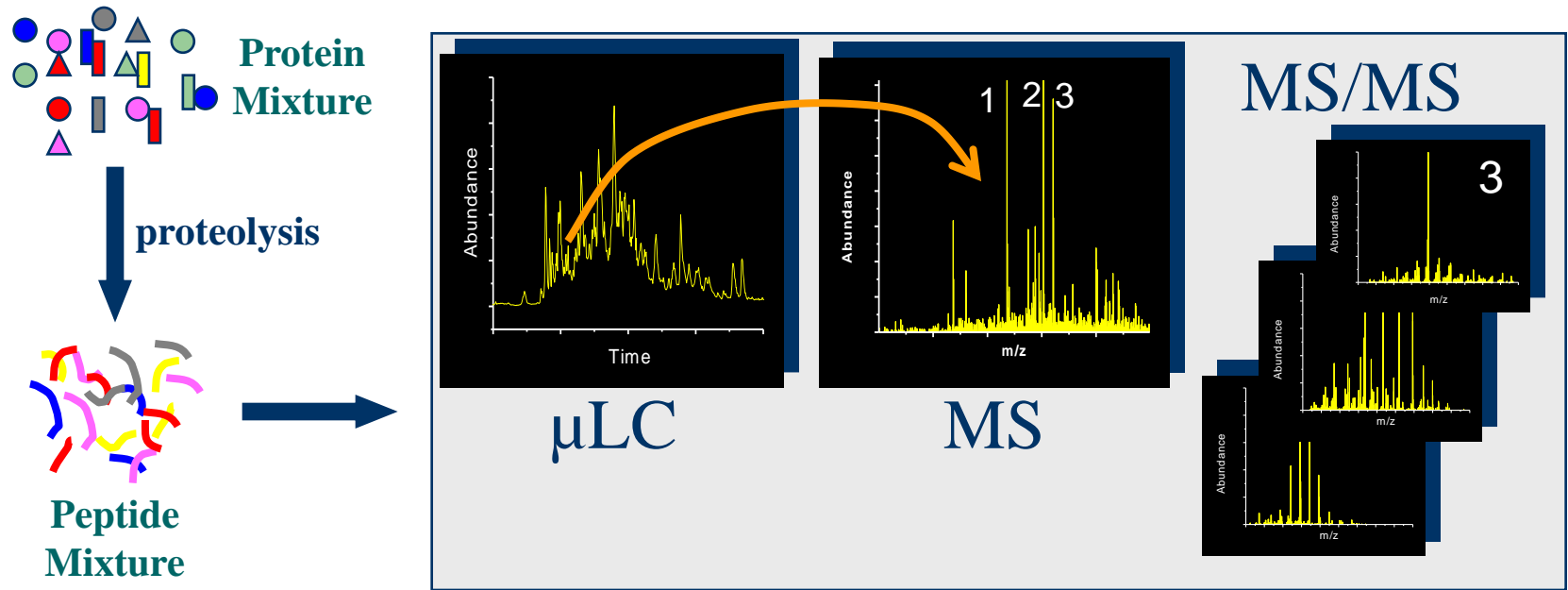
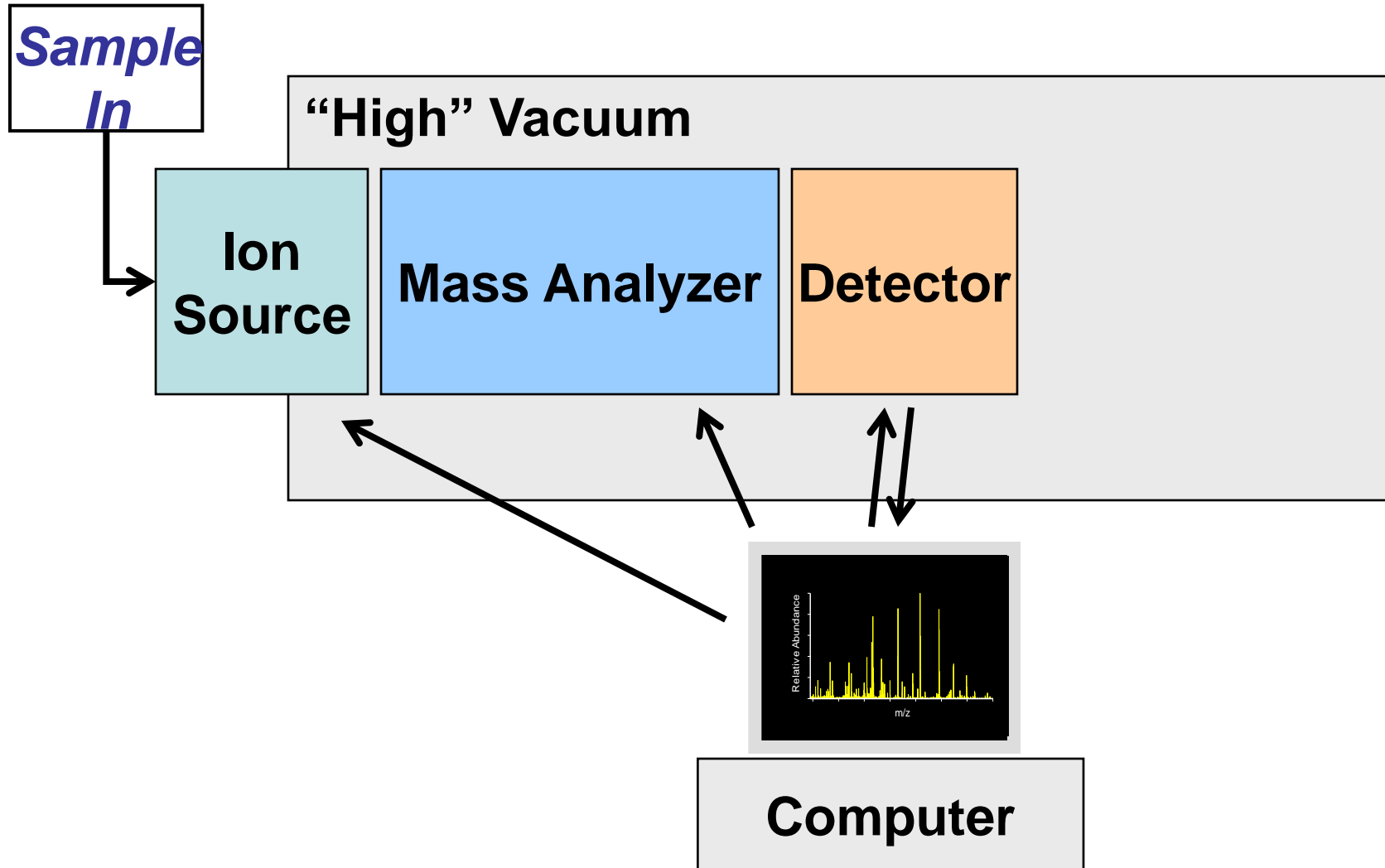


Shotgun Proteomics

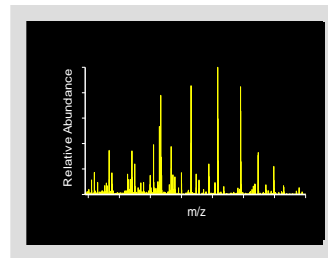
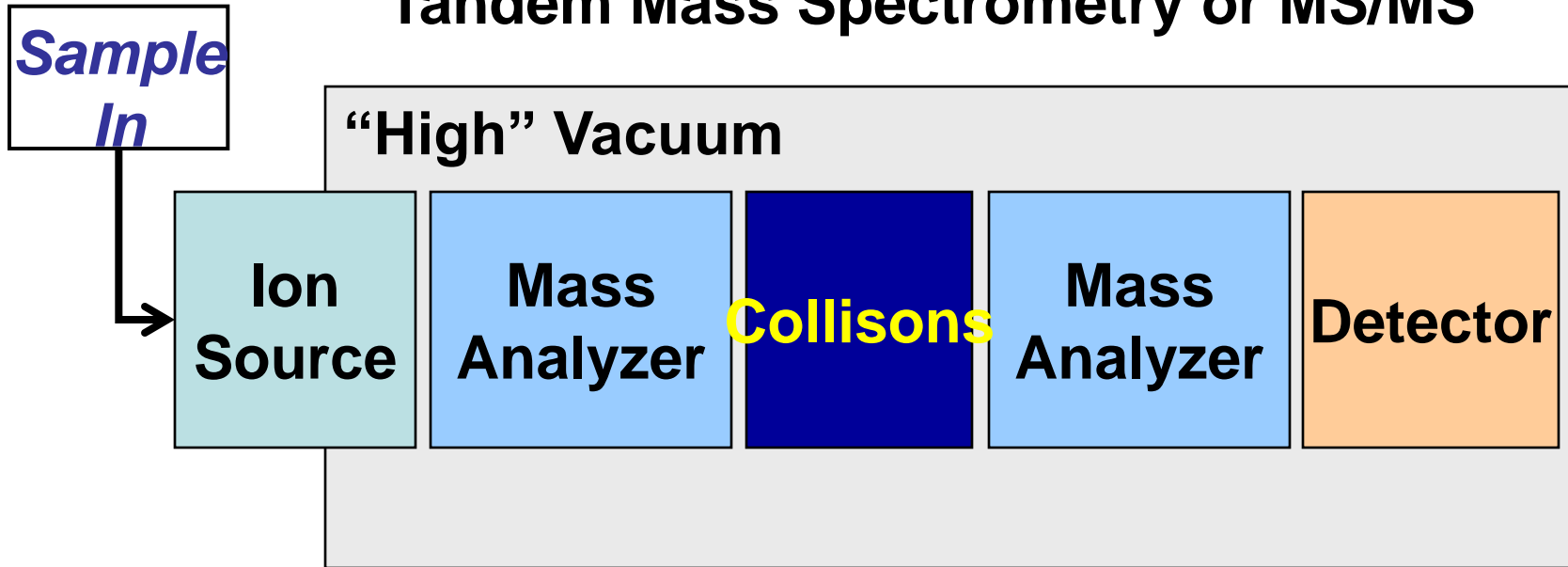


Block Diagram of a Mass Spectrometer



Block Diagram of a Mass Spectrometer

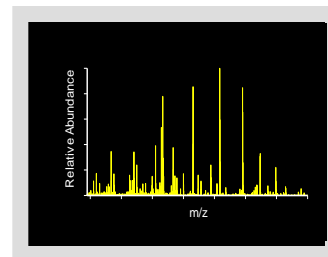
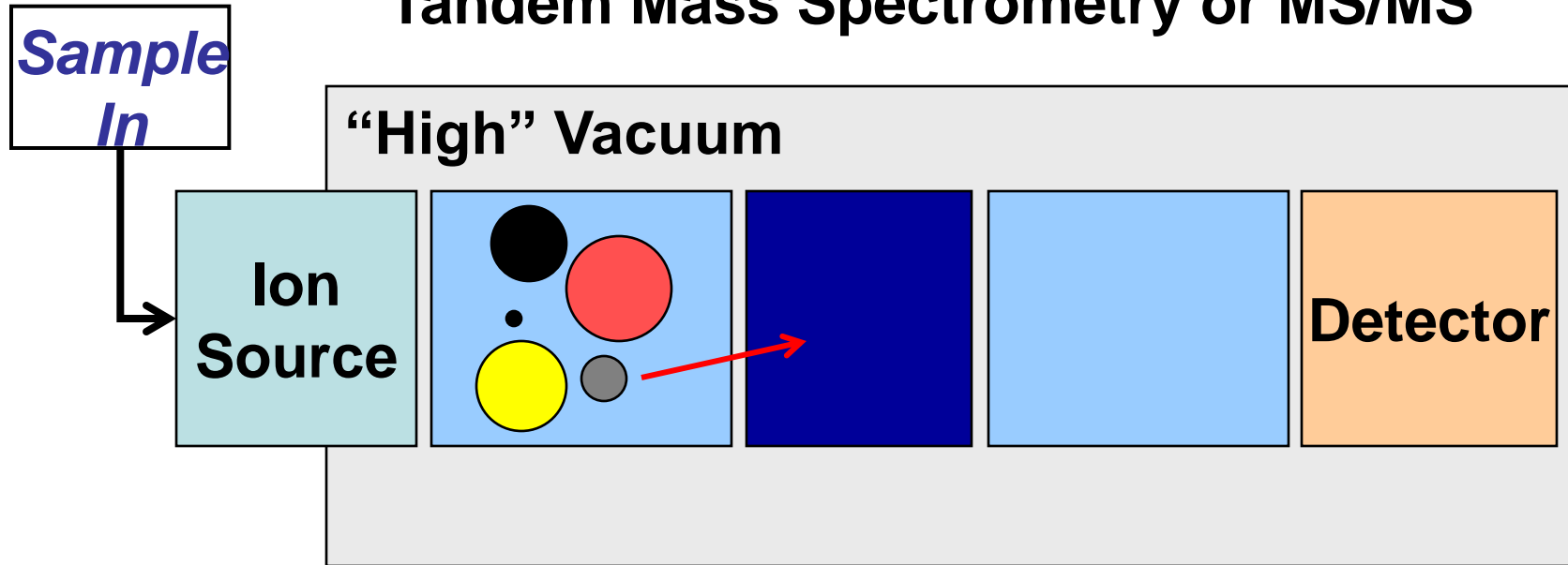
Tandem Mass Spectrometry or MS/MS



