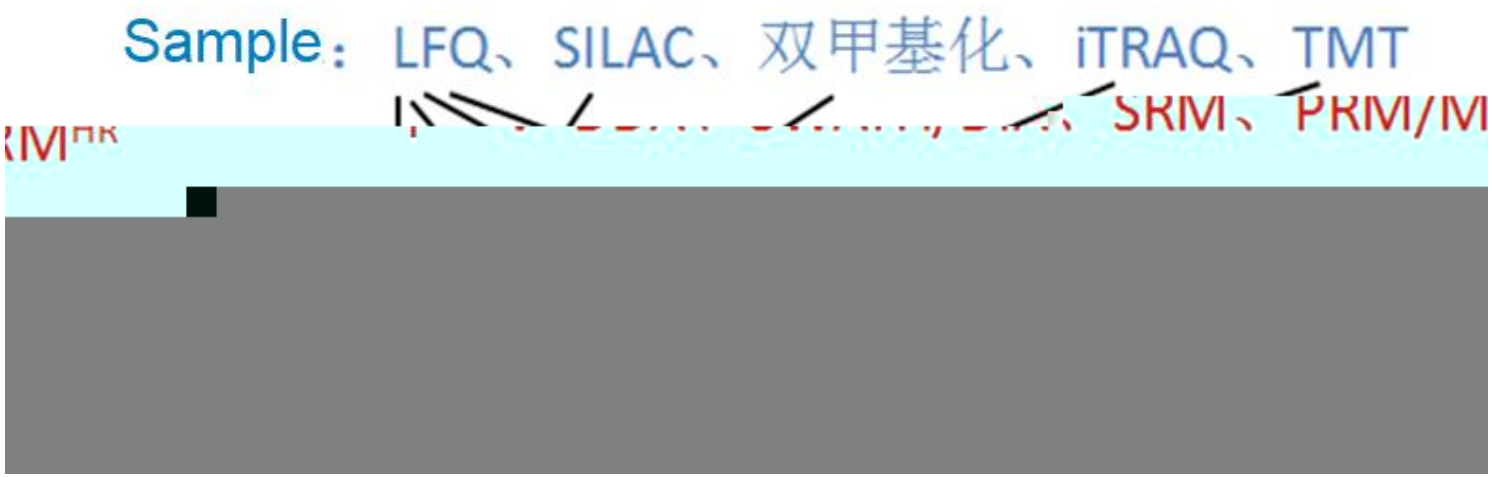
z ˝. ḫ ج´ OBJ ι. ḧ´ ‰ ‿ / ⊣

z ـ ⌊ J̌ ḧ ɲ v ‿ ι ḧ ٍ ặ ק

z ن ص‰´ ⊣ ı Ɑ ḫ א ʌ Ḿ ḧ ٍ

- DDA (Shotgun, IDA, topN)
- SWATH/DIA
- Targeted method (MRM, SRM; PRM, MRM^{HR})

