



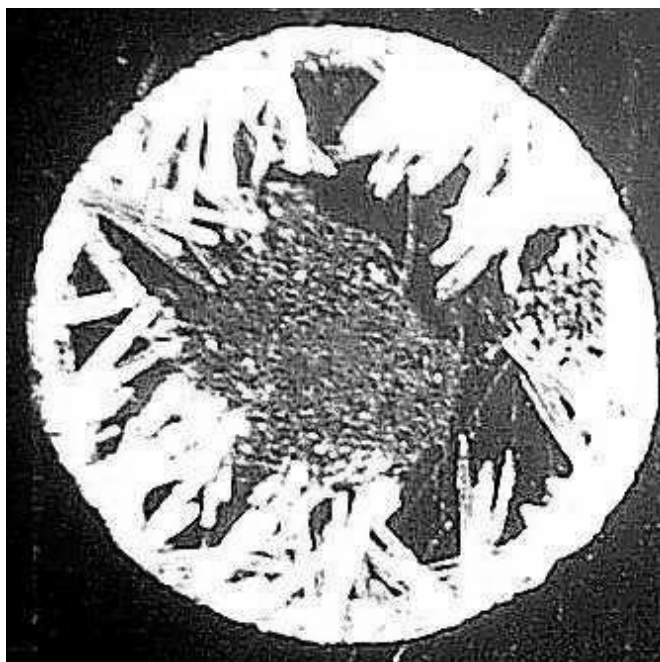
Využití TOF/TOF instrumentace v MS

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Applied Biosystems Česká republika s.r.o.

AB Applied Biosystems | **MDS** Analytical Technologies



MALDI Sample Preparation



Typical DHB dried droplet preparation on a steel target

MALDI preparation protocol:

- Dried droplet preparation: matrix solution mixed with analyte solution on the metal target and dried
- Matrices are e.g. 2,5-dihydroxy benzoic acid (DHB) or 4-hydroxy- α -cyanocinnamic acid (CHCA)

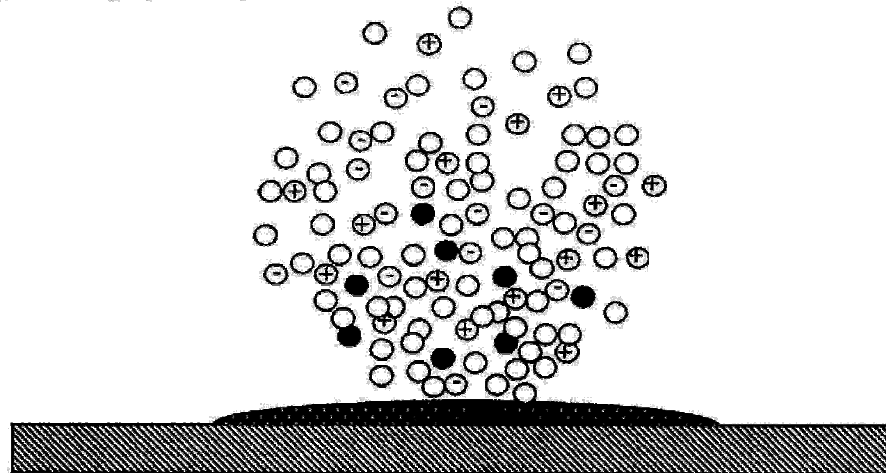
Beavis, Chaudhary, Chait, *Org. Mass Spectrom.* 1992, 27, 156.

Strupat, Karas, Hillenkamp, 1991, 111, 89.



MALDI ionization mechanism

1. Laser flash produces matrix neutrals, + and - ions, and sample neutrals.



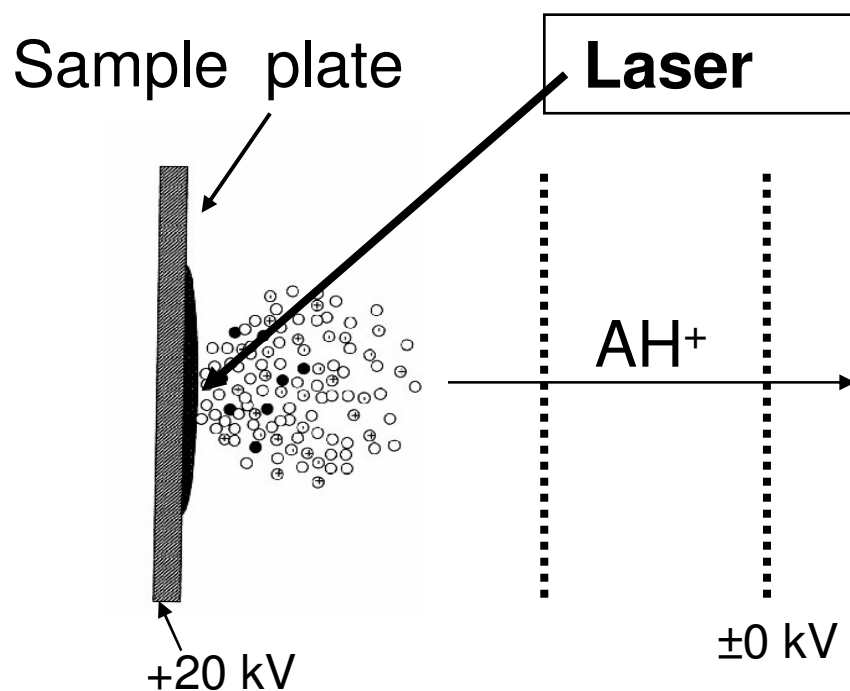
2. Sample molecules are ionized by gas-phase proton transfer.





How does MALDI-TOF MS work?

