2015 –Omics Course
Nov 13, 20 and Dec 4

Visualizing the proteome and in what form(s)

The lens system

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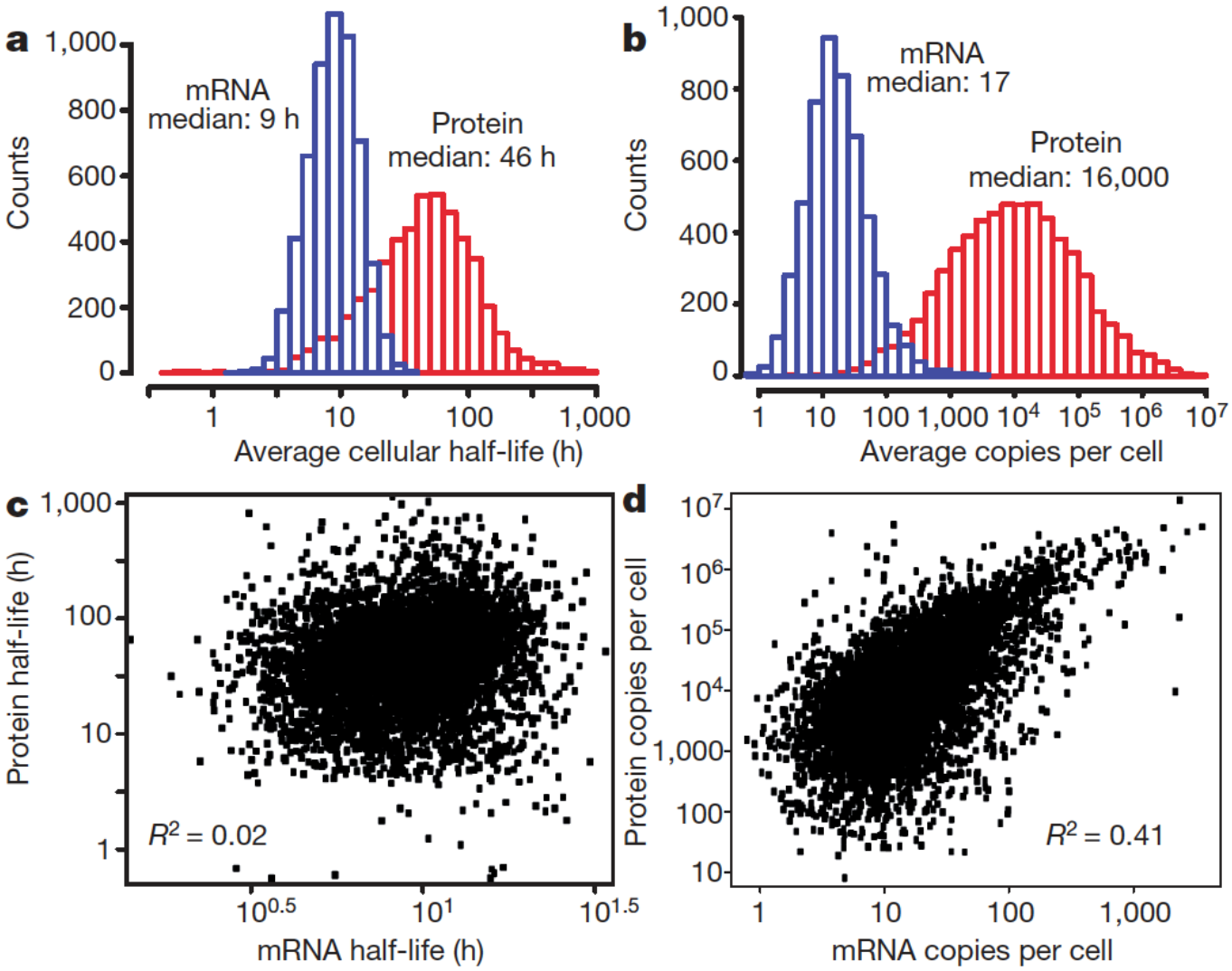
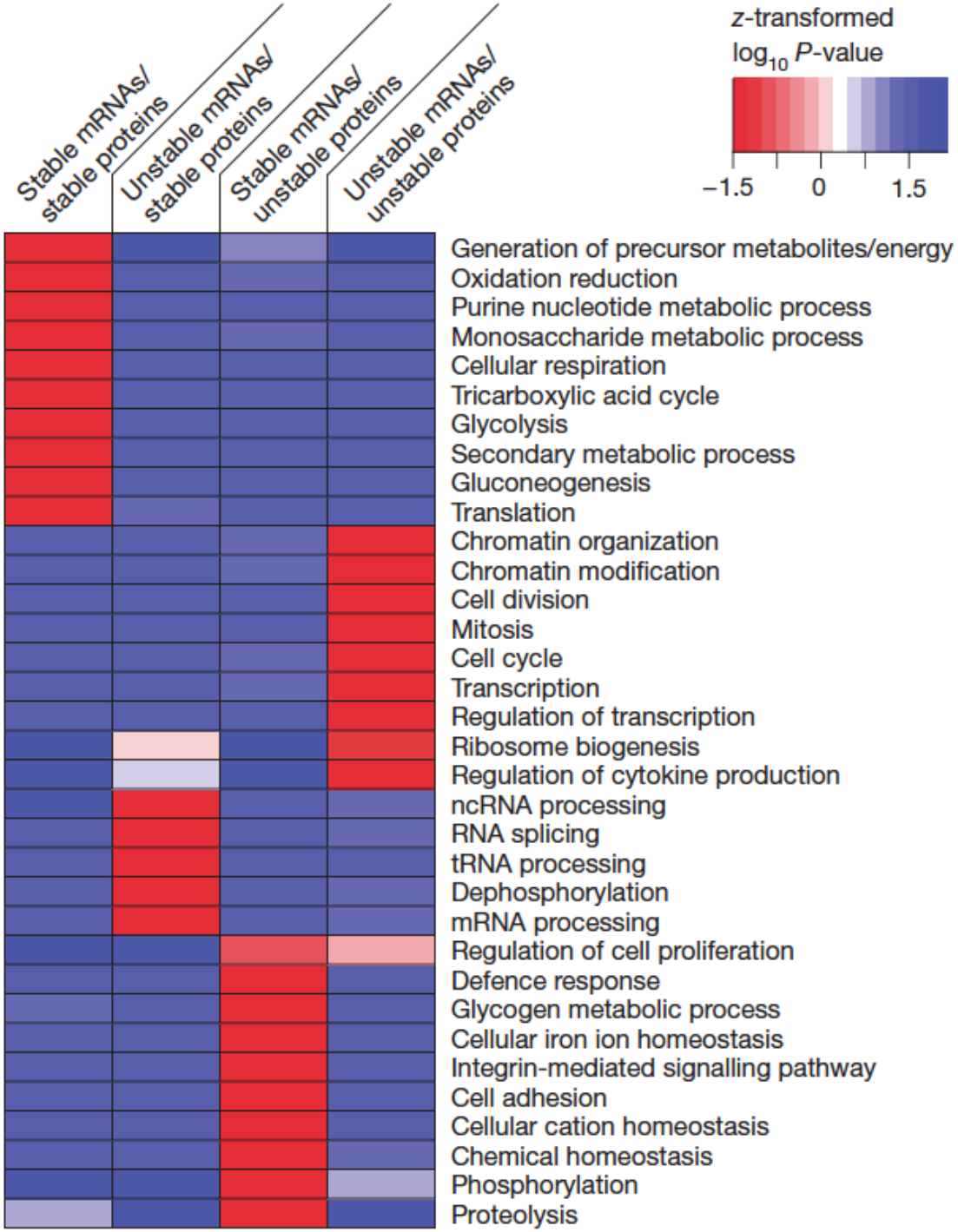
Matt Renfrow

Landon Wilson

Protein – mRNA correlations



Schwanhäusser et al.
Nature 473: 337 (2011)

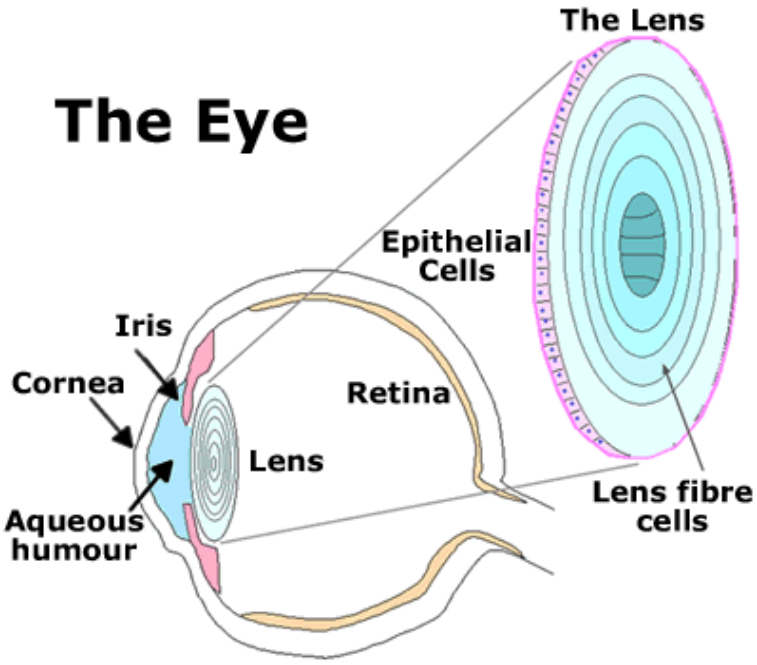


Proteins and mRNA have different stabilities and can be divided into 4 quadrants. The major cellular functions are divided as shown in the table.