Computer

Block Diagram of a Mass Spectrometer

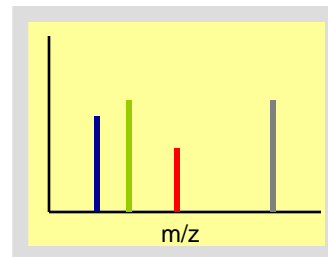
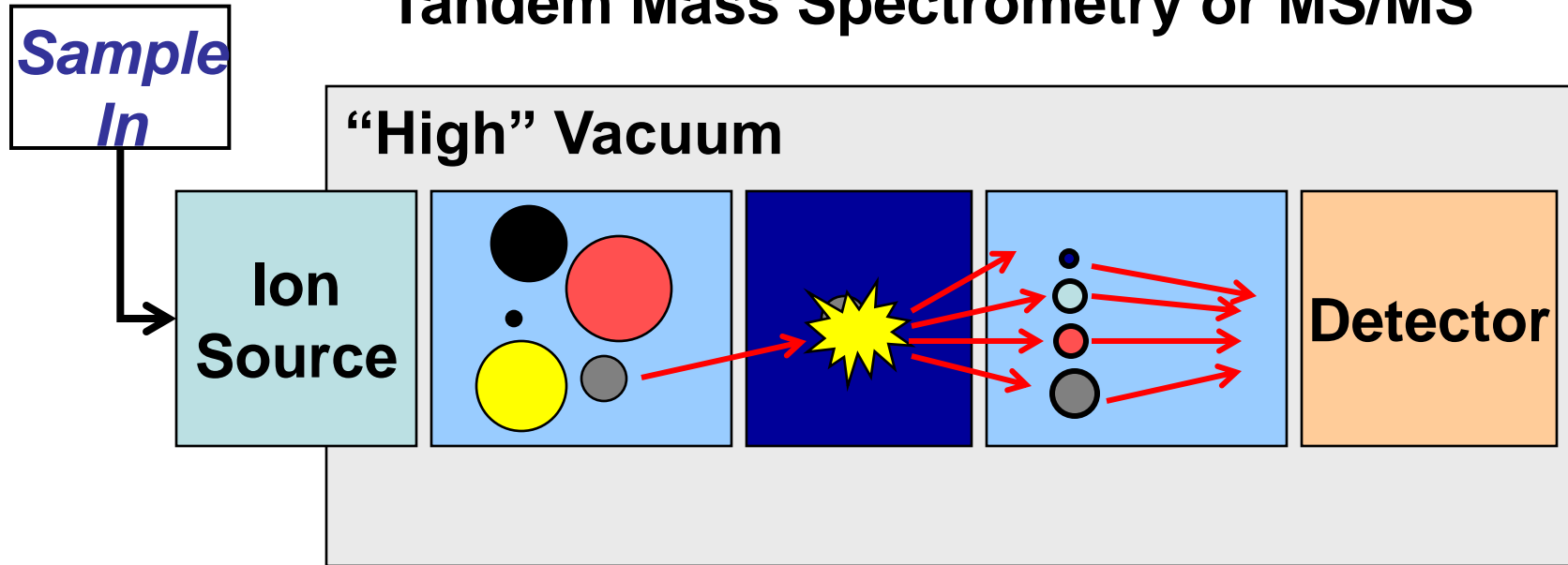
Tandem Mass Spectrometry or MS/MS



Computer

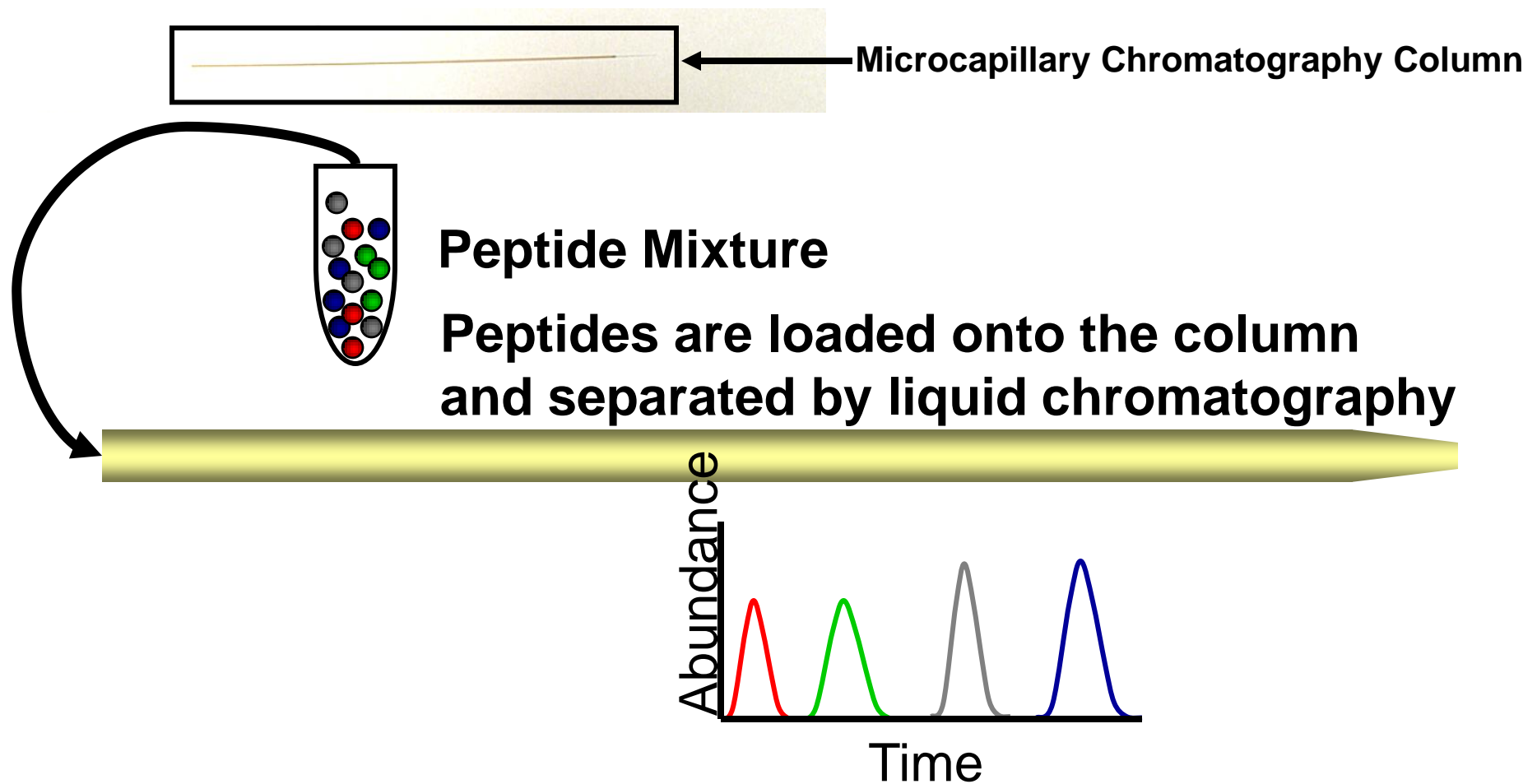
Block Diagram of a Mass Spectrometer

Tandem Mass Spectrometry or MS/MS

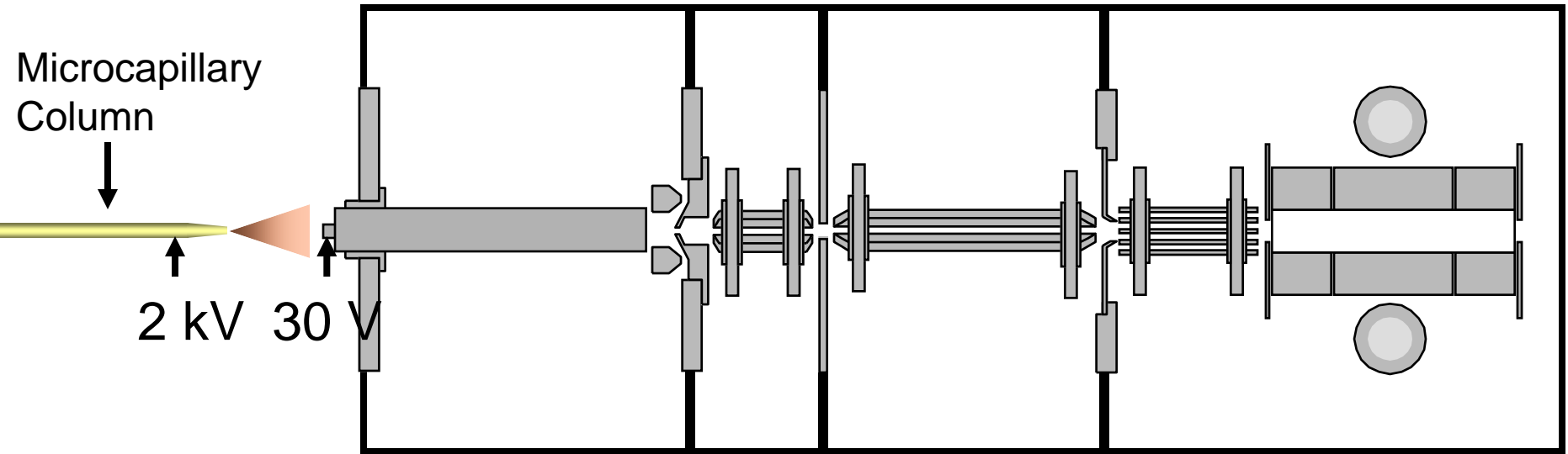


Computer

Measurement of Peptides by Microcapillary Liquid Chromatography Tandem Mass Spectrometry

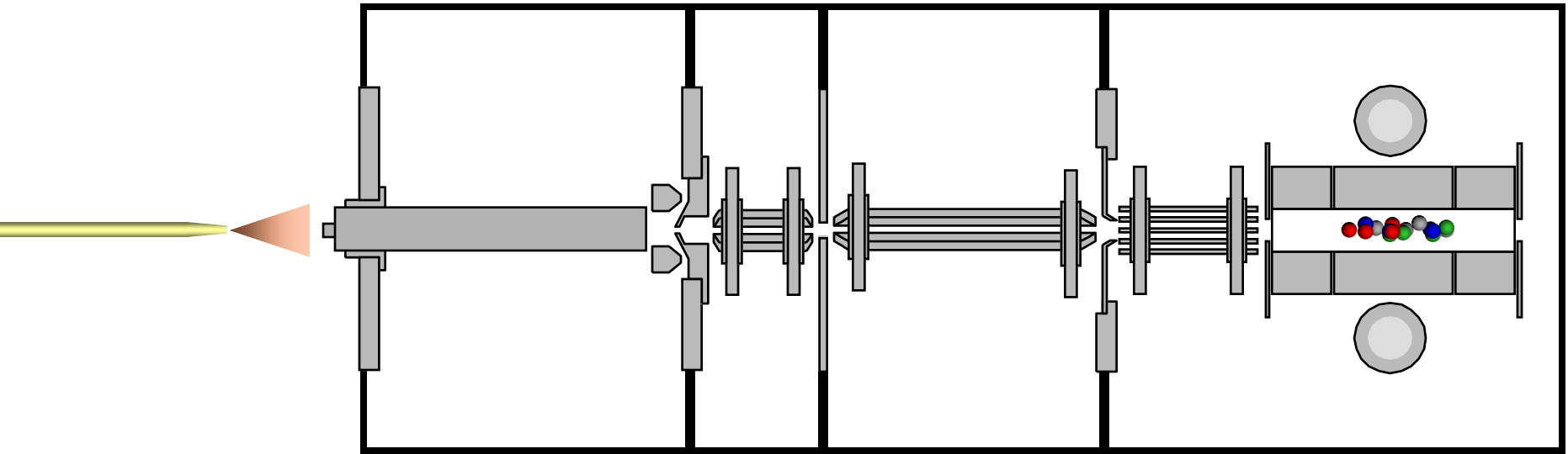


Acquiring Mass Spectrometry Data of Peptides

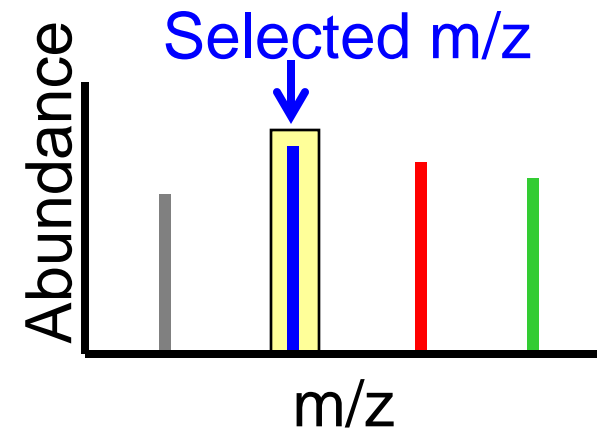


- Peptides from the column are ionized and transmitted into the vacuum system and trapped in the mass spectrometer