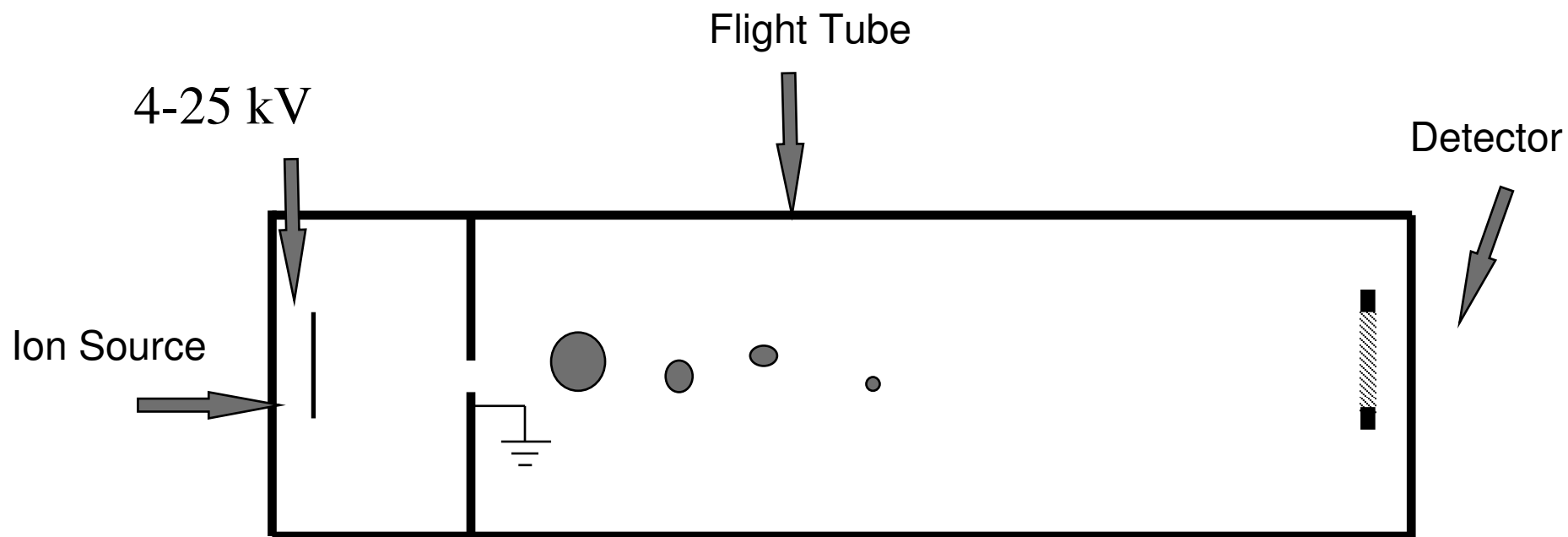
Matrix Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry



1. Sample is mixed with excess matrix and dried on a MALDI plate.
2. Laser pulse desorbes matrix molecules and embedded biomolecules.
3. Sample molecules are ionized and accelerated in the electric field.
4. Mass analysis of ions in TOF-section.



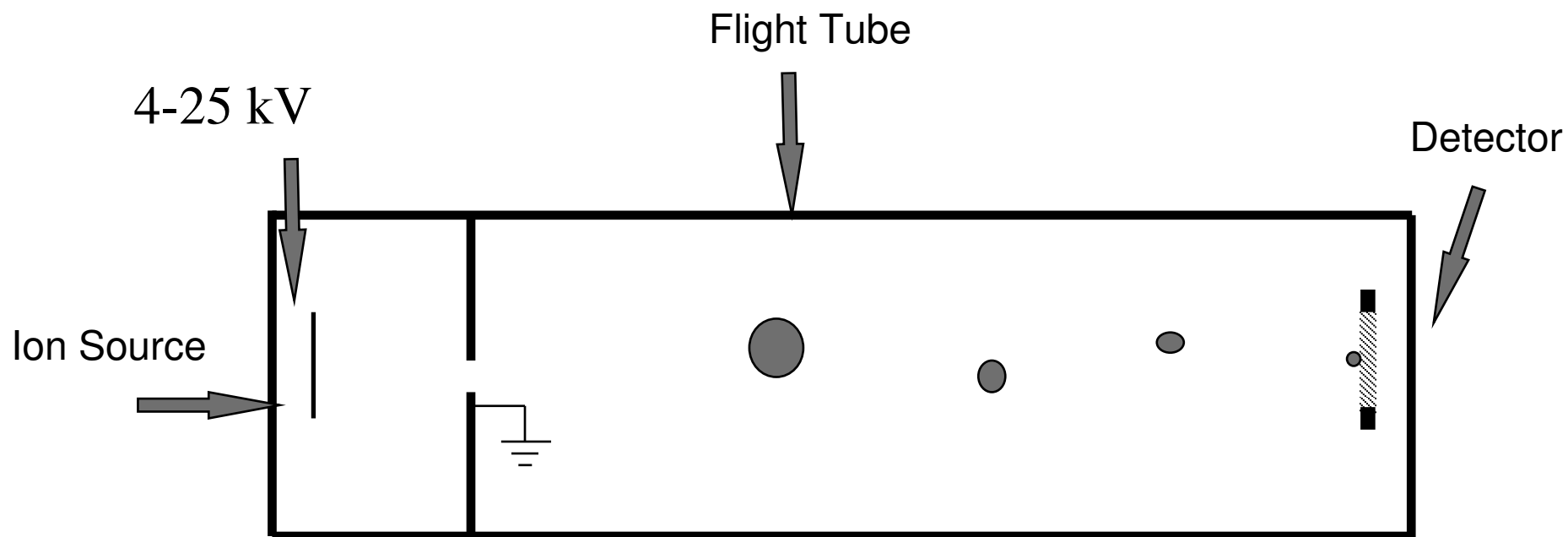
Time of Flight (TOF)



The ions enter the flight tube with the lighter ions travelling faster than the heavier ions