Schwanhäusser et al.
Nature 473: 337 (2011)

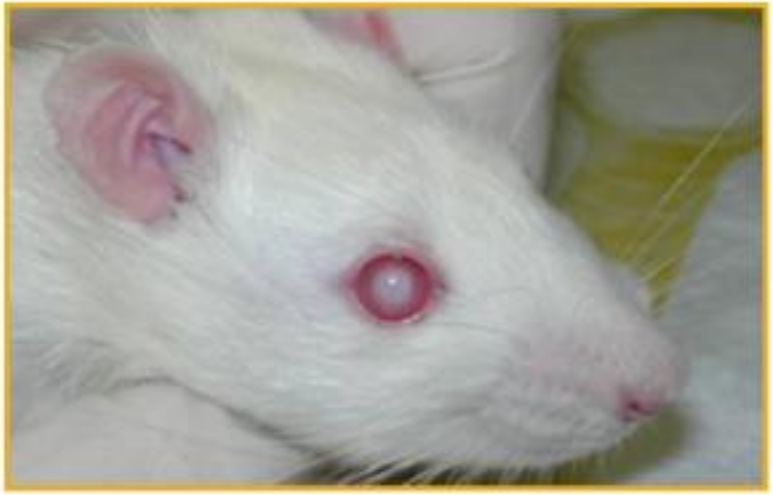
Lens (Structure and Function)

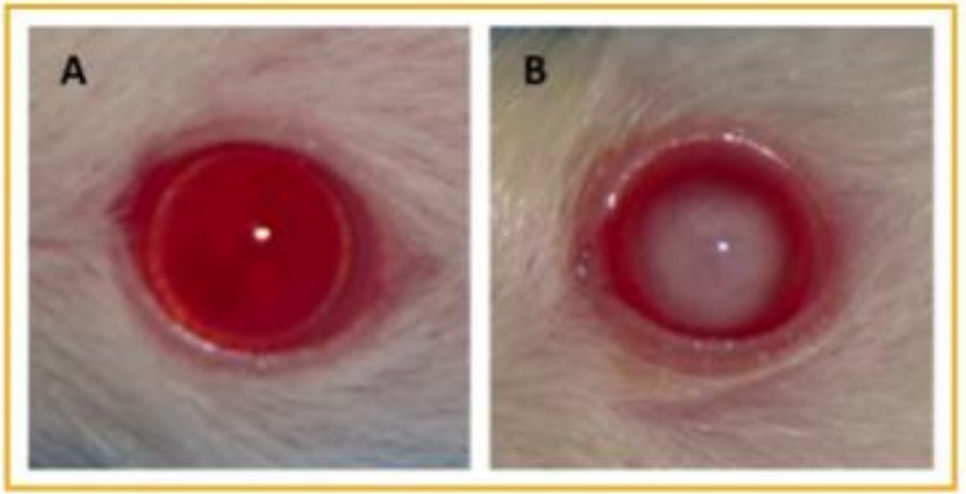
- Part of anterior segment of eye, just beyond the cornea
- Biconvex
- Focuses light onto the retina
  - Changes shape to accommodate light entering eye
- Proteins
  - Synthesis stops to allow light to pass
  - Chief proteins within the lens: <u>crystallins</u>
    - Chaperone-like proteins
    - Mammalian lens largely comprised of α and β crystallins
- Cataract
  - Opacity of the lens
  - Obstructs vision – surgical intervention necessary



Link to Science article on learning to see

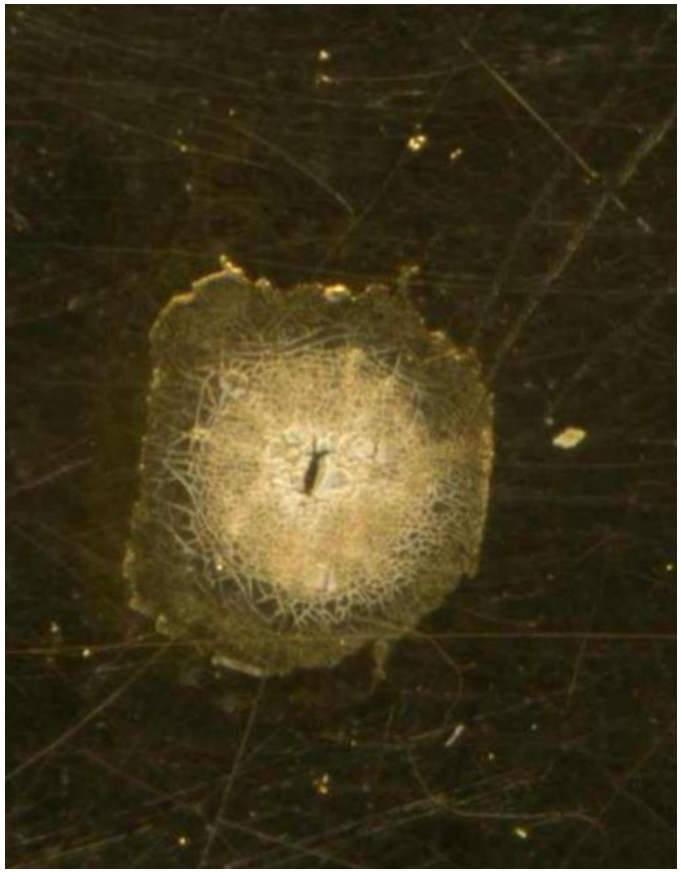
The rat model we use



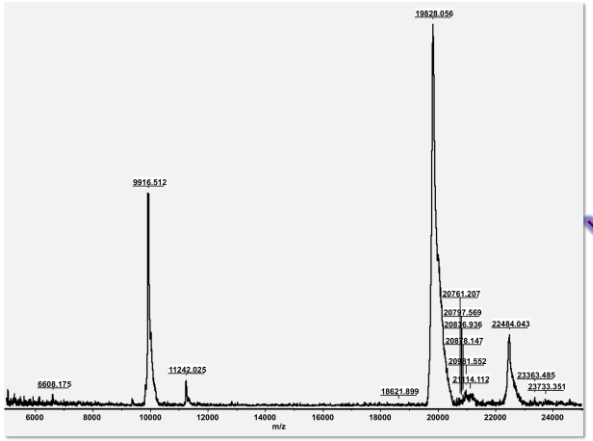


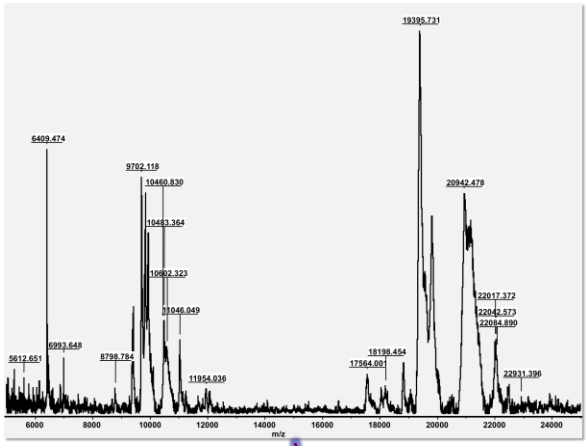
- ICR/f rat (Ihara/Inherited Cataract Rat, strain-f)
  - Model of age-related disease.
  - Spontaneously develops cataracts by 10 weeks of age.
    - Possible result of early oxidative insult.
    - Compare 21-day vs. 100-day