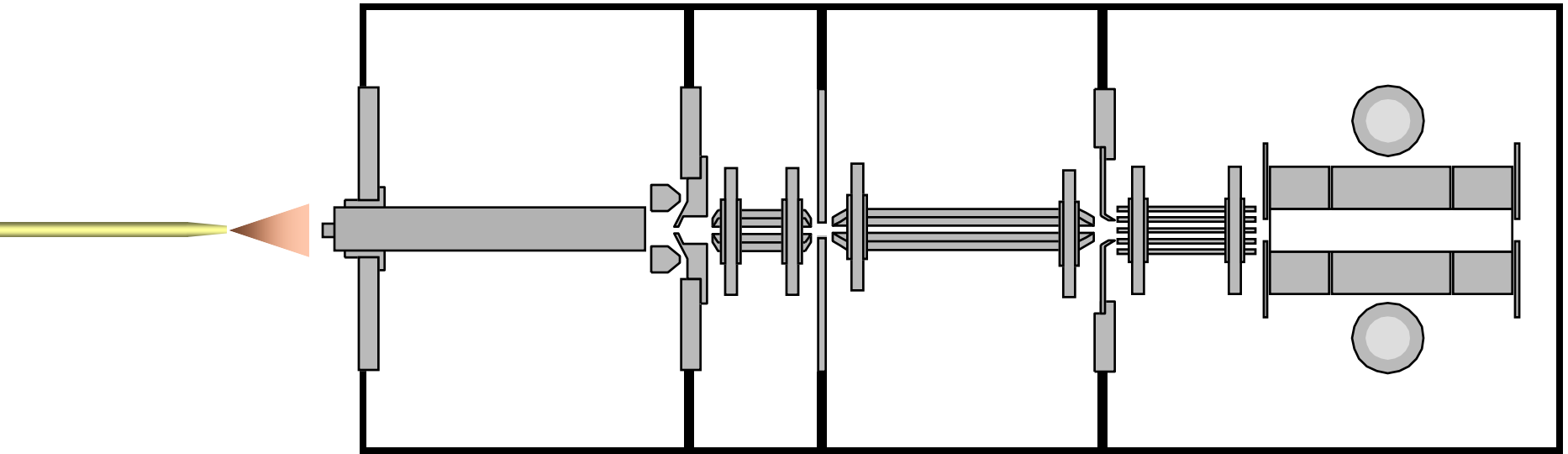
Acquiring Mass Spectrometry Data of Peptides



- Peptides from the column are ionized and transmitted into the vacuum system and trapped in the mass spectrometer
- Peptides are selectively ejected by their mass to produce a mass spectrum
- The instrument automatically selects a peptide for further analysis

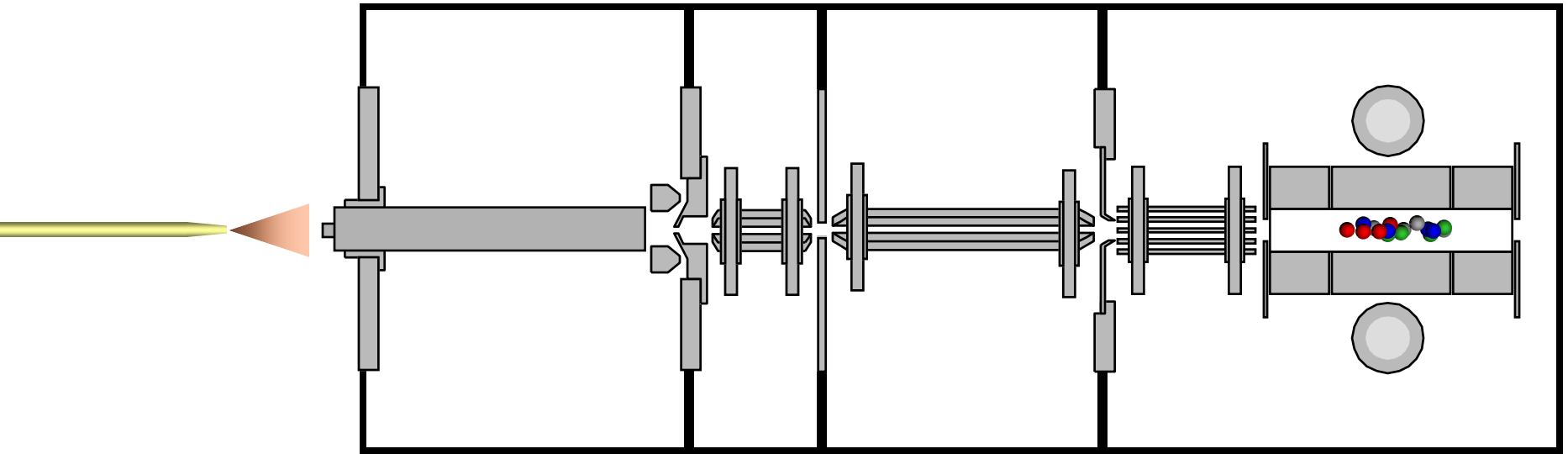


Acquiring a Fragmentation Spectrum



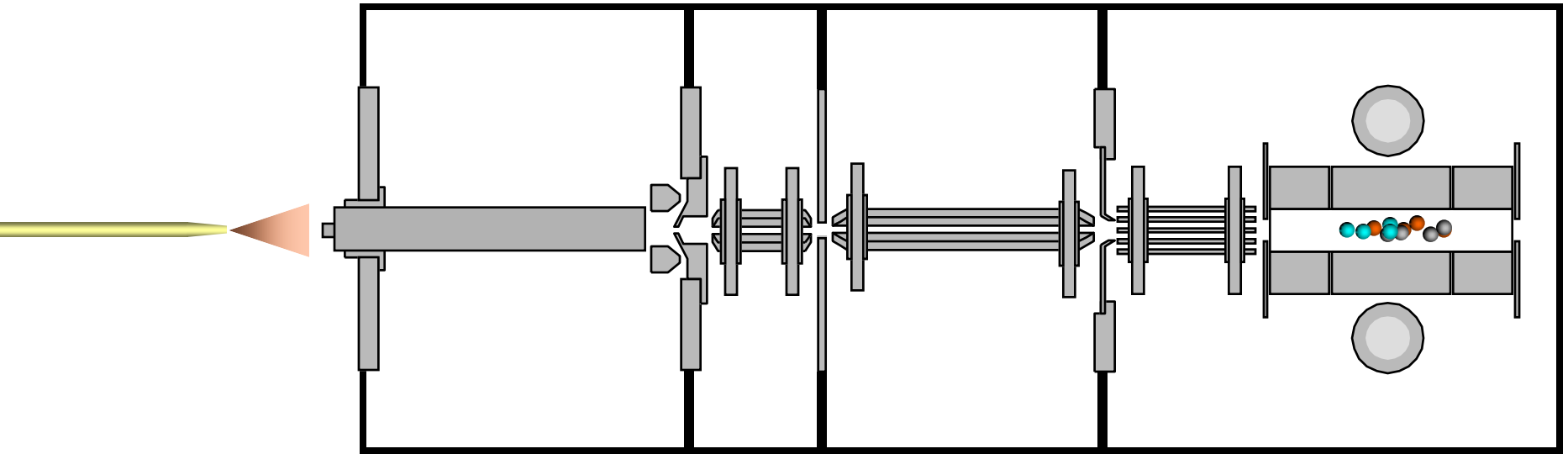
- The trap is then refilled

Acquiring a Fragmentation Spectrum

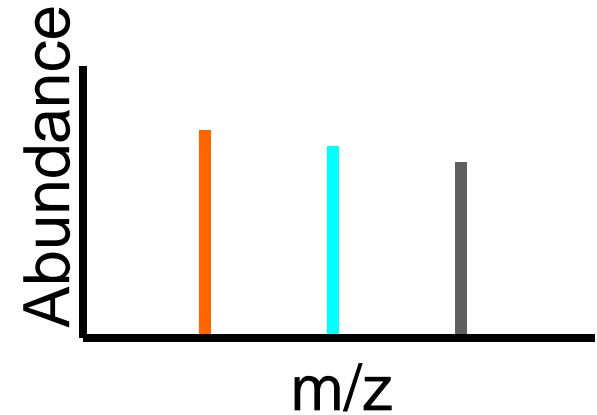


- The trap is then refilled
- All peptides are ejected from the trap except peptides with the predetermined mass
- The energy of the isolated peptide is increased resulting in collisions with a gas

Acquiring a Fragmentation Spectrum



- The collisions result in structure specific fragments
- The fragments are then selectively ejected to produce a tandem mass spectrum



Multidimensional Protein Identification Technology (MudPIT)

- 100 μm i.d. fused silica
- Tip is pulled to ~ 5 μm I.D.



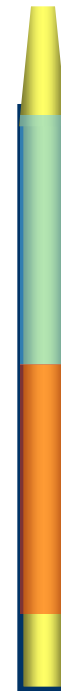
Multidimensional Protein Identification Technology (MudPIT)

- 100 μm i.d. fuse silica
- Tip is pulled to $\sim 5 \mu\text{m}$ I.D.
- Pack with **C18** material 1st



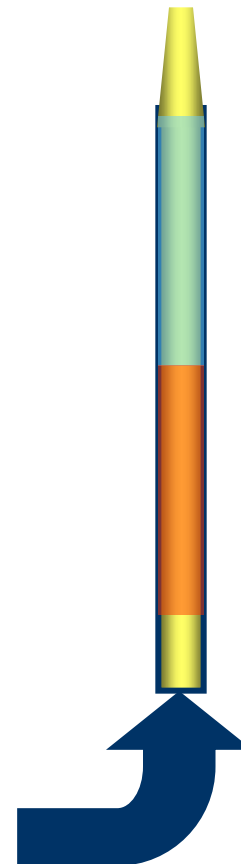
Multidimensional Protein Identification Technology (MudPIT)

- 100 μm i.d. fuse silica
- Tip is pulled to $\sim 5 \mu\text{m}$ I.D.
- Pack with **C18** material 1st
- Pack with **SCX** material 2nd



Multidimensional Protein Identification Technology (MudPIT)

- 100 μm i.d. fuse silica
- Tip is pulled to $\sim 5 \mu\text{m}$ I.D.
- Pack with **C18** material 1st
- Pack with **SCX** material 2nd
- Peptide digest is loaded off-line