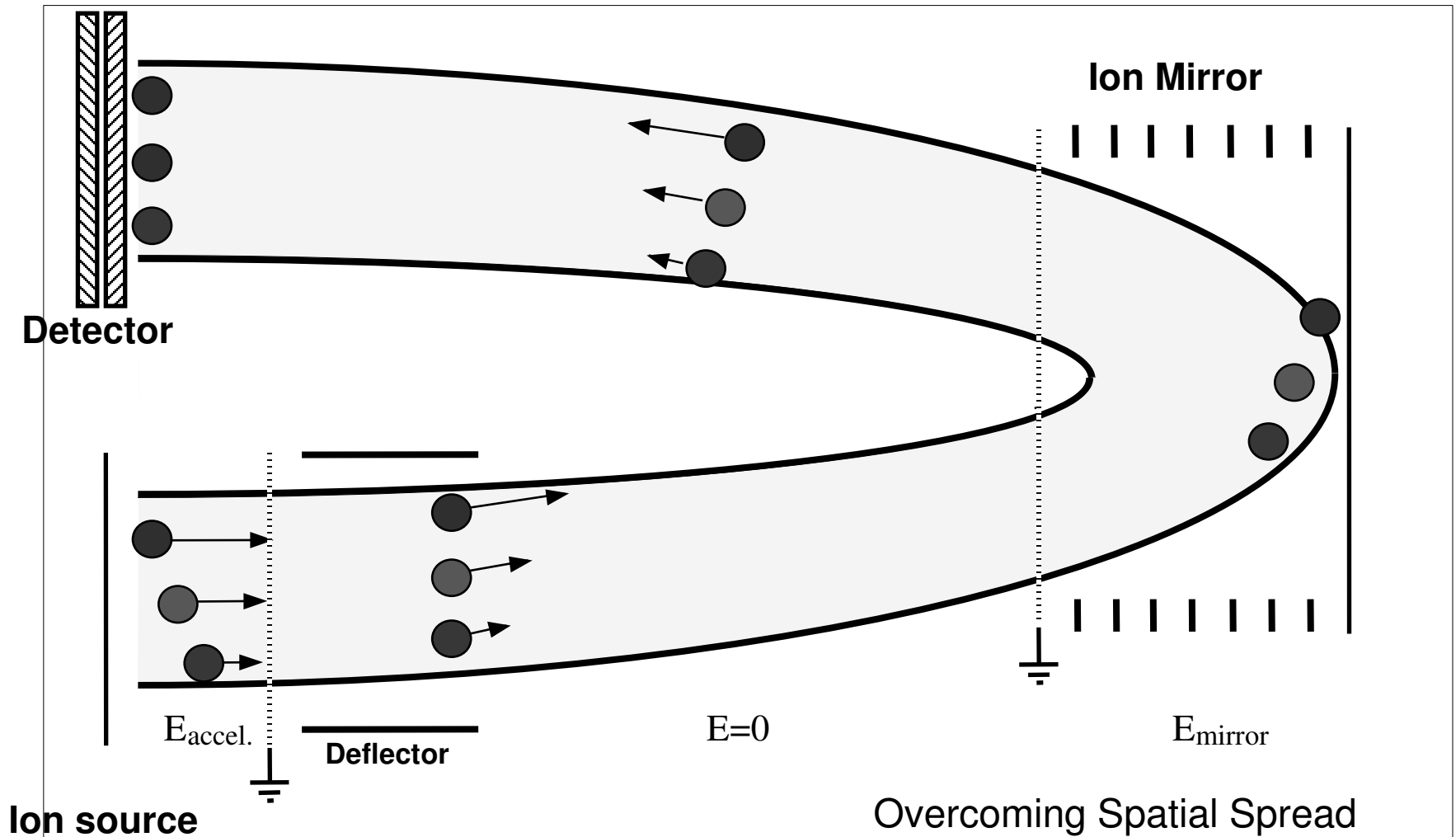
Time of Flight (TOF)



The lighter ions strike the detector before the heavier ions.
This “time of flight” can be converted to the mass ($KE=1/2mv^2$)



Reflector TOF

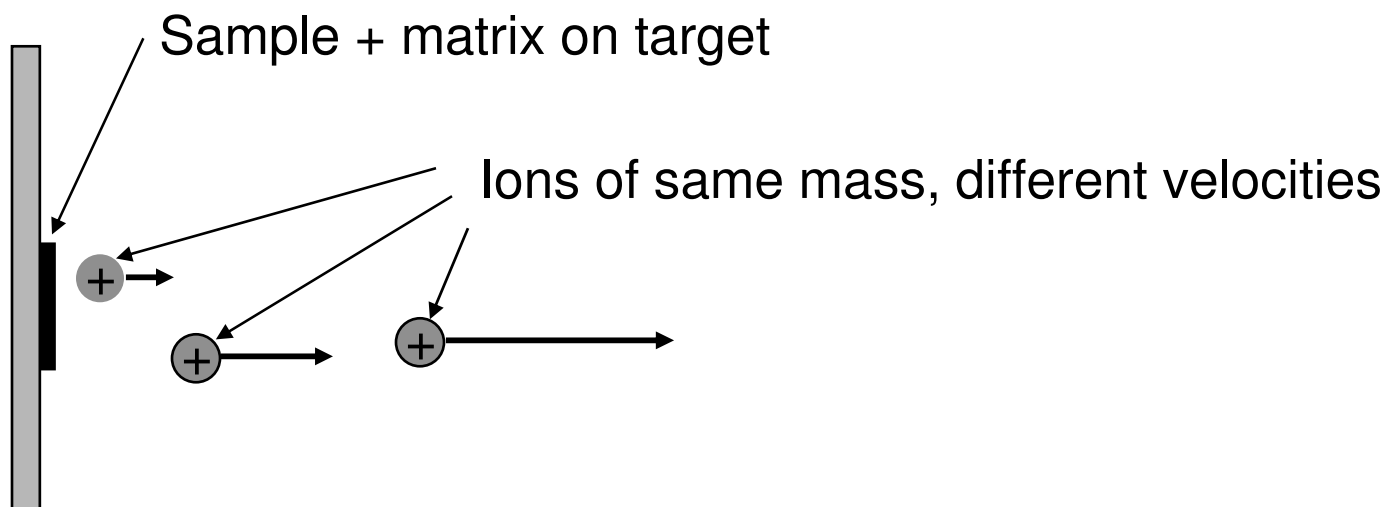




Delayed Extraction (DE)

The problem: Peaks are broad in some MALDI-TOF spectra (poor resolution).

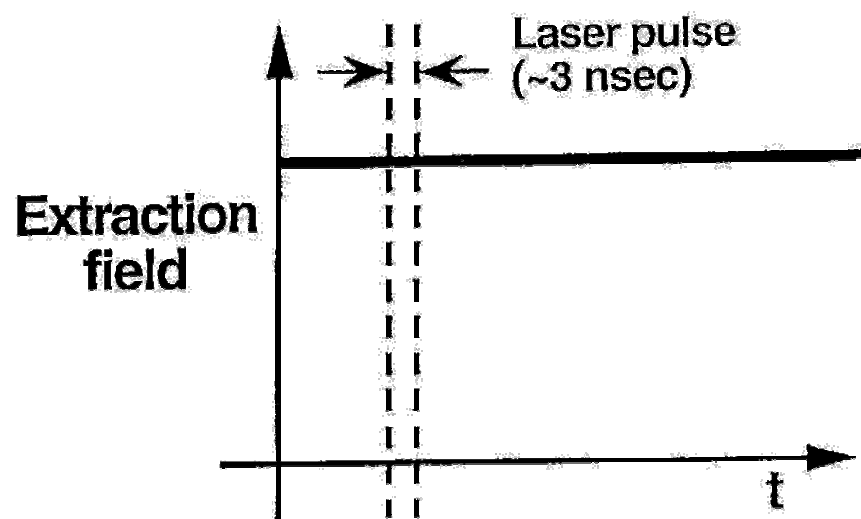
The cause: Ions coming from the target have different velocity due to the desorption process.