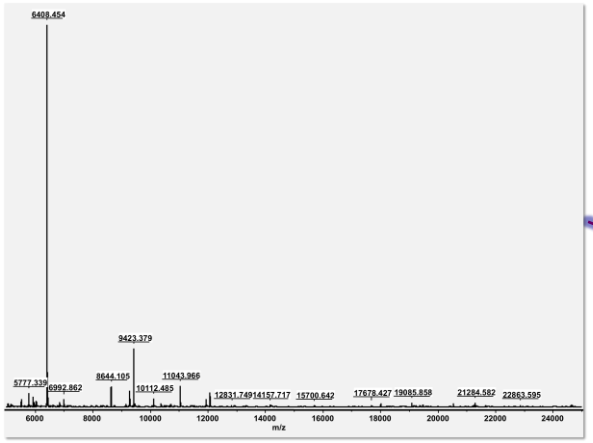
20 μm section: washed and fixed to gold MALDI target plate

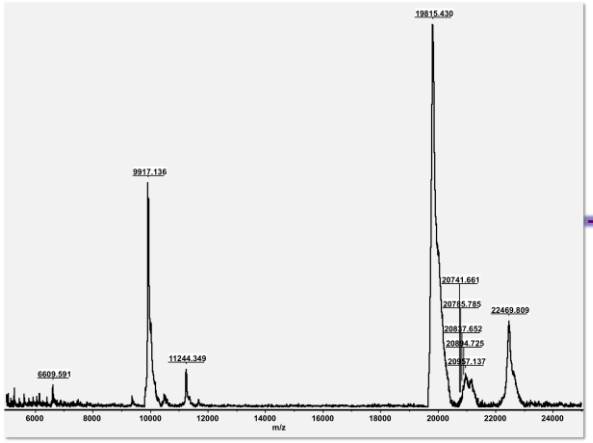


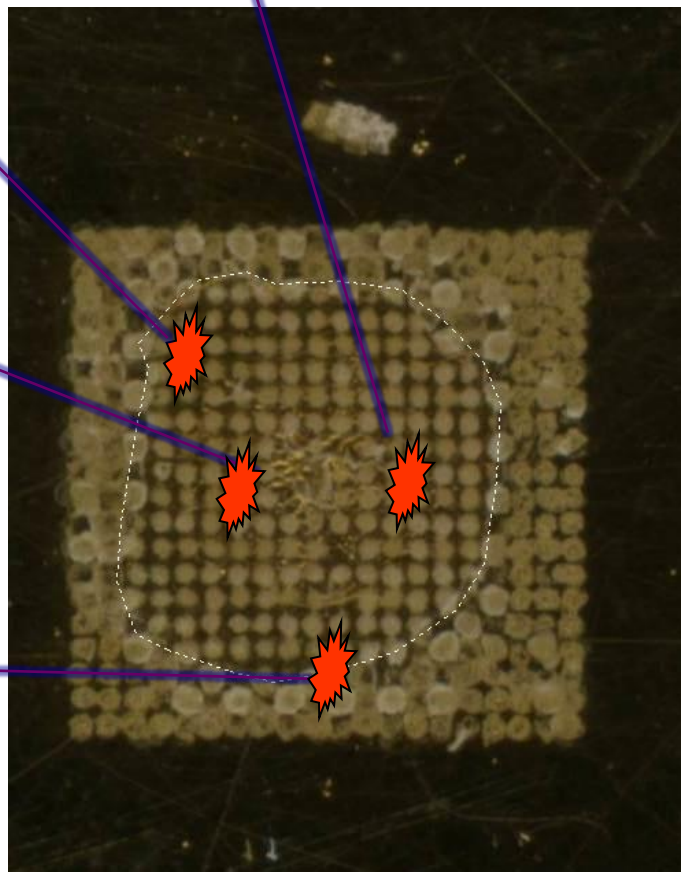
Same section with MALDI matrix spotted on top.


19828.056
9916.512
20761.207
20797.569
20836.936
20878.147
20981.552
21014.112
22484.043
23363.485
23733.351
11242.025
6608.175
18621.899
m/z

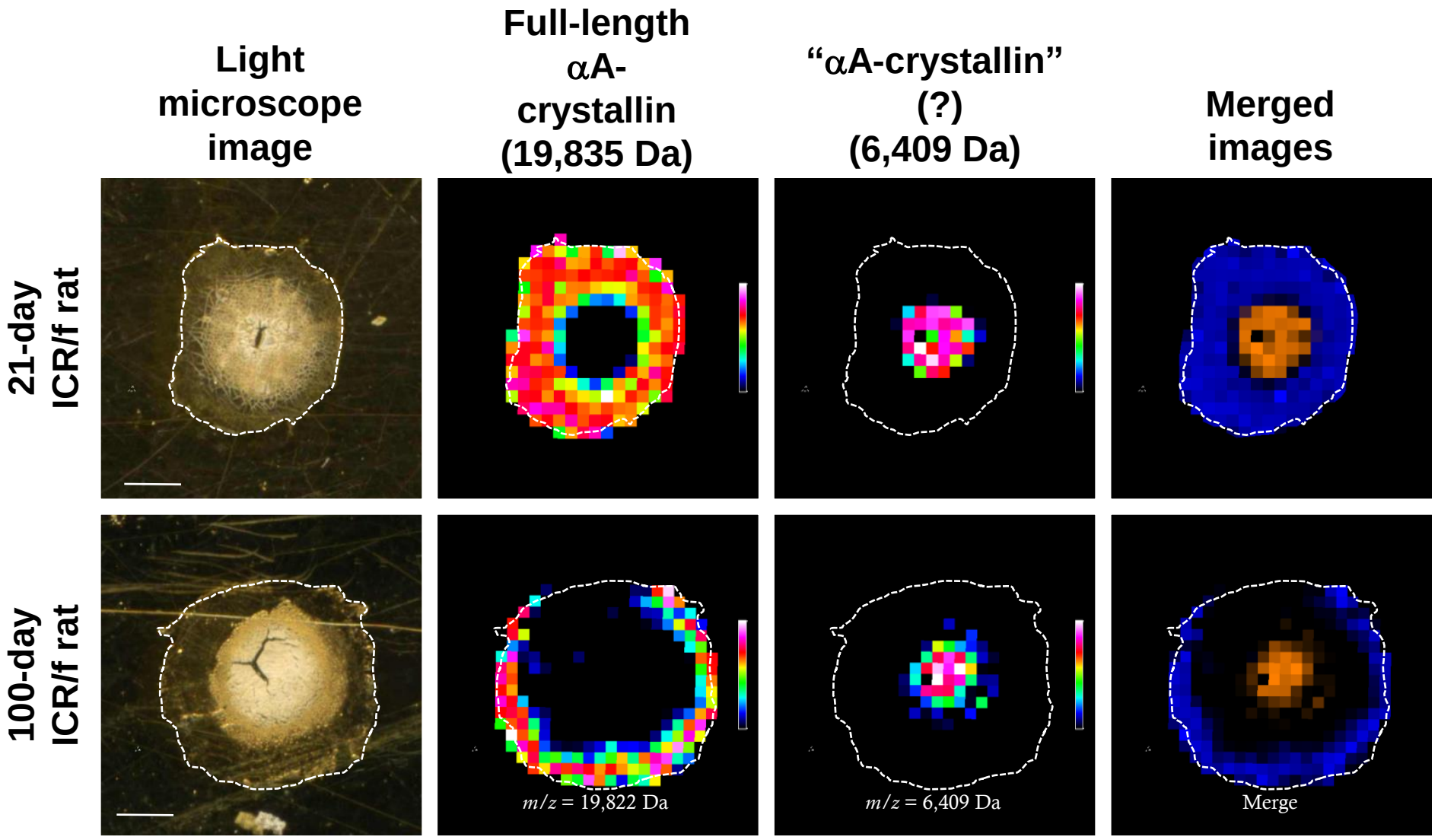

19395.731
6409.474
9702.118
10460.830
10463.364
10602.323
11046.049
20942.479
22017.372
22042.573
22084.690
6993.648
5612.651
8798.784
11954.036
18198.454
17564.001
22931.396
m/z


6408.454
9423.379
5777.339
6992.862
8644.105
10112.485
11043.966
12831.749
14157.717
15700.642
17678.427
19085.858
21284.582
22863.595
m/z