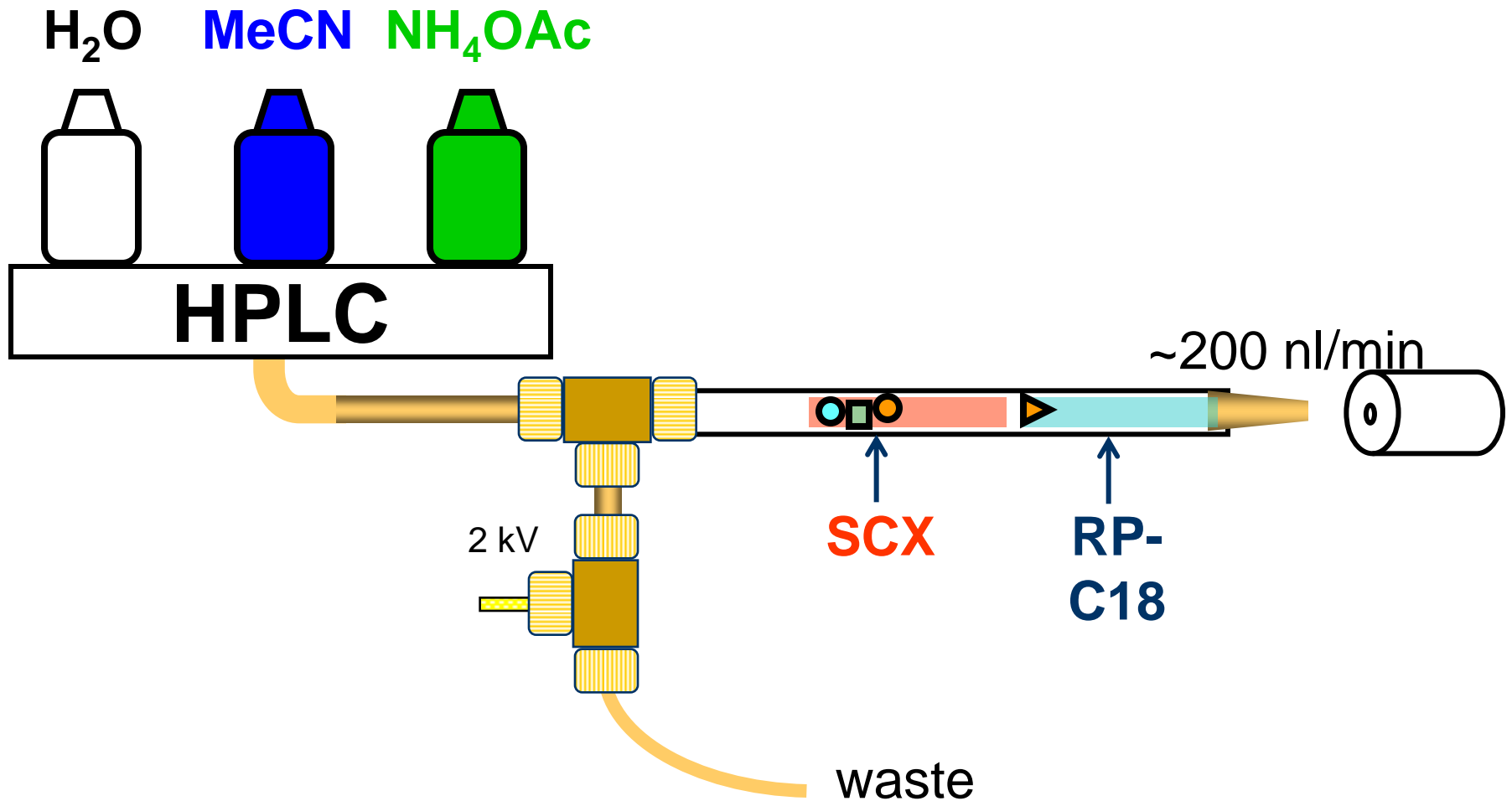


Multidimensional Protein Identification Technology (MudPIT)

- 100 μm i.d. fused silica
- Tip is pulled to $\sim 5 \mu\text{m}$ I.D.
- Pack with **C18** material 1st
- Pack with **SCX** material 2nd
- Peptide digest is loaded off-line

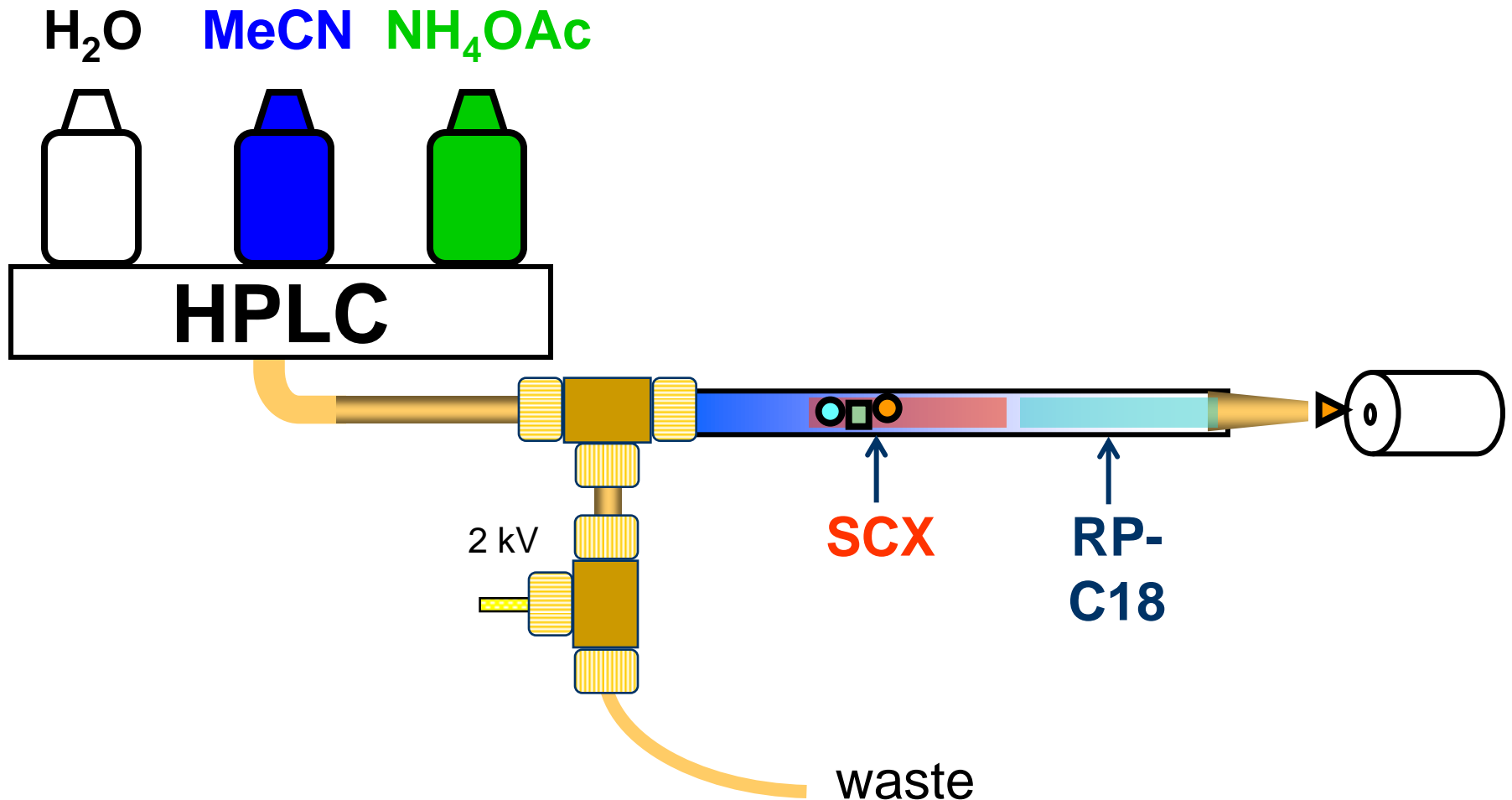


Multidimensional Protein Identification Technology (MudPIT)



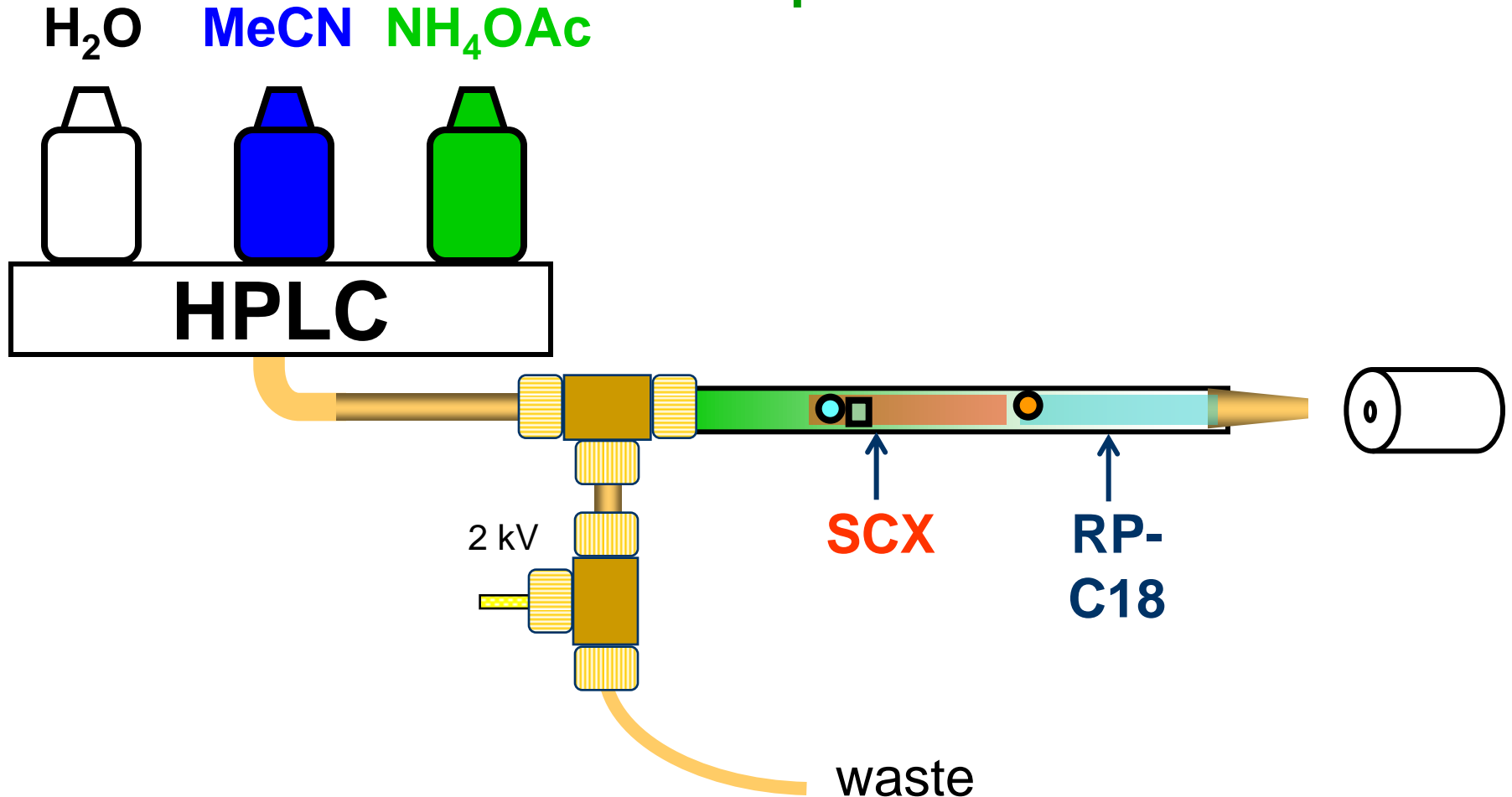
Multidimensional Protein Identification Technology (MudPIT)