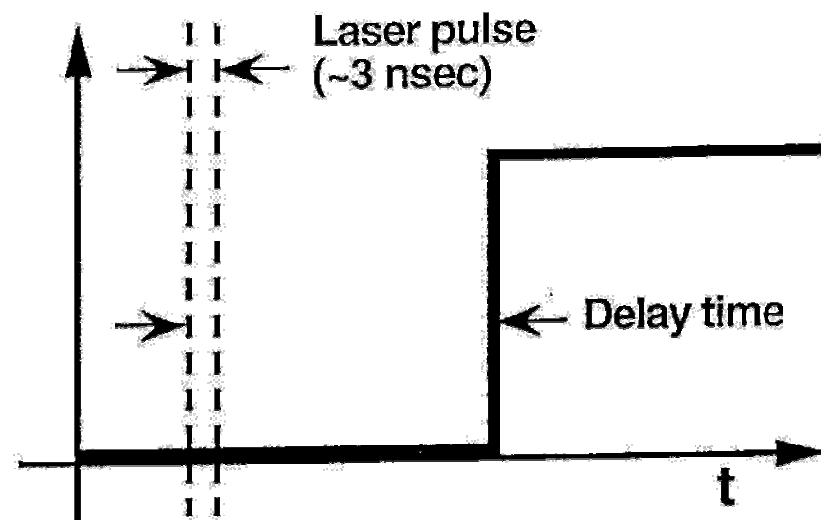
The solution: Delayed Extraction (DE)
first commercialized by Perseptive Biosystems in 1996



Continuous vs. Delayed Extraction



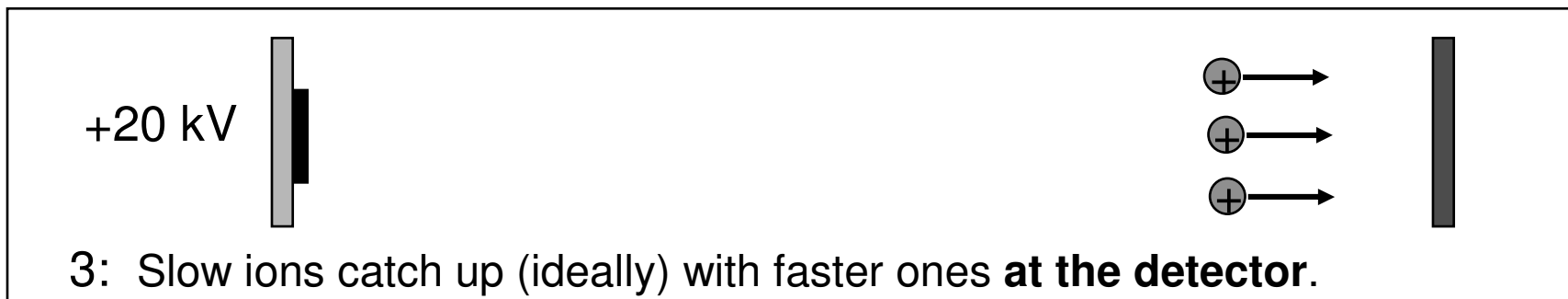
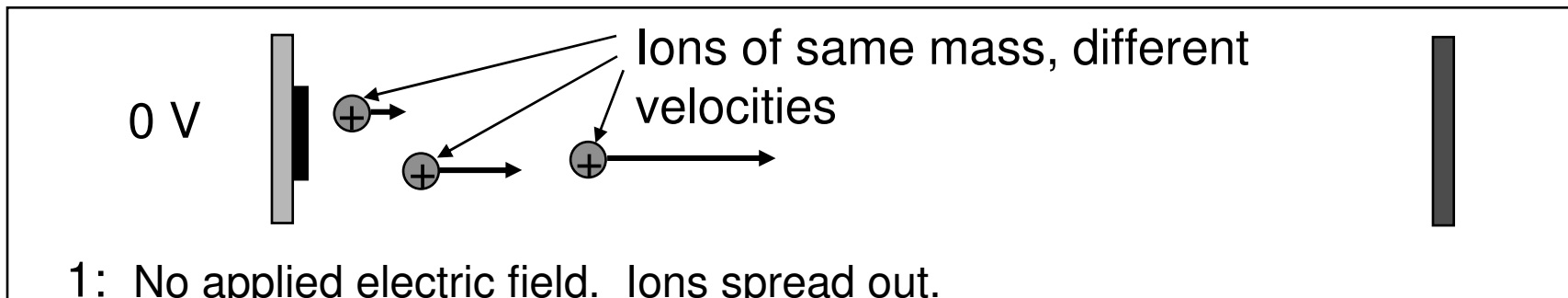
Continuous extraction



Delayed extraction



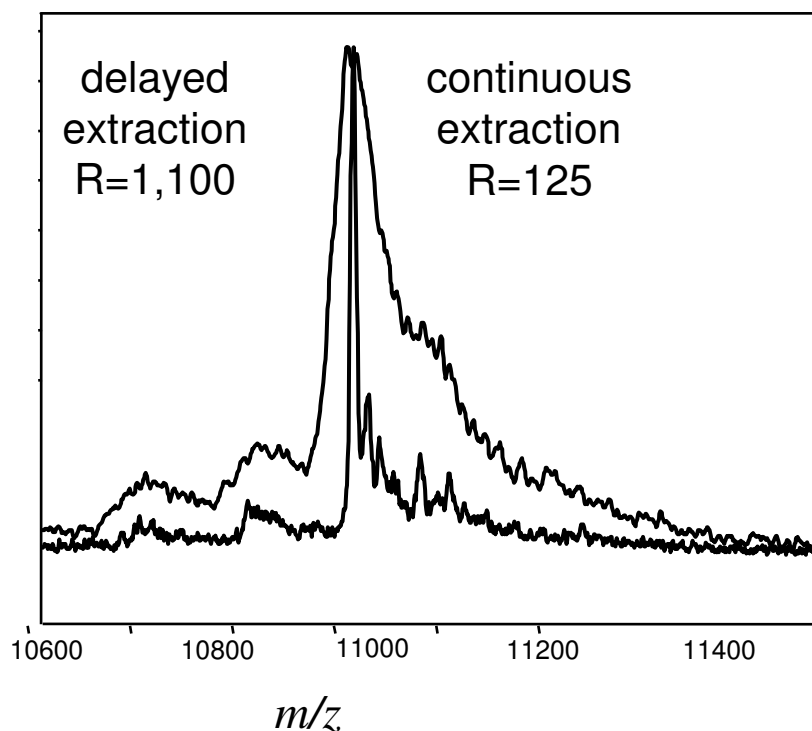
Delayed Extraction (DE)