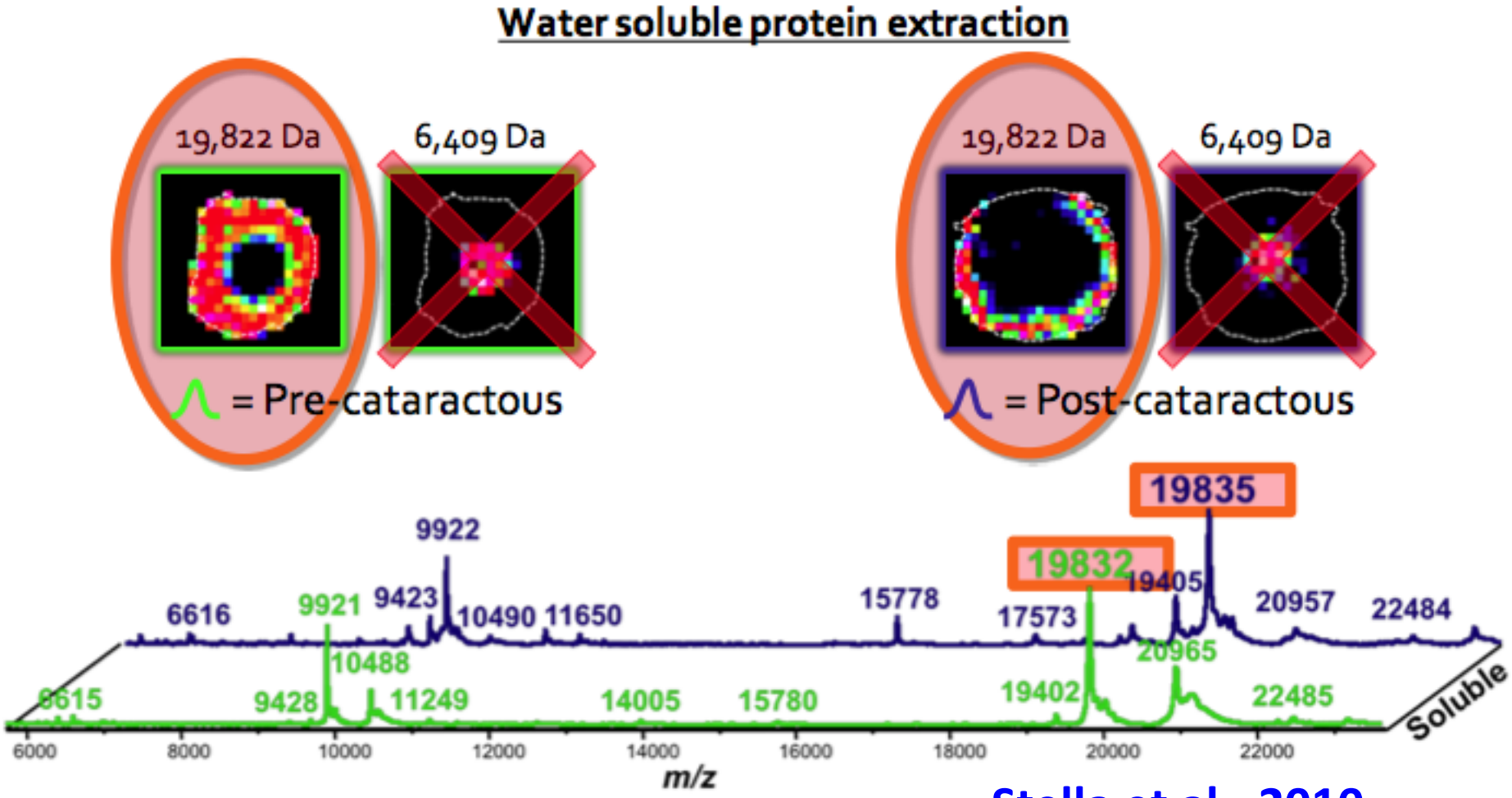

19815.430
9917.136
20741.661
20765.785
20817.652
20894.725
20967.137
22469.809
11244.349
6609.591
m/z