- **Step 1** – Reverse Phase Gradient



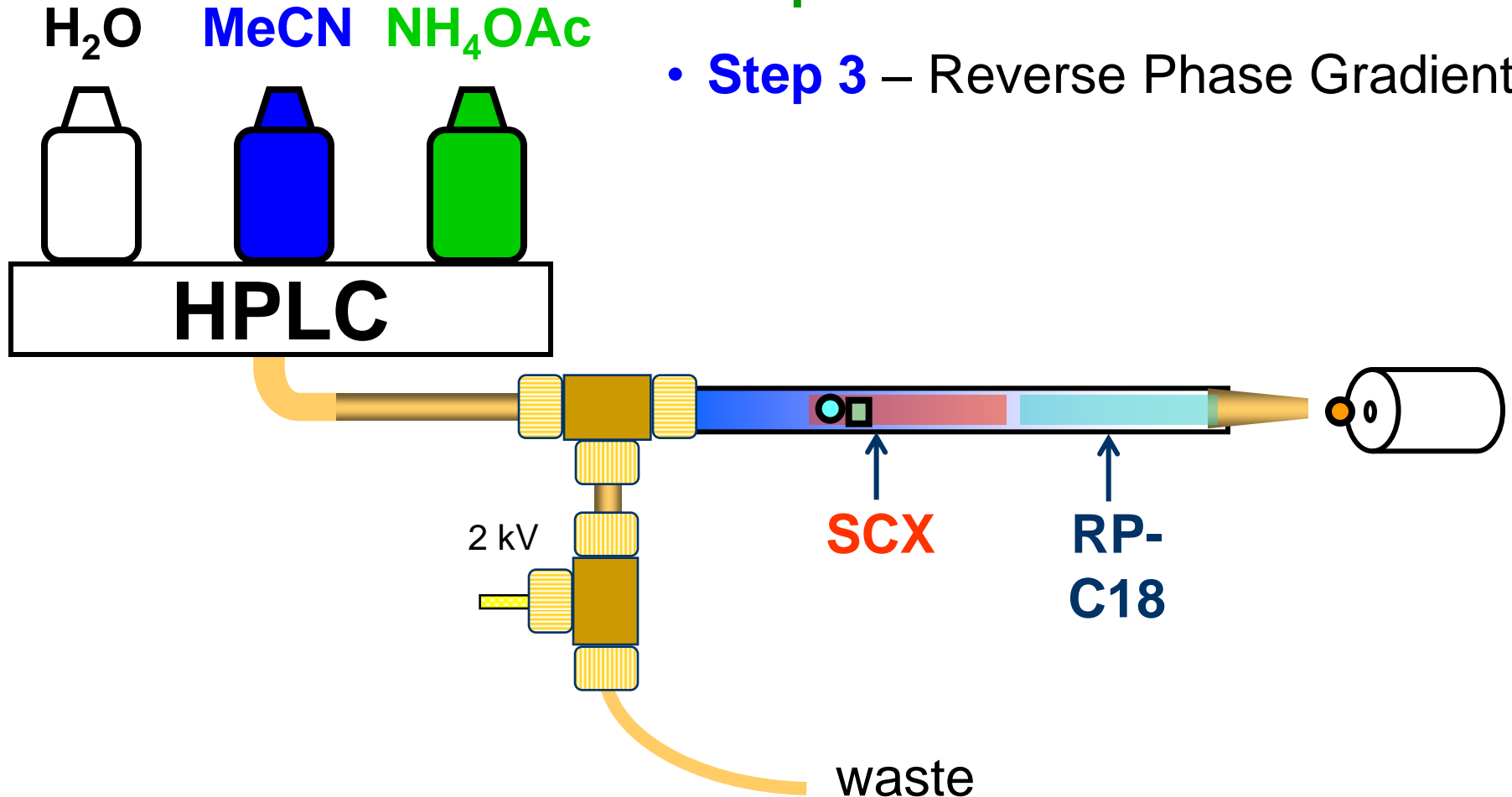
Multidimensional Protein Identification Technology (MudPIT)

- **Step 1** – Reverse Phase Gradient
- **Step 2** – Salt Pulse



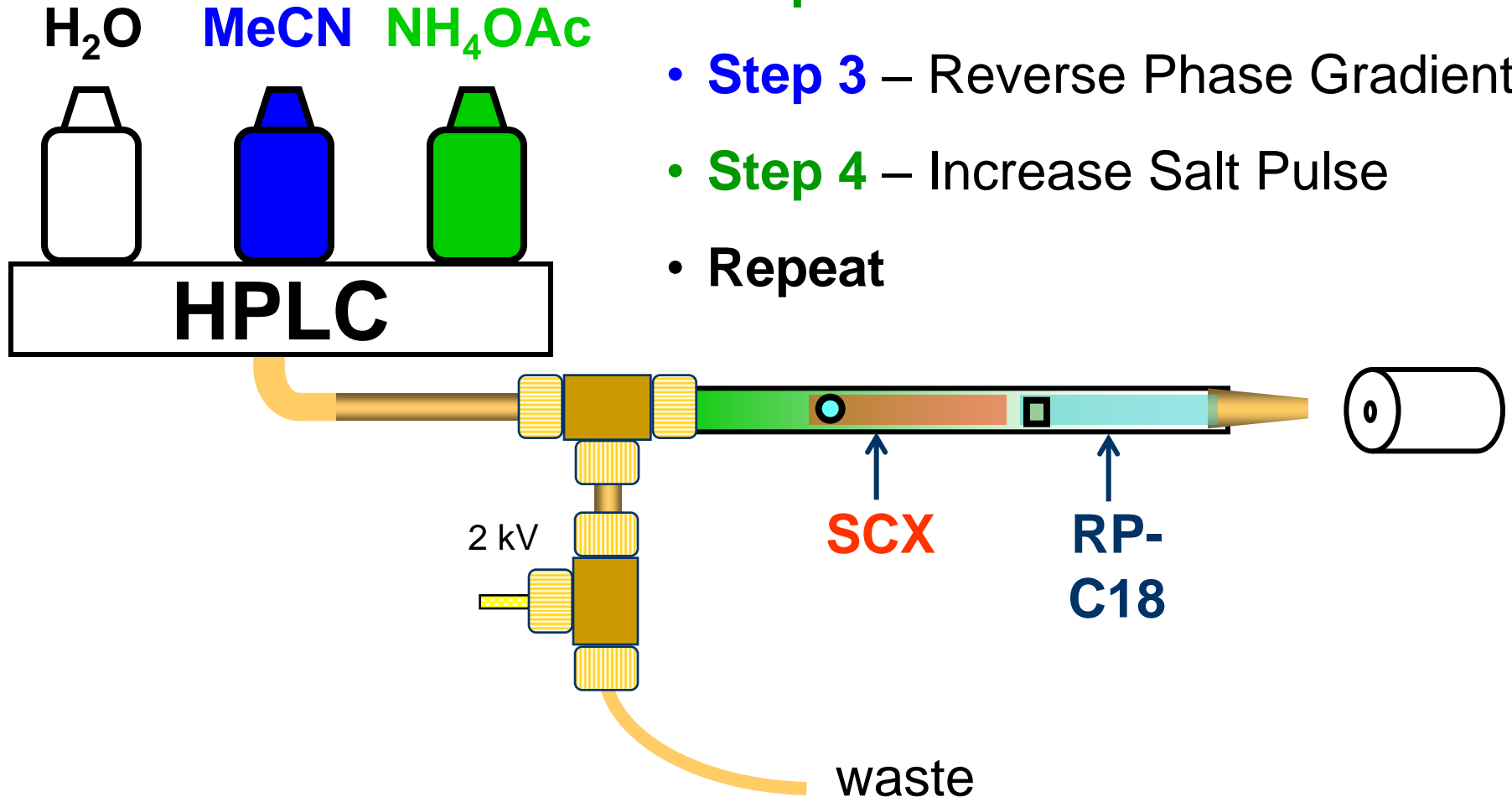
Multidimensional Protein Identification Technology (MudPIT)

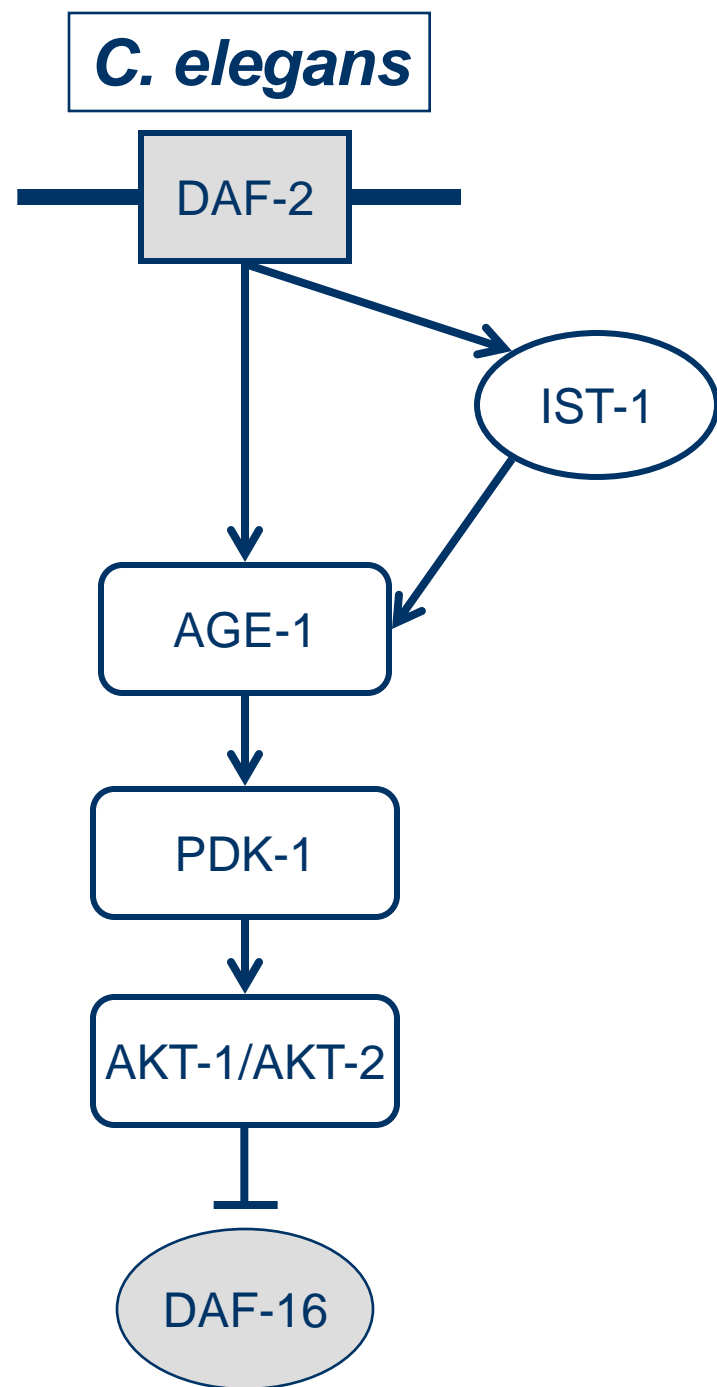
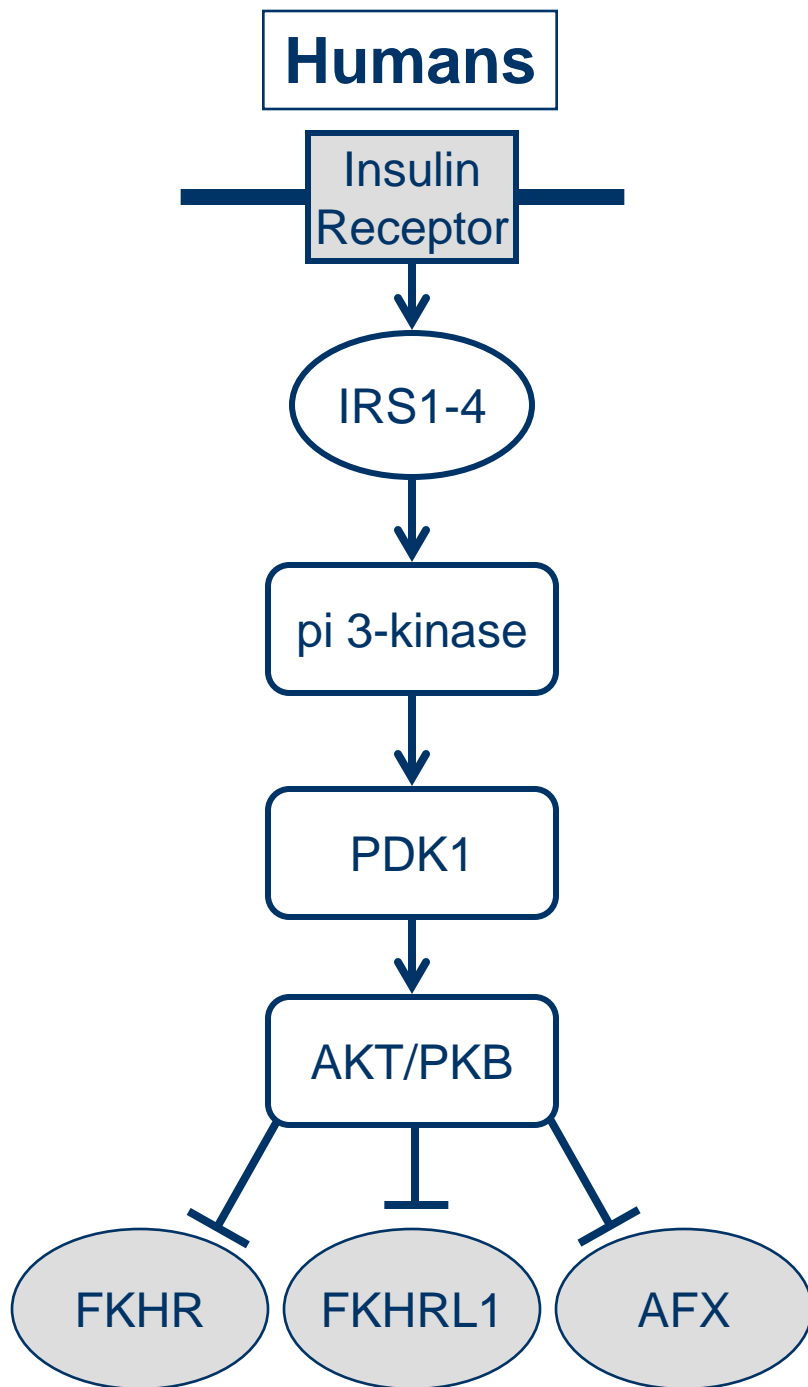
- **Step 1** – Reverse Phase Gradient
- **Step 2** – Salt Pulse
- **Step 3** – Reverse Phase Gradient