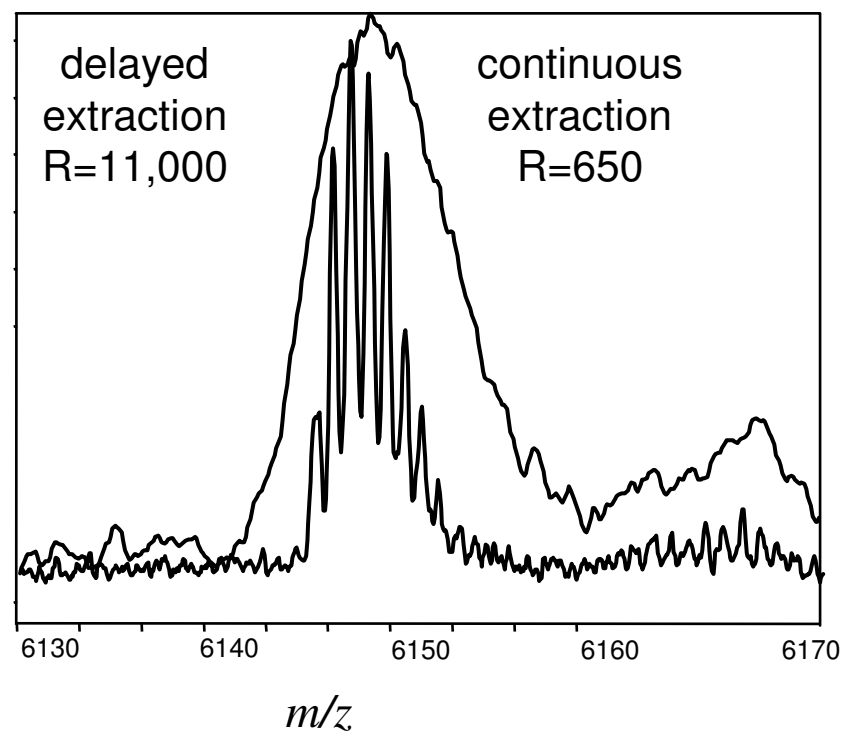
Delayed Extraction (DE): Resolution Improvements