Extracted *m/z* values: the image

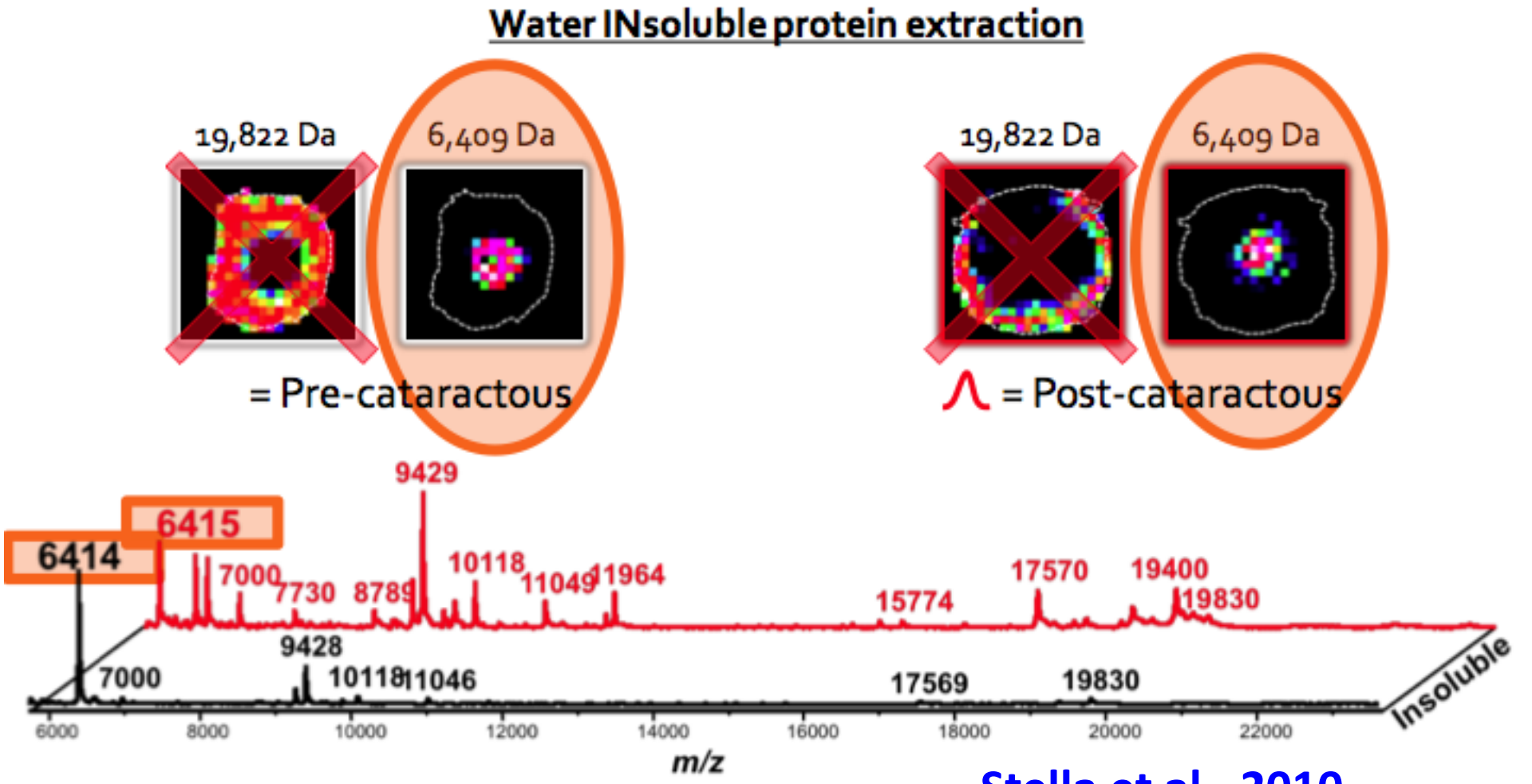


Aqueous extract of the lens



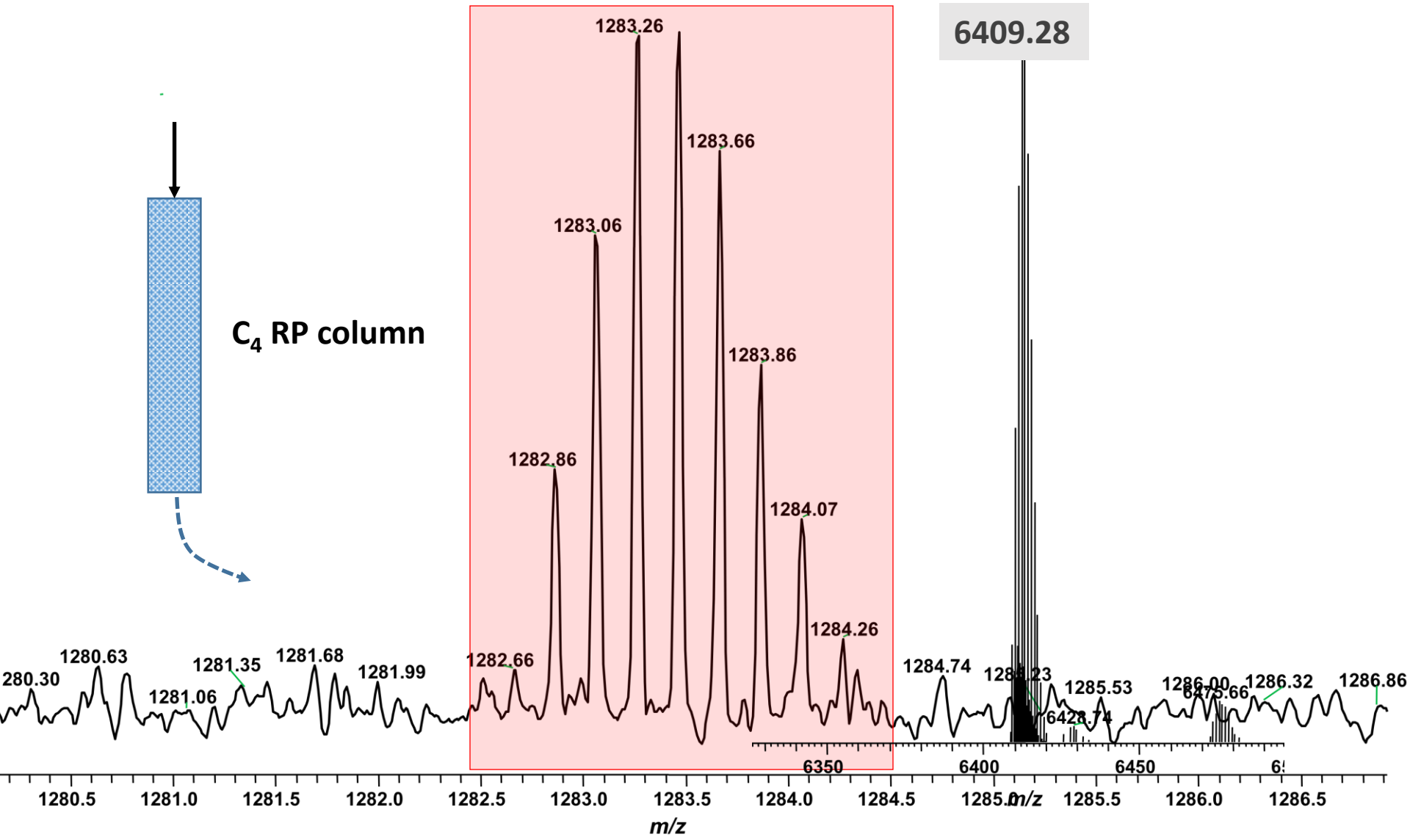
Stella et al., 2010

Water-insoluble/urea soluble

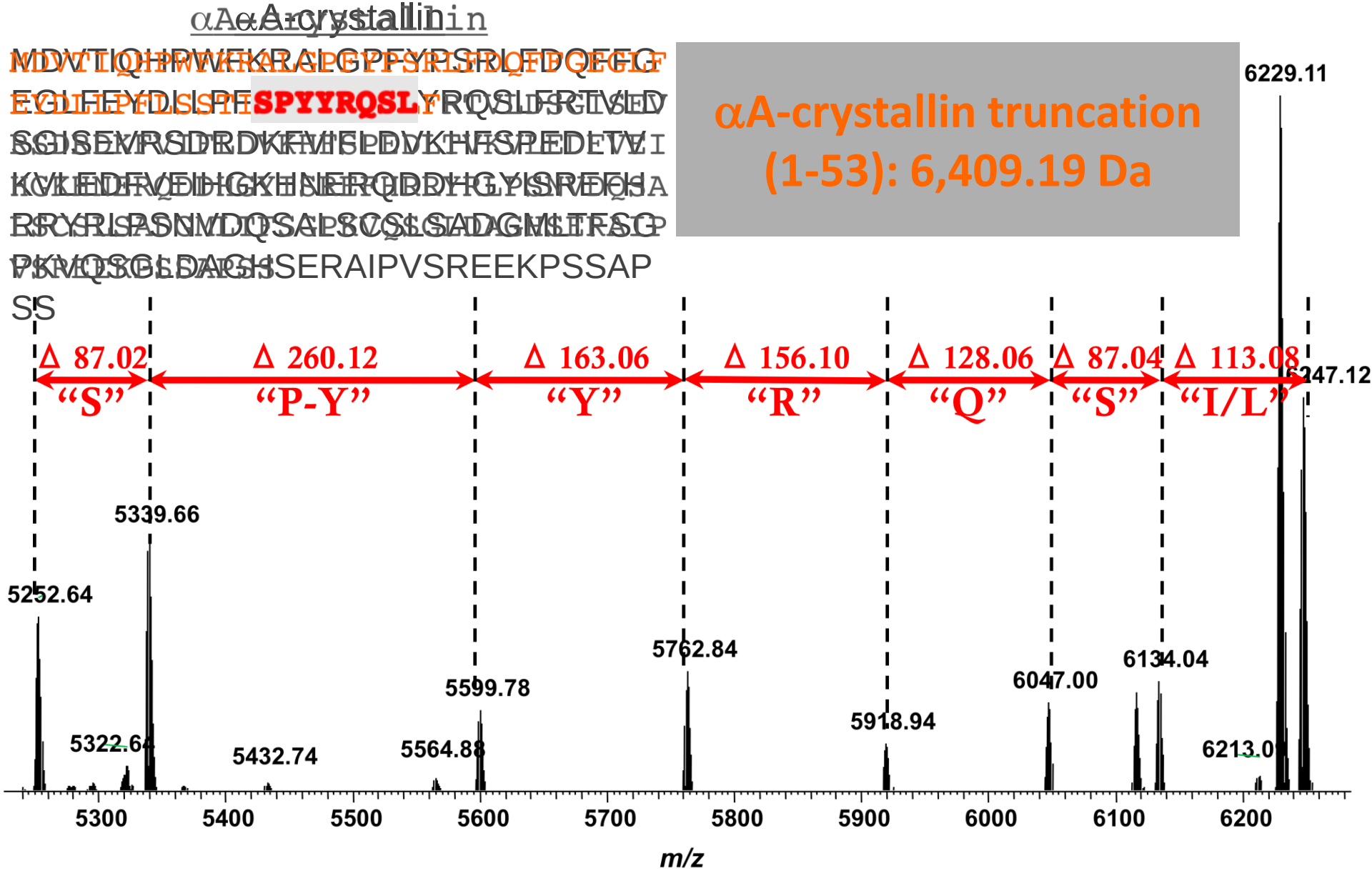


Stella et al., 2010

Top-down identification of *m/z*=1283.3 (5^+ charge state) using FT-ICR-MS



Top-down identification of $[M+H]^+$ = *6,409.28 Da*



Top-Down Summary

αA-crystallin species alignment

* = identical residues
: = conserved residue substitutions
. = semi-conserved residue substitutions
– = truncations ID'd from literature
◄ = truncations ID'd in ICR/f rat using MSI data

What we've learned

- Proteins and message are poorly related
- Proteins in tissues can have specific locations
  - We already knew this because of antibodies
- Proteins can be truncated and have multiple (different) locations
  - Unless specific antibodies are used, we would not know this
- Prior to this study, it was presumed that this only occurred after long periods of time
  - The imaging suggests that this may not be the case
  - Now need to quantify the C-terminal peptides

Verifying and quantifying C-truncation

- αA crystallin is supposedly processed to a 172aa form from the 196aa translated product. Interestingly, what we see is the removal of an interior 23aa peptide, so it must be differential splicing, not posttranslational processing.
- Processed rat αA crystallin has a chymotrypsin cleavage site at ^{141}Phe
- This peptide can be observed as a triply charged peptide
  - F|SGPKVQSGLDAGHSERAIPVSREEKPSSAPSS
- The C-truncations observed by mass spectrometry imaging are the following:
  - SGPKVQSGLD (truncation at 151)
  - SGPKVQSGLDAGHSE (truncation at 156)
  - SGPKVQSGLDAGHSER (truncation at 157)
  - SGPKVQSGLDAGHSERAIPVSR (truncation at 163)
  - SGPKVQSGLDAGHSERAIPVSREEKPS (truncation at 168)
- S,K,R are residues that carry positive charges in 0.1% formic acid