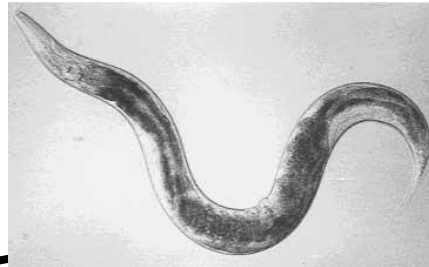
Multidimensional Protein Identification Technology (MudPIT)

- **Step 1** – Reverse Phase Gradient
- **Step 2** – Salt Pulse
- **Step 3** – Reverse Phase Gradient
- **Step 4** – Increase Salt Pulse
- Repeat





Fractionation to Improve the Coverage of Proteins Involved in Insulin / IGF-1 Signaling



0 Proteins Identified that are Known to be Involved in the Insulin / IGF-1 Signaling Pathway

Rev
Pr

s
tion

10 Fractions

8 Fractions

10 Fractions



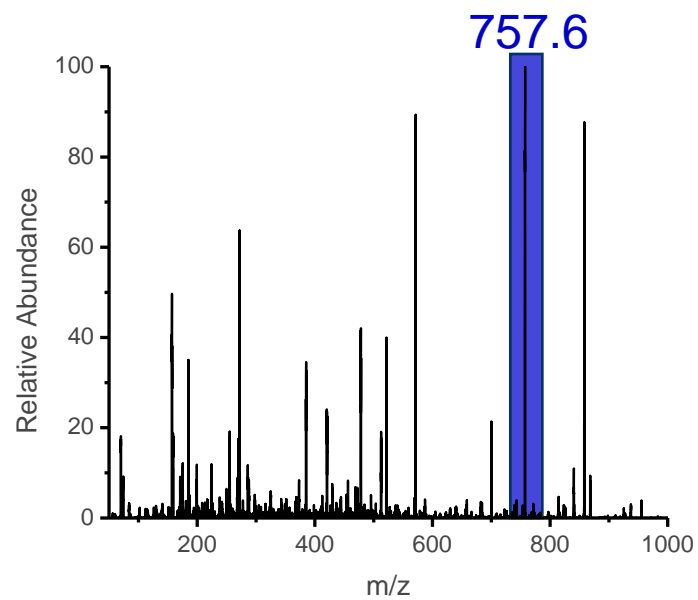
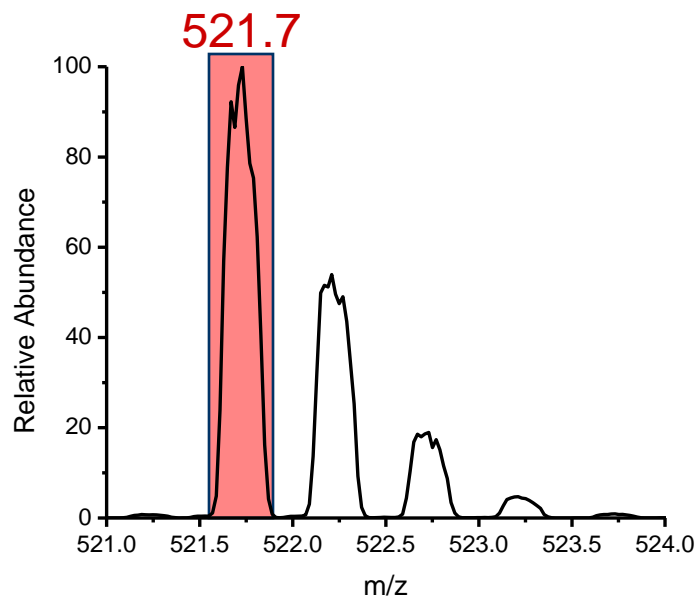
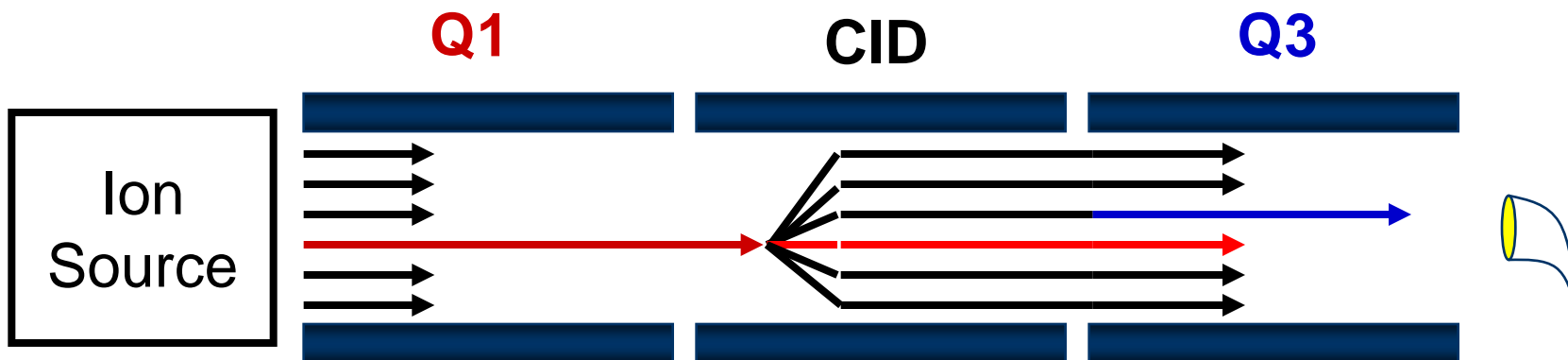
10 MudPITs

8 MudPITs

10 MudPITs

>6,000,000 MS/MS Spectra, 21,264 Unique Peptide Identifications, and 4,373 Unique Protein Identifications

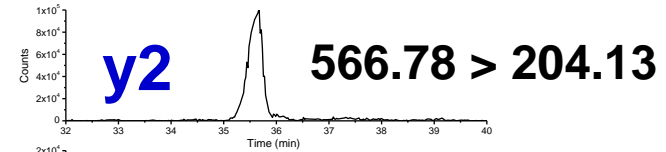
Targeted Proteomics using nanoLC-hSRM-MS



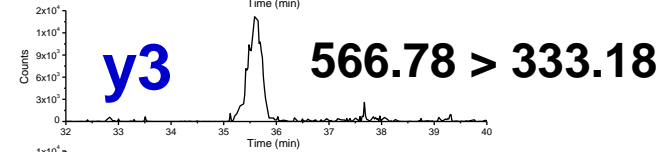
Precursor Ion > Product Ion

SRM Chromatograms

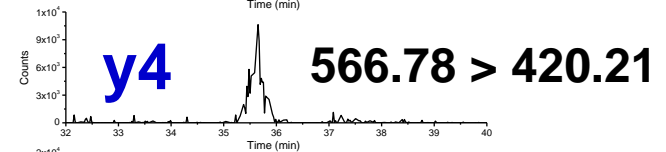
TGASEAVPSEGK > GK



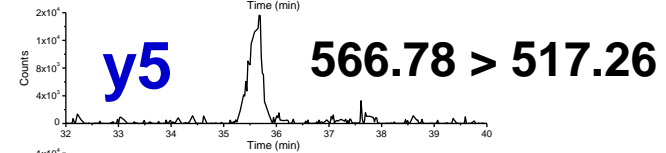
TGSAEAVPSEGK > EGK



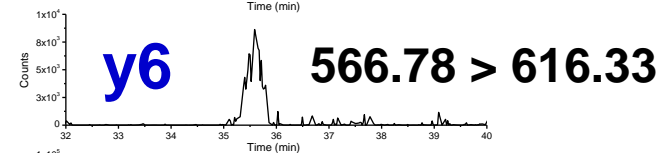
TGSAEAVPSEGK > SEGK



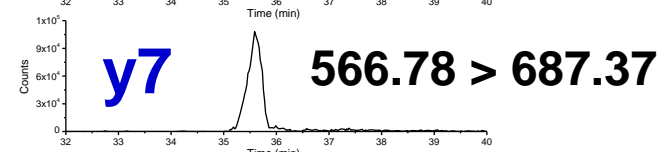
TGSAEAVPSEGK > PSEGK



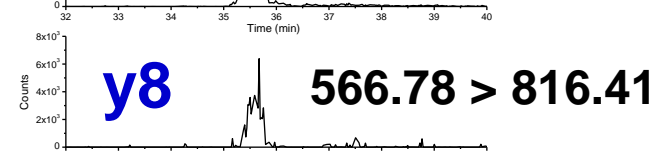
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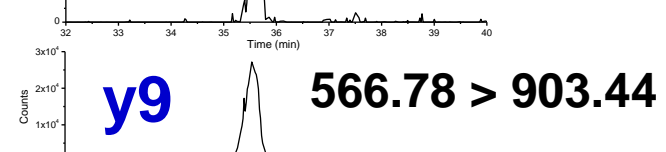
TGSAEAVPSEGK > AVPSEGK



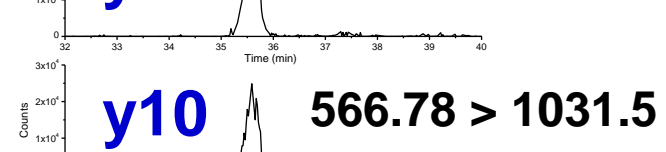
TGSAEAVPSEGK > EAVPSEGK



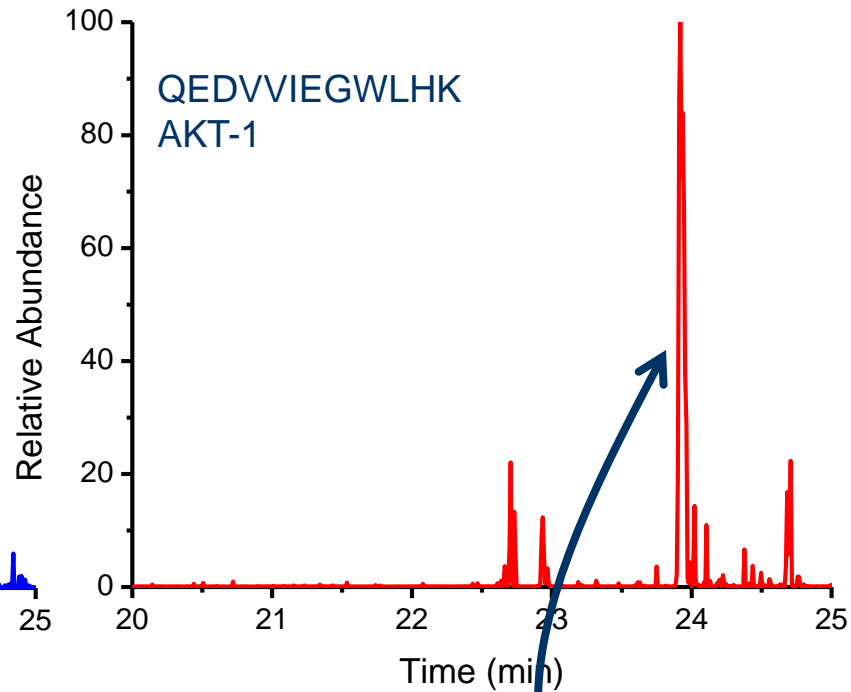
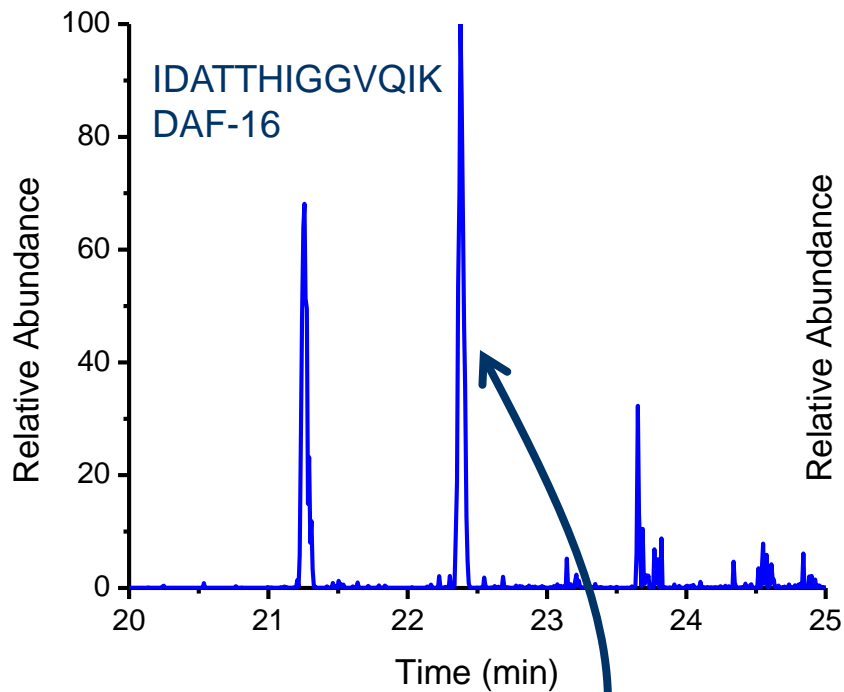
TGSAEAVPSEGK > AEAVPSEGK