Linear mode



Sample: mixed base DNA 36-mer

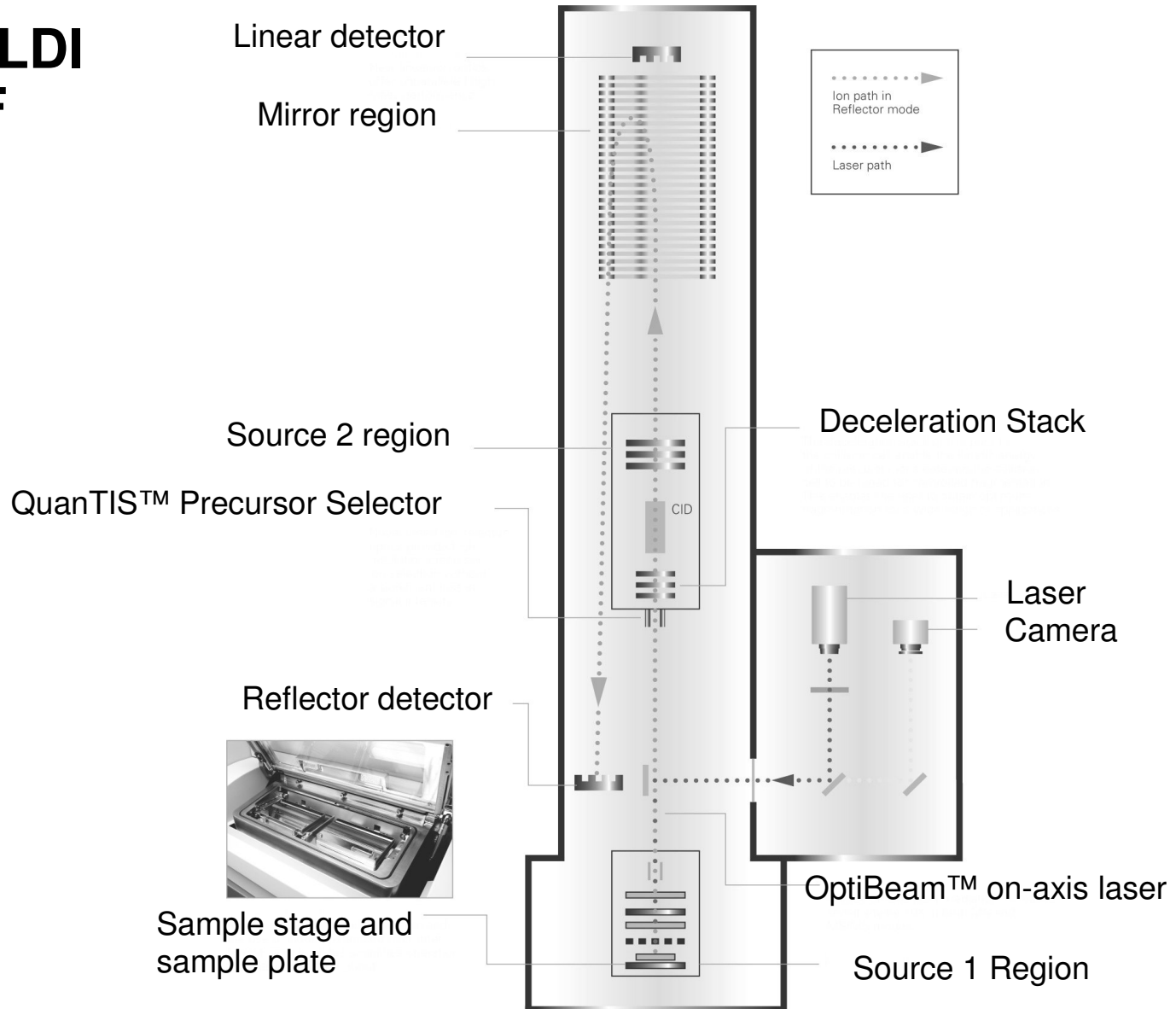
Reflector mode



Sample: mixed base DNA 20-mer

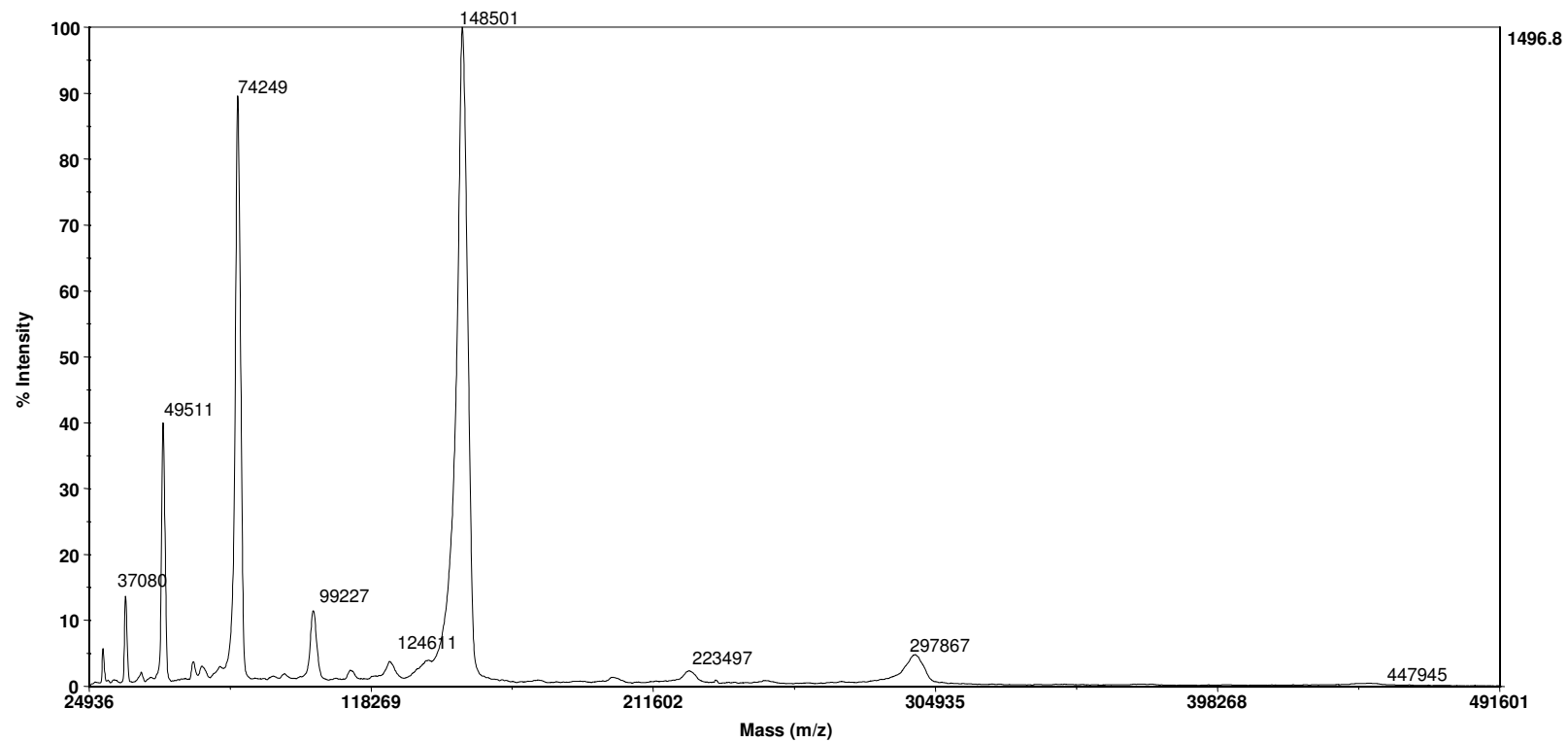


4800 MALDI TOF/TOF System





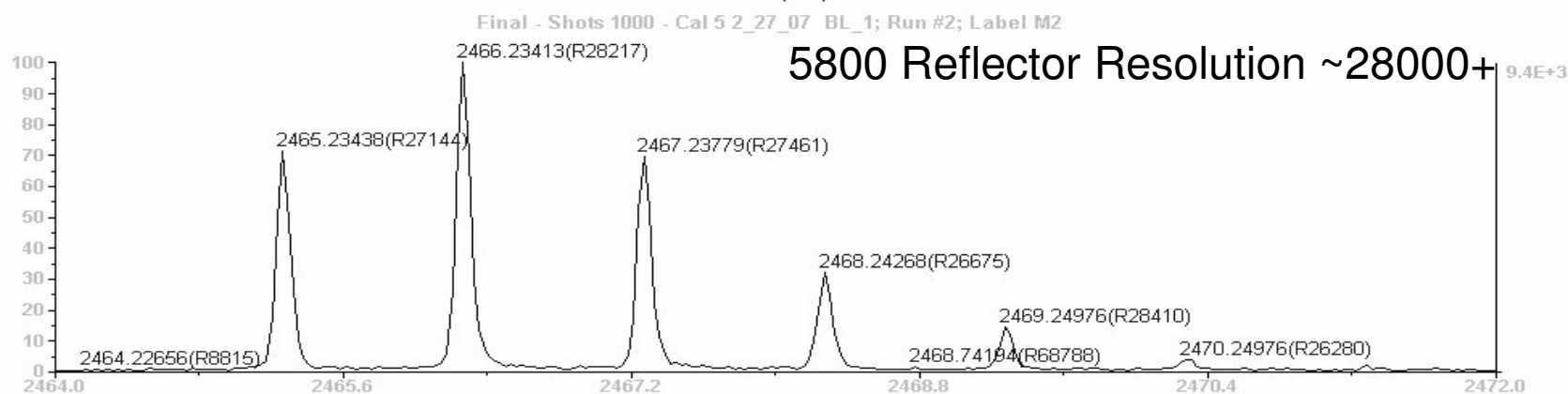
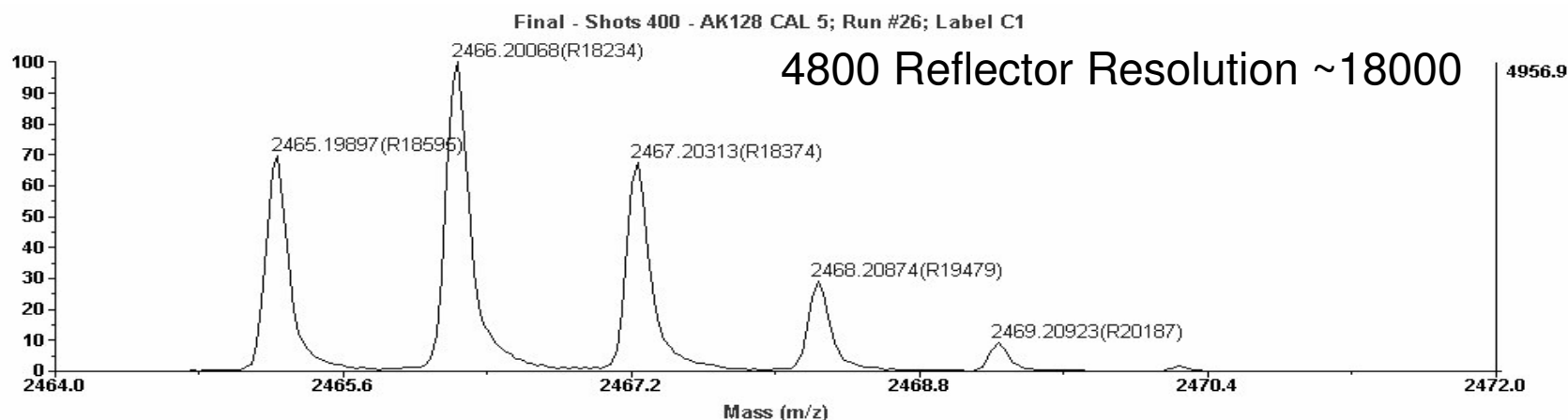
IgG Linear Mode