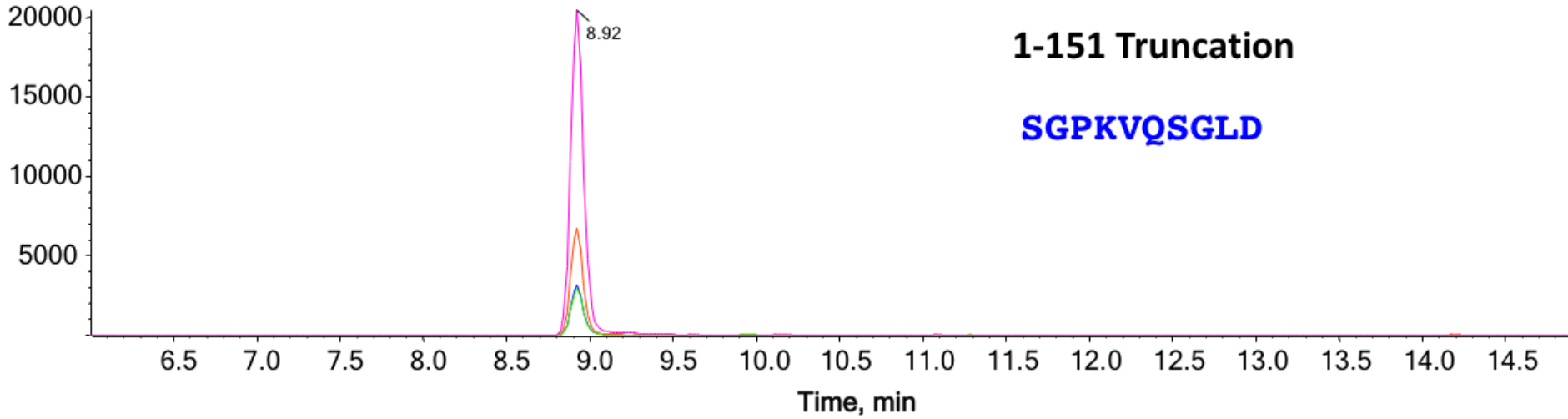
Expected ions in MSMS spectrum

- Let's take the simplest chymotryptic peptide
  - NH_2-SGPKVQSGLD-COOH
- We consider two types, b-ions and y-ions
  - Come from dissociation of the peptide bond
  - b-ions contain the N-terminal amino acid
    - Sum of the residue masses + 1
  - y-ions contain the C-terminal amino acid
    - Sum of the residue masses + H_2O (18) + 1

	b_2	b_3	b_4	b_5	b_6	b_7	b_8	b_9	
-	145	242	370	469	597	684	741	854	-
S	G	P	K	V	Q	S	G	L	D
-	900	843	746	618	519	391	304	247	134
	y_9	y_8	y_7	y_6	y_5	y_4	y_3	y_2	y_1

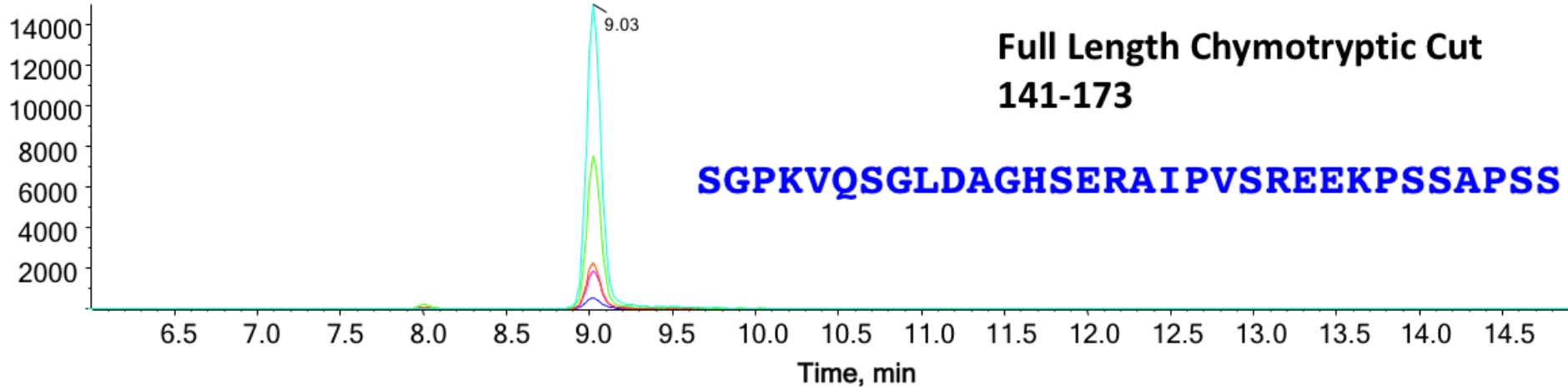
MS-Product

Truncation of αA-crystallin in ICR/F rat on Day 21



1-151 Truncation

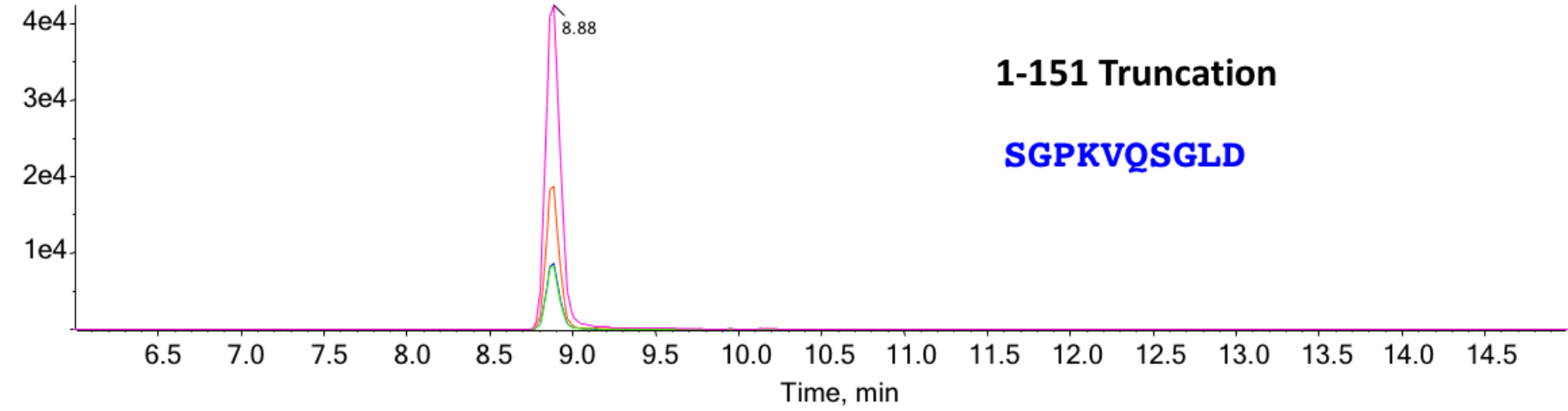
SGPKVQSGLD



Full Length Chymotryptic Cut
141-173

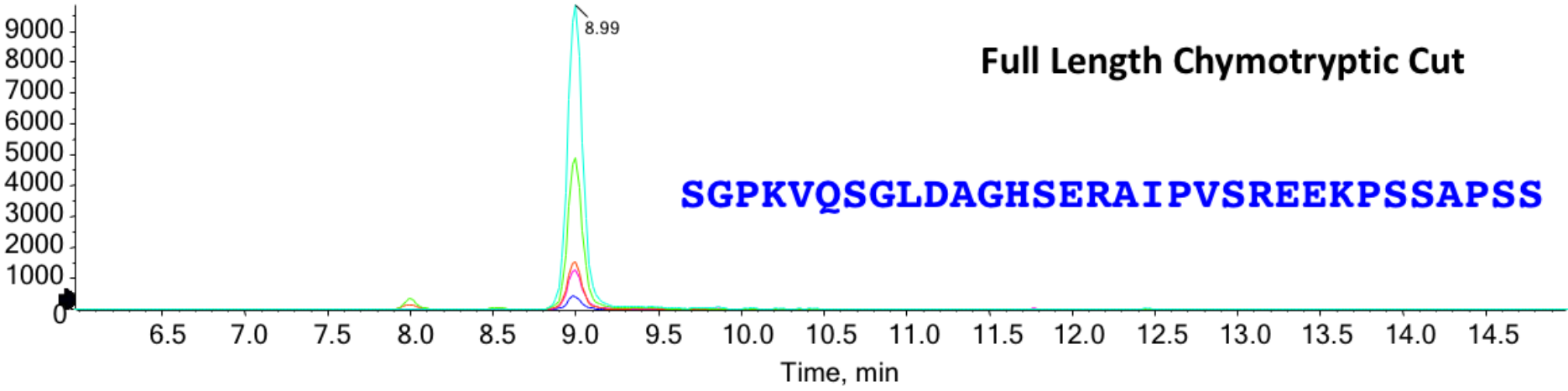
SGPKVQSGLDAGHSERAIPVSREEKPSSAPSS

Truncation of αA-crystallin in ICR/F rat on Day 100



1-151 Truncation

SGPKVQSGLD



Full Length Chymotryptic Cut

SGPKVQSGLDAGHSERAIPVSREEKPSSAPSS

How to quantify peptides

- **Prepare synthetic peptide standards**
  - **~$20/residue**
  - **Important to check purity**
  - **Some available at Aldrich-Sigma**
- **Better approach**
  - **Prepare $^{13}C/^{15}N$-labeled peptides**
  - **Add a single amount of each to unknowns and standards**
- **Best approach (for tryptic peptides)**
  - **Prepared a $^{13}C/^{15}N$-concatenated, artificial protein formed from the peptides of interest**
  - **Controls for digestion efficiency and recovery**