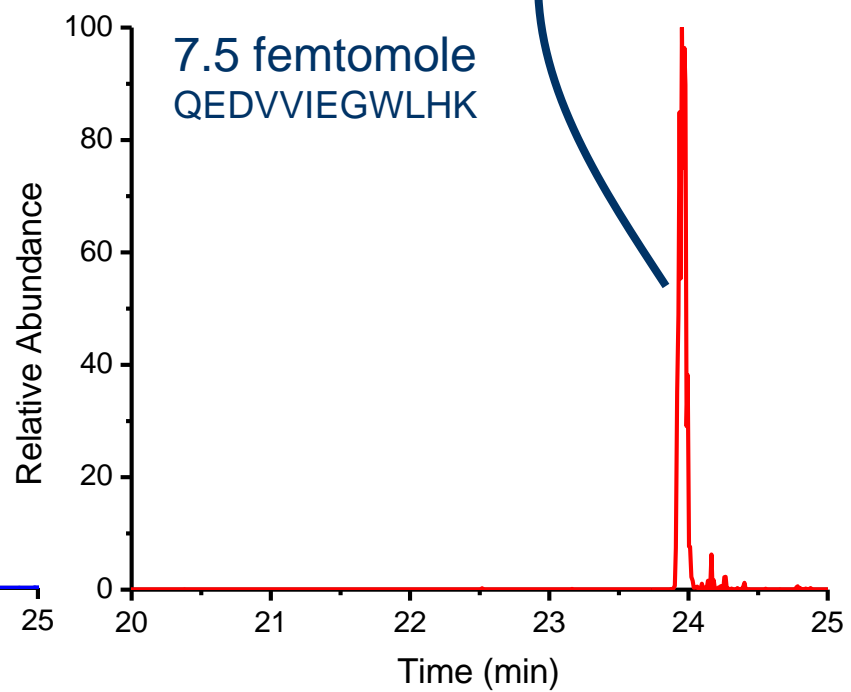
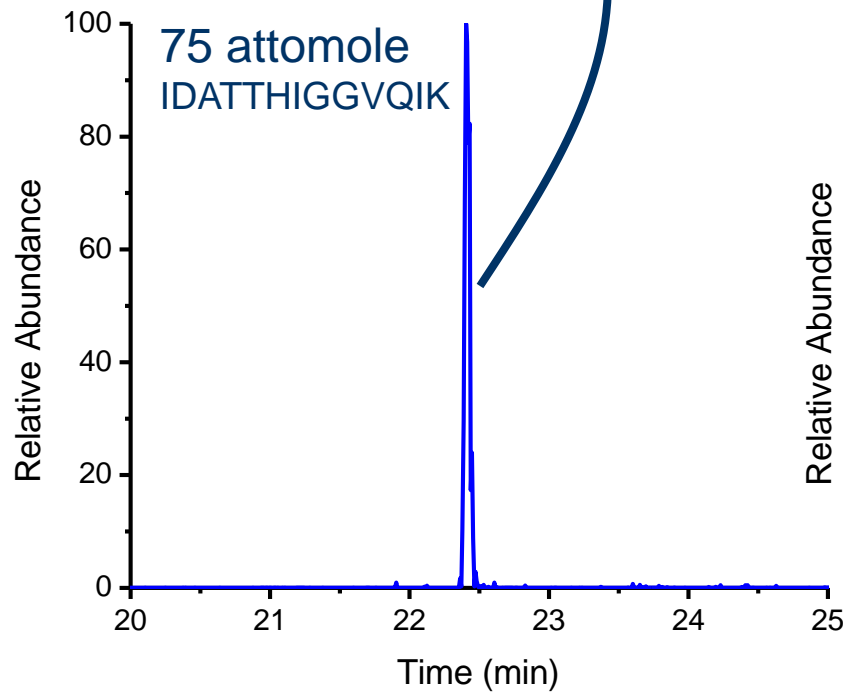
TGSAEAVPSEGK > SAEAVPSEGK



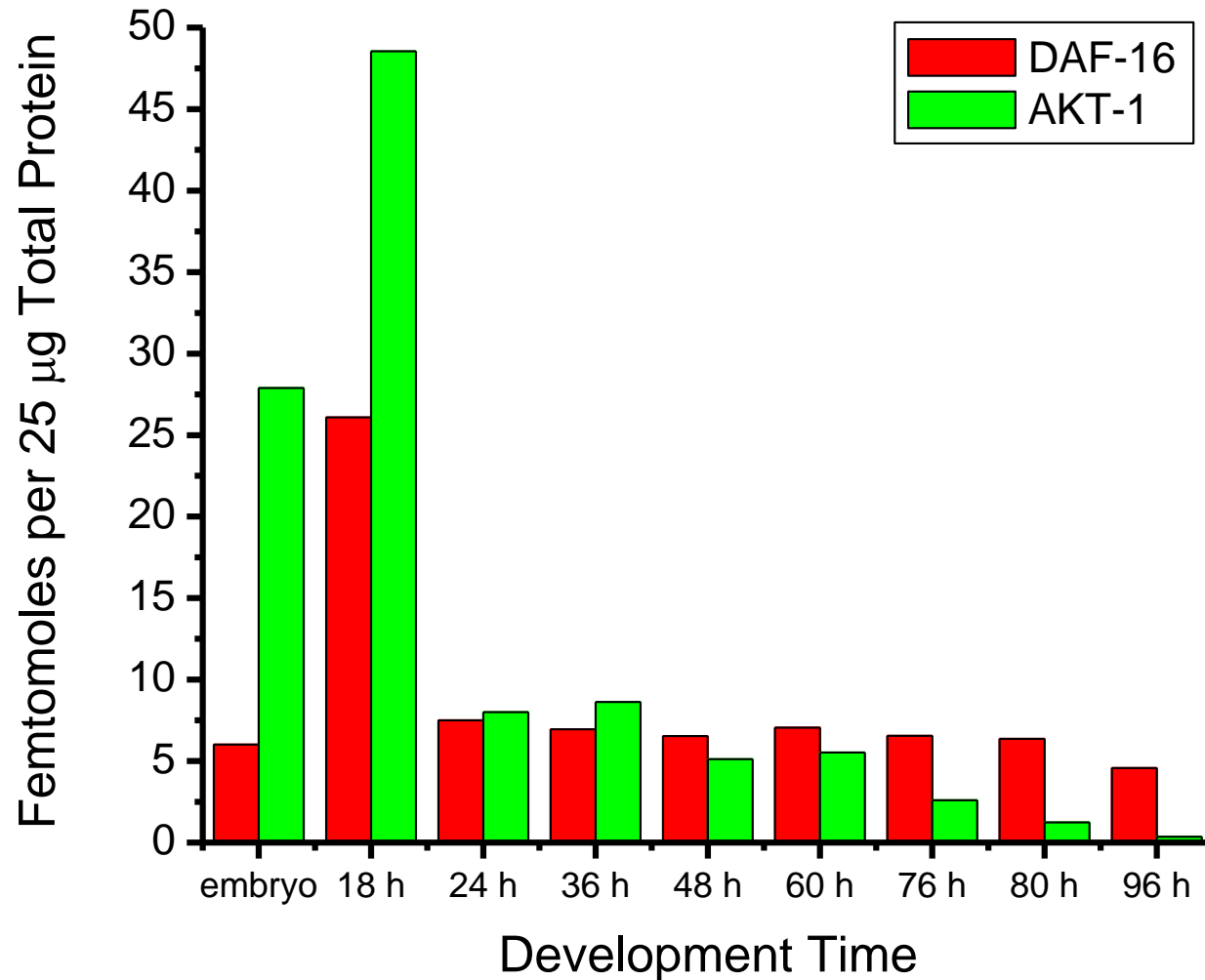
Worm Homogenate 25 μ g



Synthetic Peptide Standards



Measurement of Absolute Levels of DAF-16 and AKT-1 During Development Using Standard Addition



General Approach for Targeted Identification of Proteins by hSRM

Protein of Interest

```
1  MAESHLIQWL LLLLPTLCGP GTAAWTTSSL ACAQGPEFWC QSLEQALQCR
51  ALGHCLQEVW GHVGADDLCQ ECEDIVHILN KMAKEAIFQD TMRKFLEQEC
101 NVLPLKLLMP QCNQVLDDYF PLVIDYFQNQ TDSNGICMHL GLCKSRQPEP
151 EQEPGMSDPL PKPLRDPLPD PLLDKLVLPV LPGALQARPG PHTQDLSEQQ
201 FPIPLPYCWL CRALIKRIQA MIPKGALAVA VAQVCRVVPL VAGGICQCLA
251 ERYSVILLDT LLGRMLPQLV CRLVLRCSMD DSAGPRSPTG EWLPRDSECH
301 LCMSVTTQAG NSSEQAIPQA MLQACVGSWL DREKCKQFVE QHTPQLLTLV
351 PRGWDAAHTTC QALGVCGTMS SPLQCIHSPD L
```

Any Peptide
Identified Previously?
(LTQ)