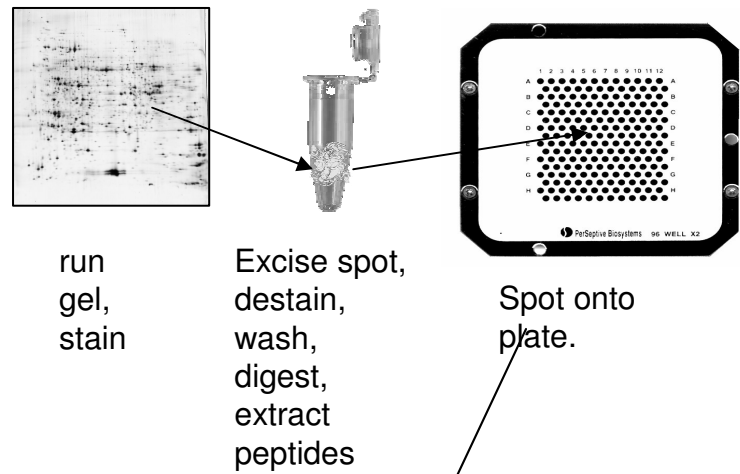
Improved Ion Optics, Detector and Digitizer

Faster Acquisition with 50% better MS Resolution





PMF result dependent analysis

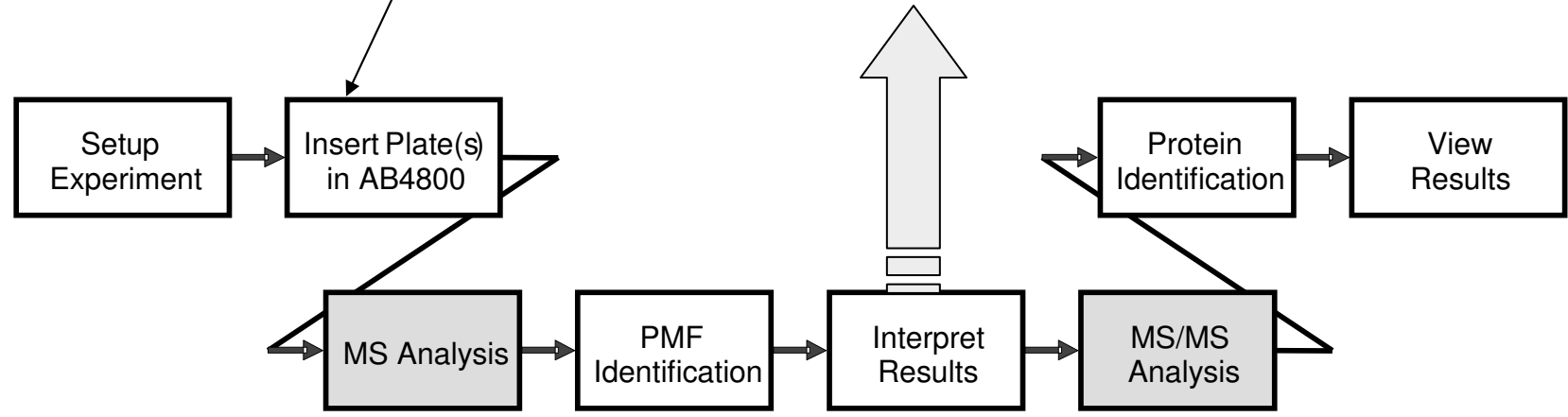


PMF confirmation mode

Peptides matching a protein(s) identified by PMF search are automatically fed back to 4800 for MS/MS acquisition and protein ID confirmed

Low confidence investigation mode

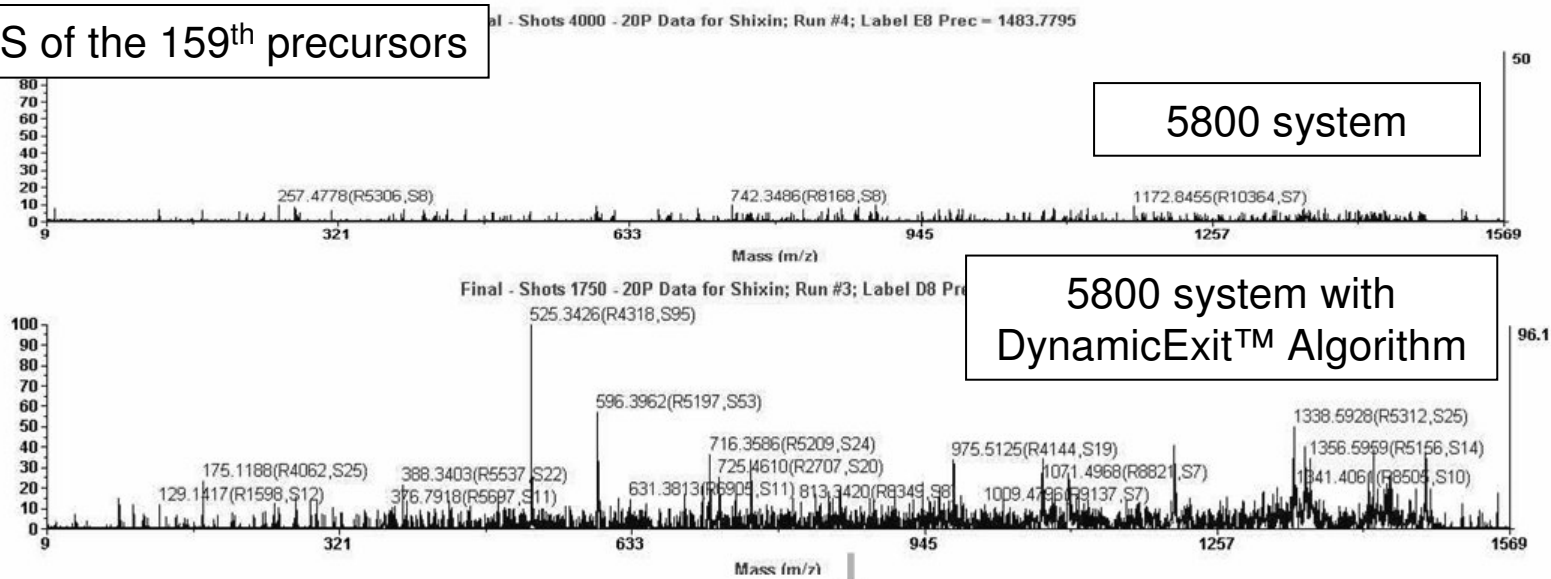
Peptides that do not match to a top protein identified by PMF search are automatically selected for MS/MS. This intelligent feed-back routine identifies multiple proteins in a mixture