-Omics-wide quantification

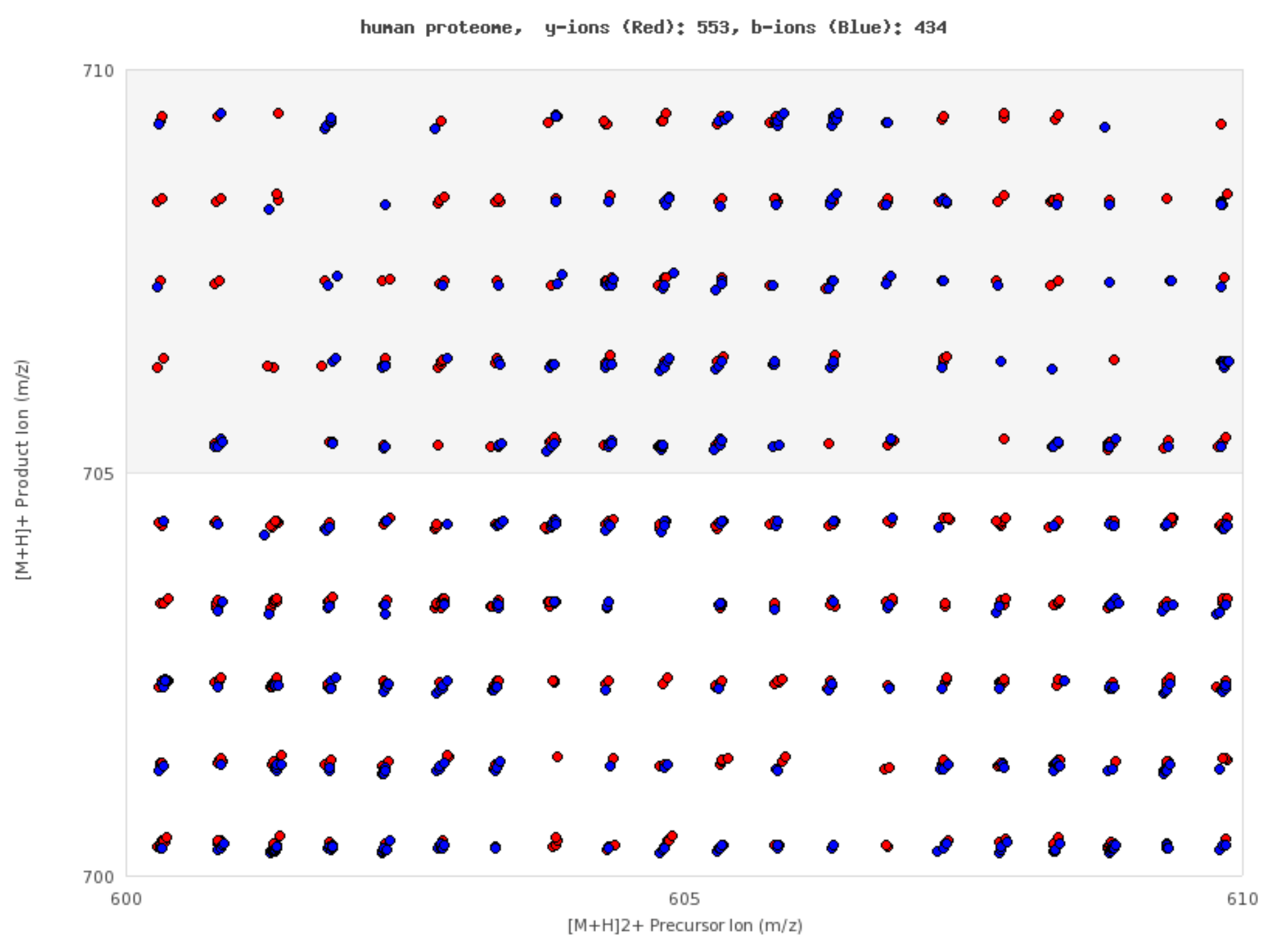
- **iTRAQ and TMT reagents**
  - **React with lysine groups**
  - **Use of $^{12}C/^{13}C$, $^{14}N/^{15}N$, $^{16}O/^{18}O$ isotopes**
  - **Have a reporter region, a (mass) balancing region and a reactive group**
  - **All have the same mass (very close), but different reporter ions**
  - **Each sample to be compared is labeled with a different form of the reagent**
  - **Samples are combined and analyzed at one time**