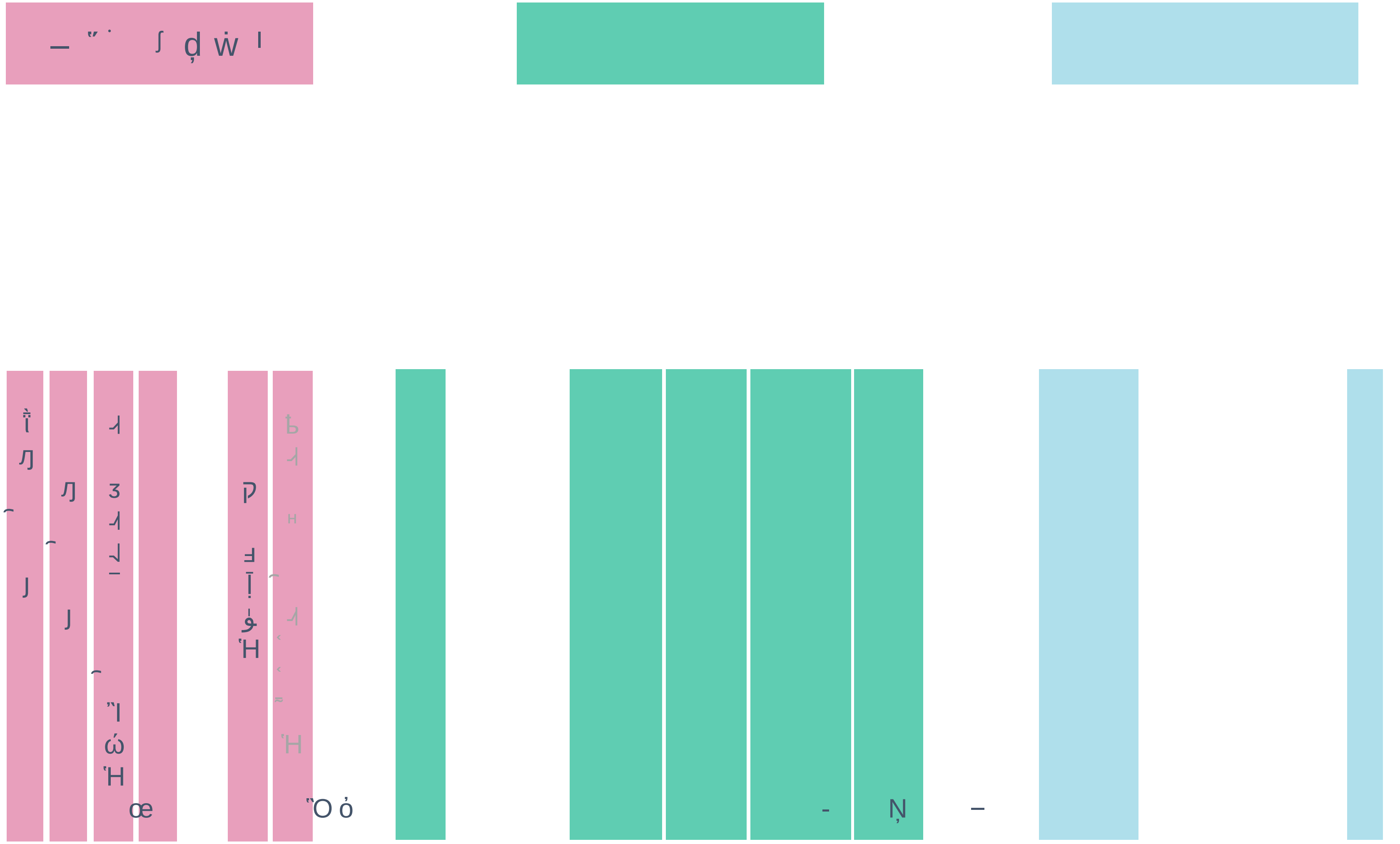
ỹ



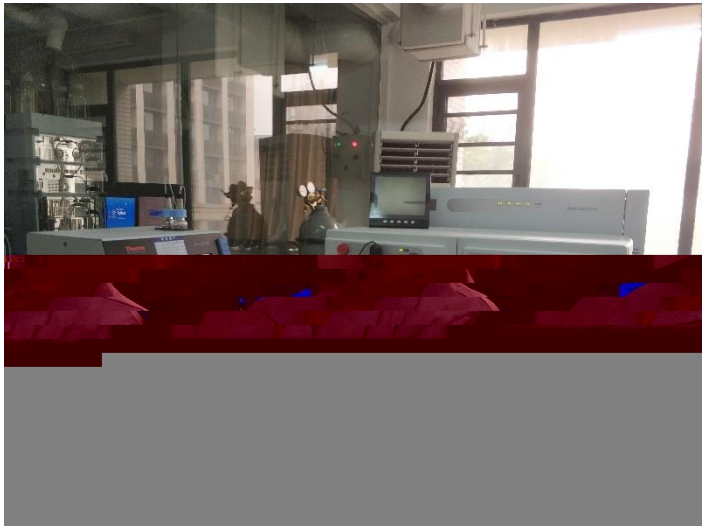
¾ ḧ ớ ͡ و ˧˩ e̯

¾ ḧ ɟ̤ ͡ و ˧˩ e̯

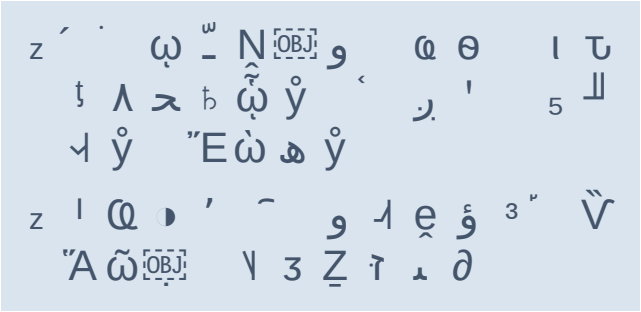
¾ b ͡ و ˧˩ e̯

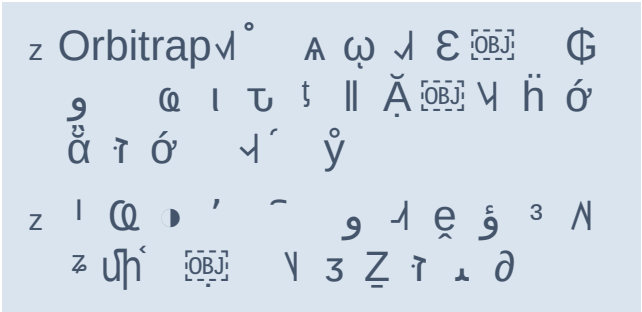


Thermo Ʒ ᴺ ᵘ Q Exactive HF-X



Thermo Ʒ ᴺ ᵘ LTQ Orbitrap Elite





謝謝