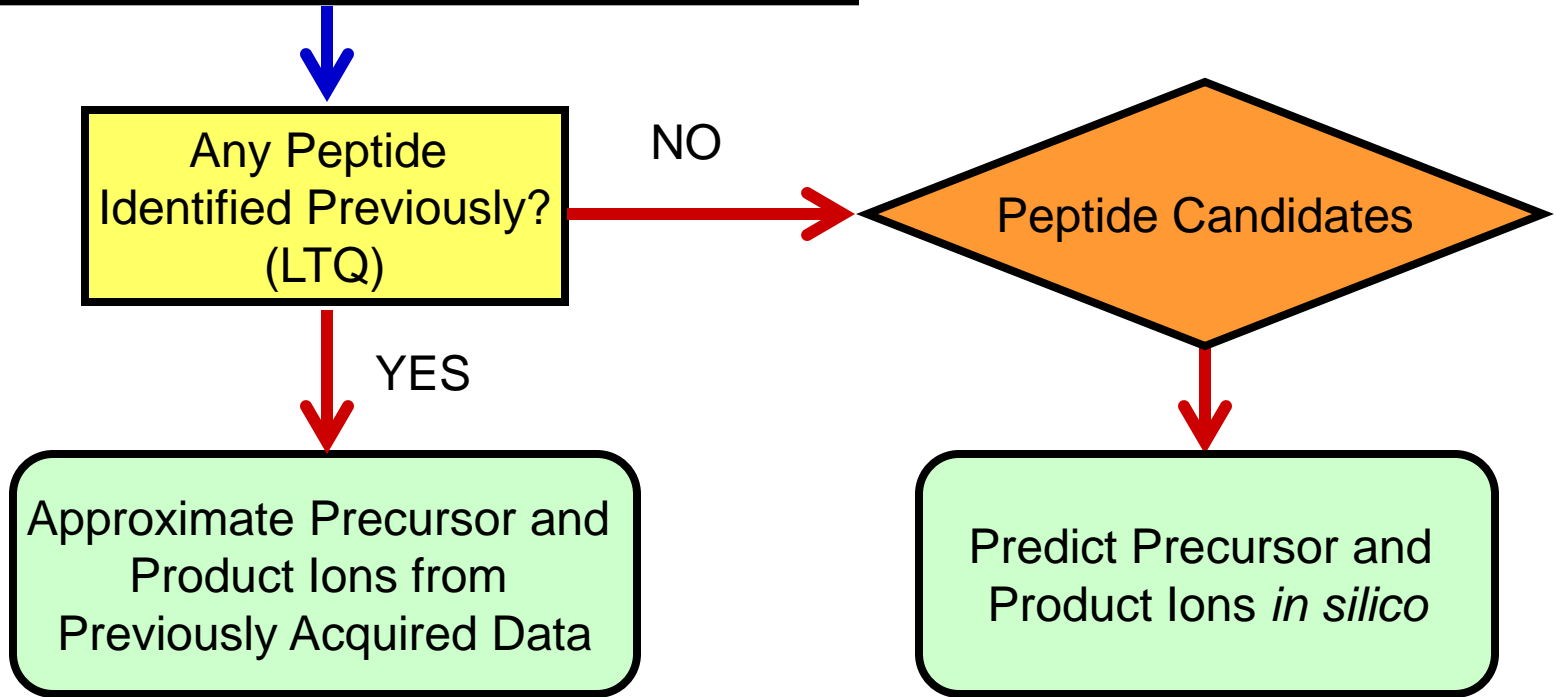
YES

Approximate Precursor and
Product Ions from
Previously Acquired Data

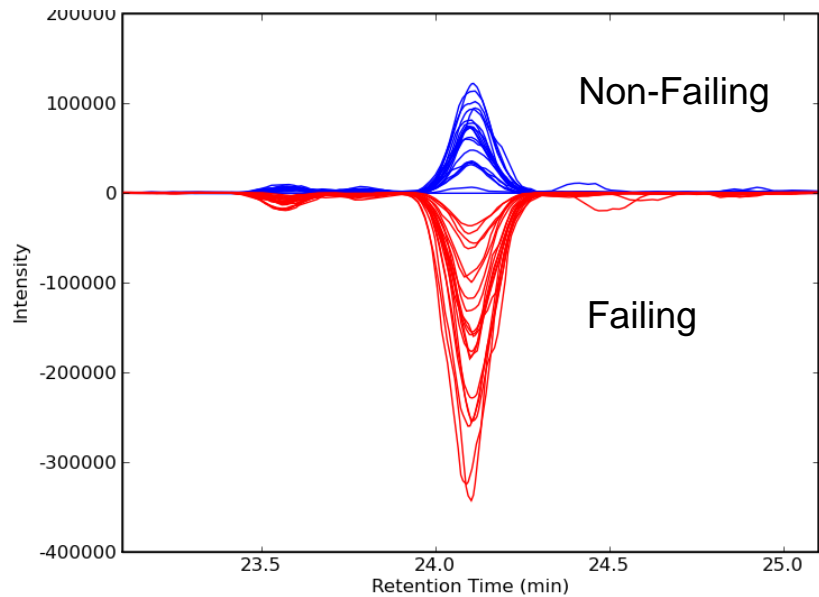
General Approach for Targeted Identification of Proteins by hSRM

Protein of Interest

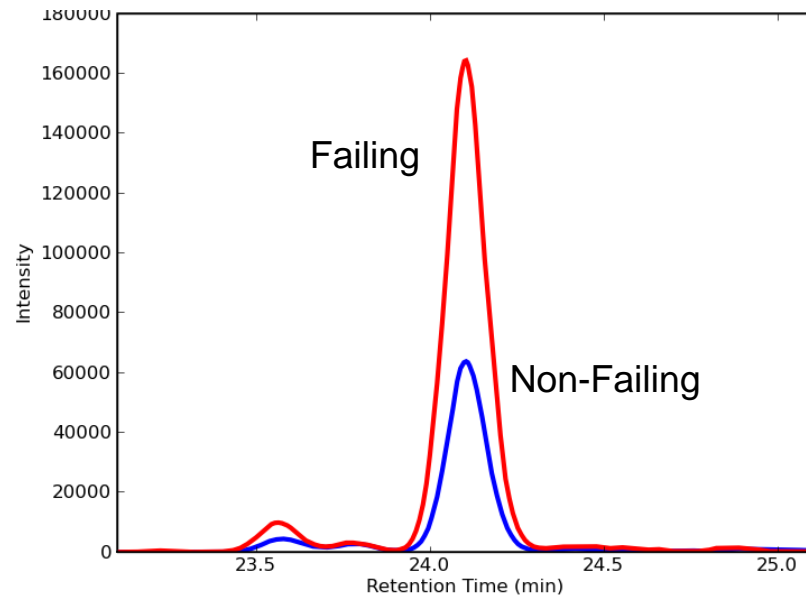
1	MAESHLLQWL	LLLLPTLCGP	GTAAWTTSSL	ACAQGPEFWC	QSLEQALQCR
51	ALGHCLQEVW	GHVGADDLCQ	ECEDIVHILN	KMAKEAIFQD	TMRKFLEQEC
101	NVLPLKLLMP	QCNQVLDDYF	PLVIDYFQNQ	TDSNGICMHL	GLCKSRQPEP
151	EQEPGMSDPL	PKPLRDPLPD	PLLDKLVLPV	LPGALQARPG	PHTQDLSEQQ
201	FPIPLPYCWL	CRALIKRIQA	MIPK GALAVA	VAQVCR VVPL	VAGGICQCLA
251	ERYSVILLDT	LLGR MPLQLV	CRLVLRCSMD	DSAGPR SPTG	EWLPR DSECH
301	LCMSVTTQAG	NSSEQAIPQA	MLQACVGSWL	DREKCKQFVE	QHTPQLLTLV
351	PRGWAHTTC	QALGVCGTMS	SPLQCIHSPD	L	



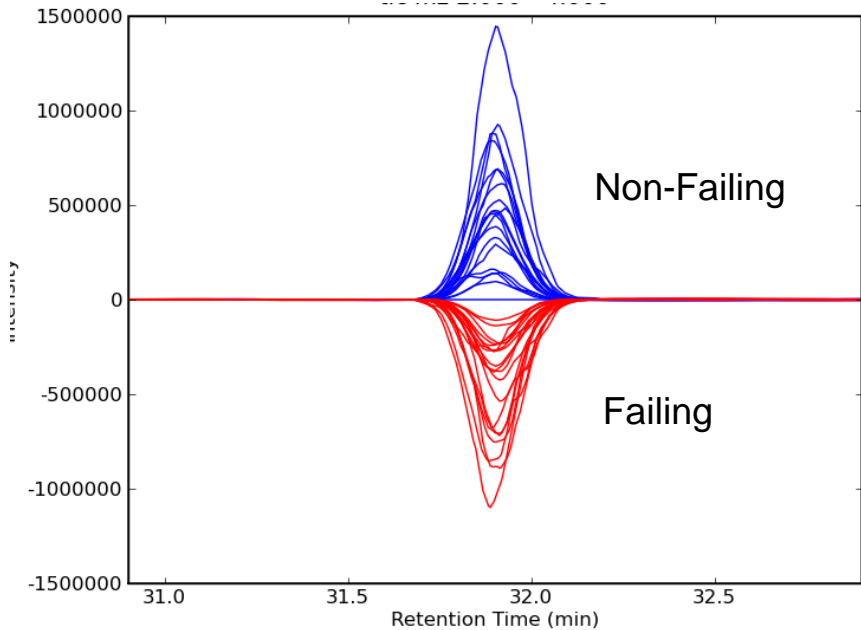
B2-AR



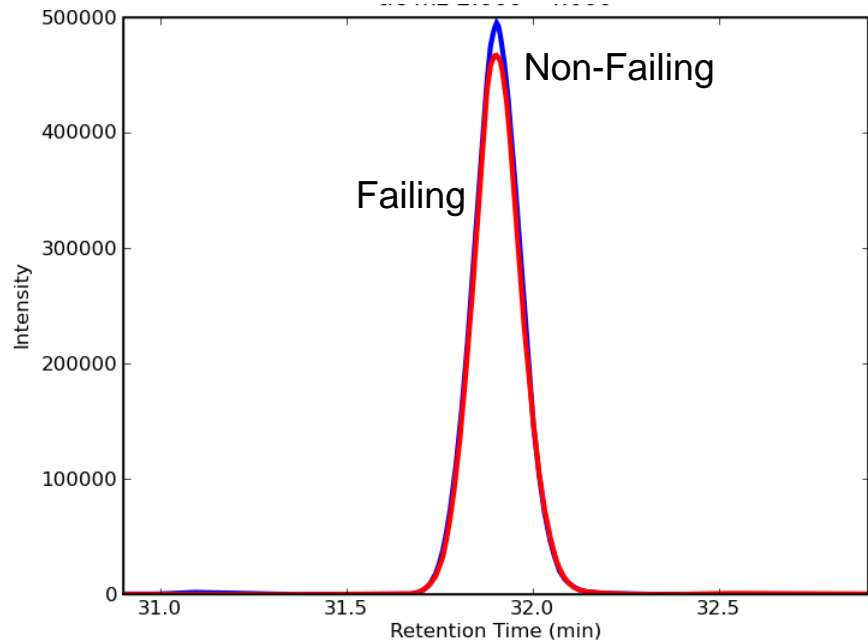
B2-AR Group Means



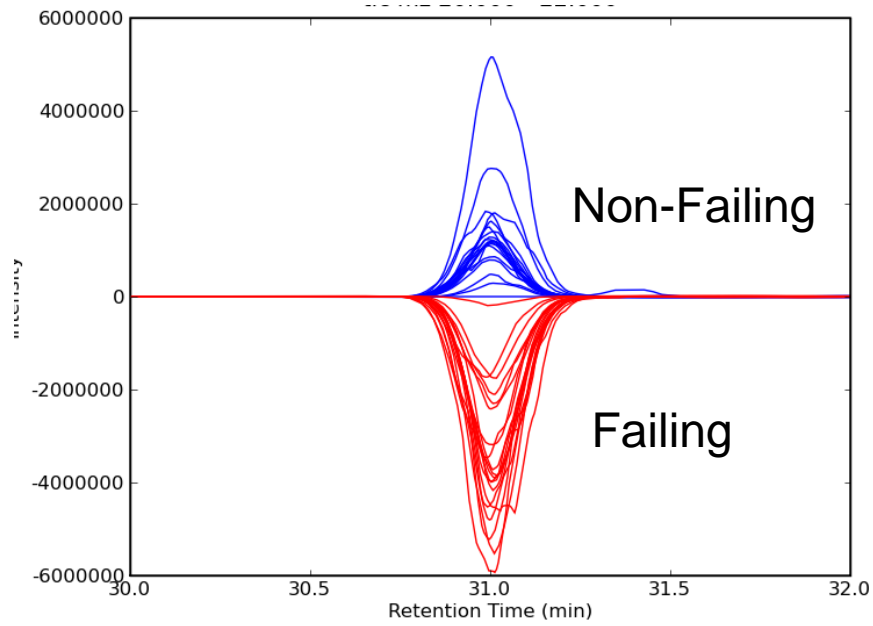
Calsequestrin



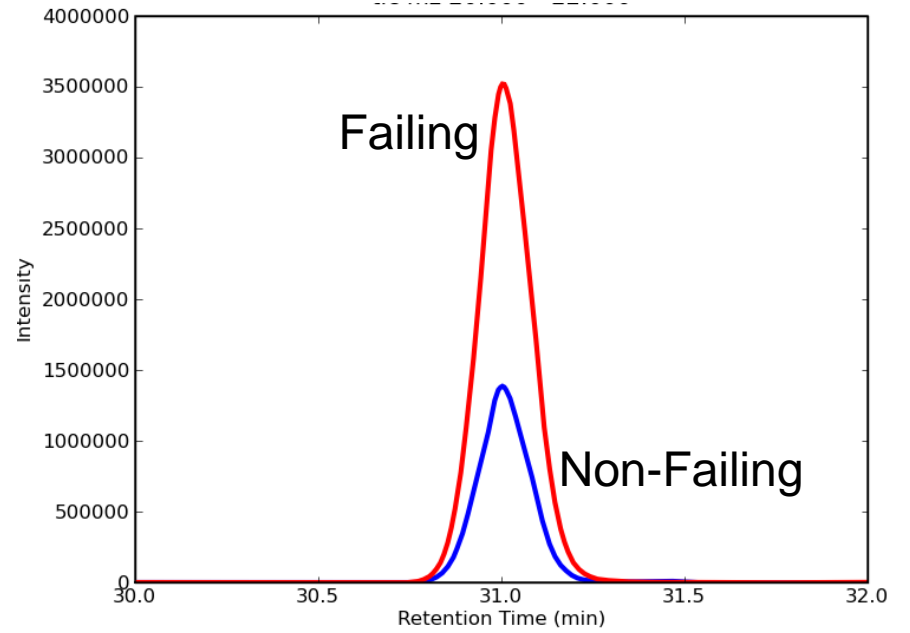
Calsequestrin Group Means



MLC2



MLC Group Means



Troponin-C: Regulation Protein of Contraction