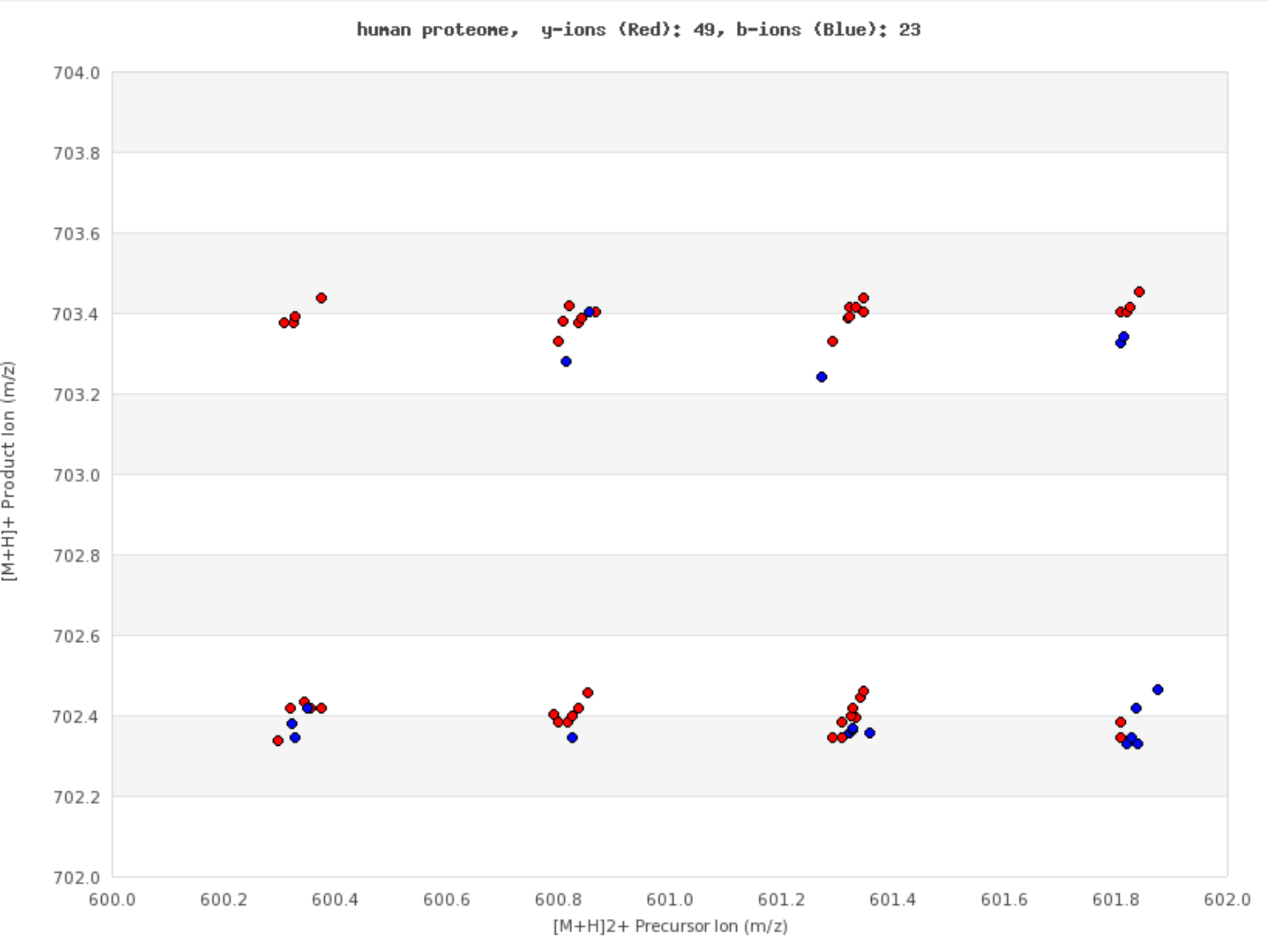
SWATH-MS

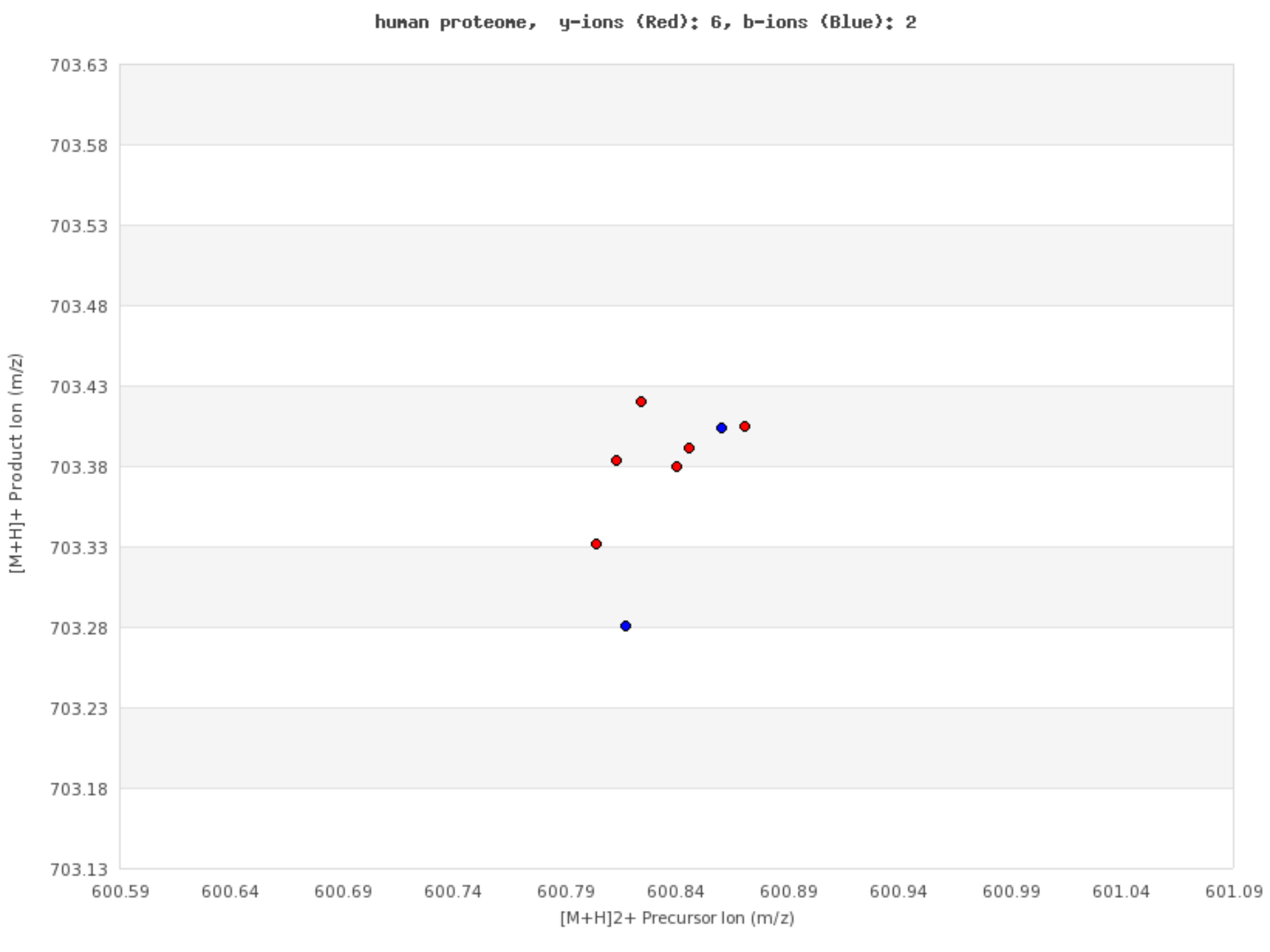
- Unlike where precursors ions are recorded and then a few of them selected for MSMS
- In SWATH, ALL ions are fragmented
- Requires generation of sample-specific database of observable proteins
- Collected ions represent a digital database of the peptides in the sample
- Can be re-searched when new databases are generated
- IMPORTANT difference – it is thoroughly quantitative
  - Many, many quantitative MSMS experiments in one

MRMPATH

http://tmpl.uab.edu/MRMPath/


human proteome, y-ions (Red): 553, b-ions (Blue): 434
710
705
700
600
605
610
[M+H]+ Product Ion (m/z)
[M+H]2+ Precursor Ion (m/z)


human proteome, y-ions (Red): 49, b-ions (Blue): 23
704.0
703.8
703.6
703.4
703.2
703.0
702.8
702.6
702.4
702.2
702.0
[M+H]+ Product Ion (m/z)
600.0
600.2
600.4
600.6
600.8
601.0
601.2
601.4
601.6
601.8
602.0
[M+H]2+ Precursor Ion (m/z)


human proteome, y-ions (Red): 6, b-ions (Blue): 2
703.63
703.58
703.53
703.48
703.43
703.38
703.33
703.28
703.23
703.18
703.13
[M+H]+ Product Ion (m/z)
600.59
600.64
600.69
600.74
600.79
600.84
600.89
600.94
600.99
601.04
601.09
[M+H]2+ Precursor Ion (m/z)

Peptide/protein records and link

Precursor ion (x)	Product ion (y)	info	link
600.7993	703.3310	GNVLNSPEDQKI>sp|Q8IYD8|FANCM_HUMAN Fanconi anemia group M protein OS=Homo sapiens GN=FANCM PE=1 SV=2	http://www.uniprot.org/uniprot/Q8IYD8
600.8089	703.3825	WPVDAWEVAKI>sp|Q8TB03|CX038_HUMAN Uncharacterized protein CXorf38 OS=Homo sapiens GN=CXorf38 PE=1 SV=1	http://www.uniprot.org/uniprot/Q8TB03
600.8195	703.4190	GPVDETGWVIKI>sp|P51160|PDE6C_HUMAN Cone cGMP-specific 3",5"-cyclic phosphodiesterase subunit alpha" OS=Homo sapiens GN=PDE6C PE=1 SV=2	http://www.uniprot.org/uniprot/P51160
600.8357	703.3786	LTVSPEPSSKRI>sp|Q969F2|NKD2_HUMAN Protein naked cuticle homolog 2 OS=Homo sapiens GN=NKD2 PE=1 SV=1	http://www.uniprot.org/uniprot/Q969F2
600.8413	703.3898	ALELASQANRKI>sp|P09681|GIP_HUMAN Gastric inhibitory polypeptide OS=Homo sapiens GN=GIP PE=1 SV=1	http://www.uniprot.org/uniprot/P09681
600.8665	703.4038	AVLITDQSILKI>sp|P26374|RAE2_HUMAN Rab proteins geranylgeranyltransferase component A 2 OS=Homo sapiens GN=CHML PE=1 SV=2	http://www.uniprot.org/uniprot/P26374
600.8125	703.2800	EHHPDSPLLRI>sp|O14753|OVOL1_HUMAN Putative transcription factor Ovo-like 1 OS=Homo sapiens GN=OVOL1 PE=2 SV=3	http://www.uniprot.org/uniprot/O14753
600.8559	703.4030	LFVETLHITKI>sp|Q8WZ42|TITIN_HUMAN Titin OS=Homo sapiens GN=TTN PE=1 SV=4	http://www.uniprot.org/uniprot/Q8WZ42

These are peptides that would conform to a *m/z* 601 doubly charged tryptic peptide with a singly charged, *m/z* 703 product ion using the quadrupole windows of 0.7 *m/z*