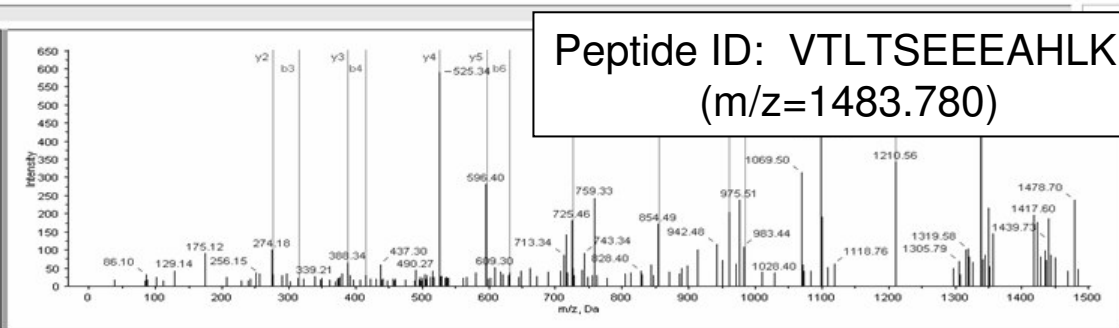
Intelligent MS/MS Acquisition DynamicExit™ Algorithm

MSMS of the 159th precursors



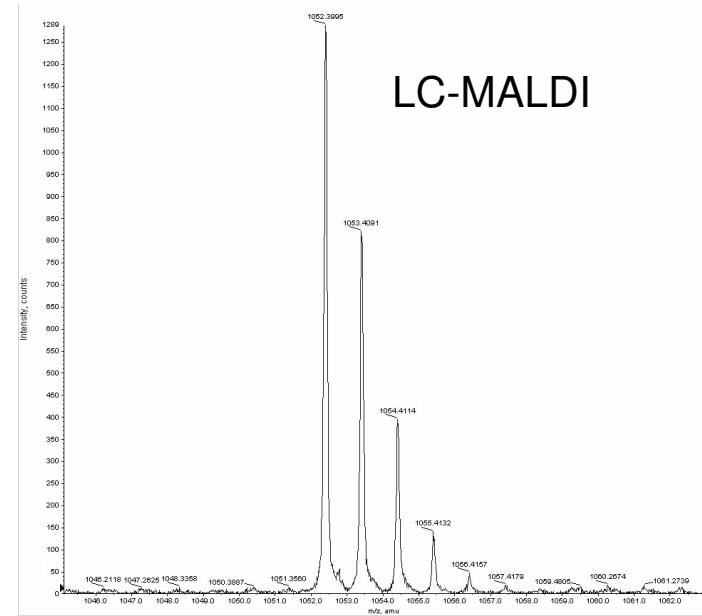
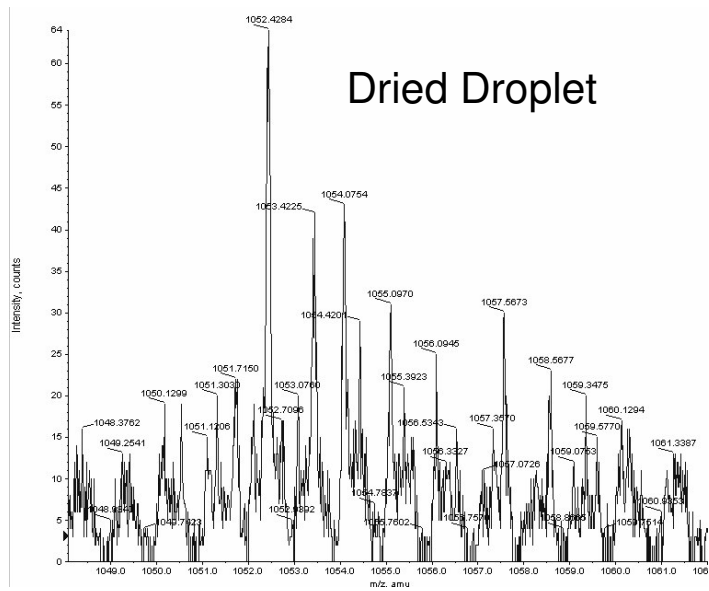
Fragmentation Evidence for Peptide

Residue	b	y
V	100.0757	1484.7955
T	201.1234	1385.7271
L	314.2074	1284.6794
T	415.2551	1171.5953
S	502.2071	1070.5477
E	631.3297	983.5156
E	760.3723	884.4738
E	809.4149	725.4384
A	960.4520	596.3879
H	1097.5109	525.3567
L	1210.5950	388.2918
K	1338.6900	275.2078
K	1466.7049	147.1128





Why LC-MALDI for proteomics ?



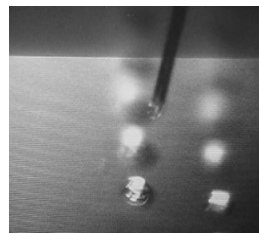


The Power of LC-MALDI

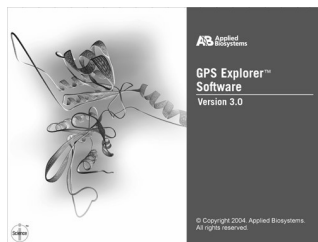


•Tempo™ LC MALDI

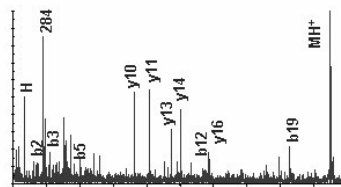
Direct Spotting onto MALDI target



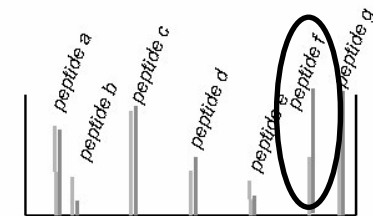
TOF/TOF MALDI System



GPS Explorer for Data Analysis



MS/MS Analysis and Quantitation (iTRAQ™)



MS Analysis and Quantitation



The Power of LC/MALDI

match MS/MS analytical power to the LC resolution
true “results-dependent” analysis (MS followed by MS/MS)
sample is frozen in time – the ultimate “stop-flow” technology

LC-MS/MS

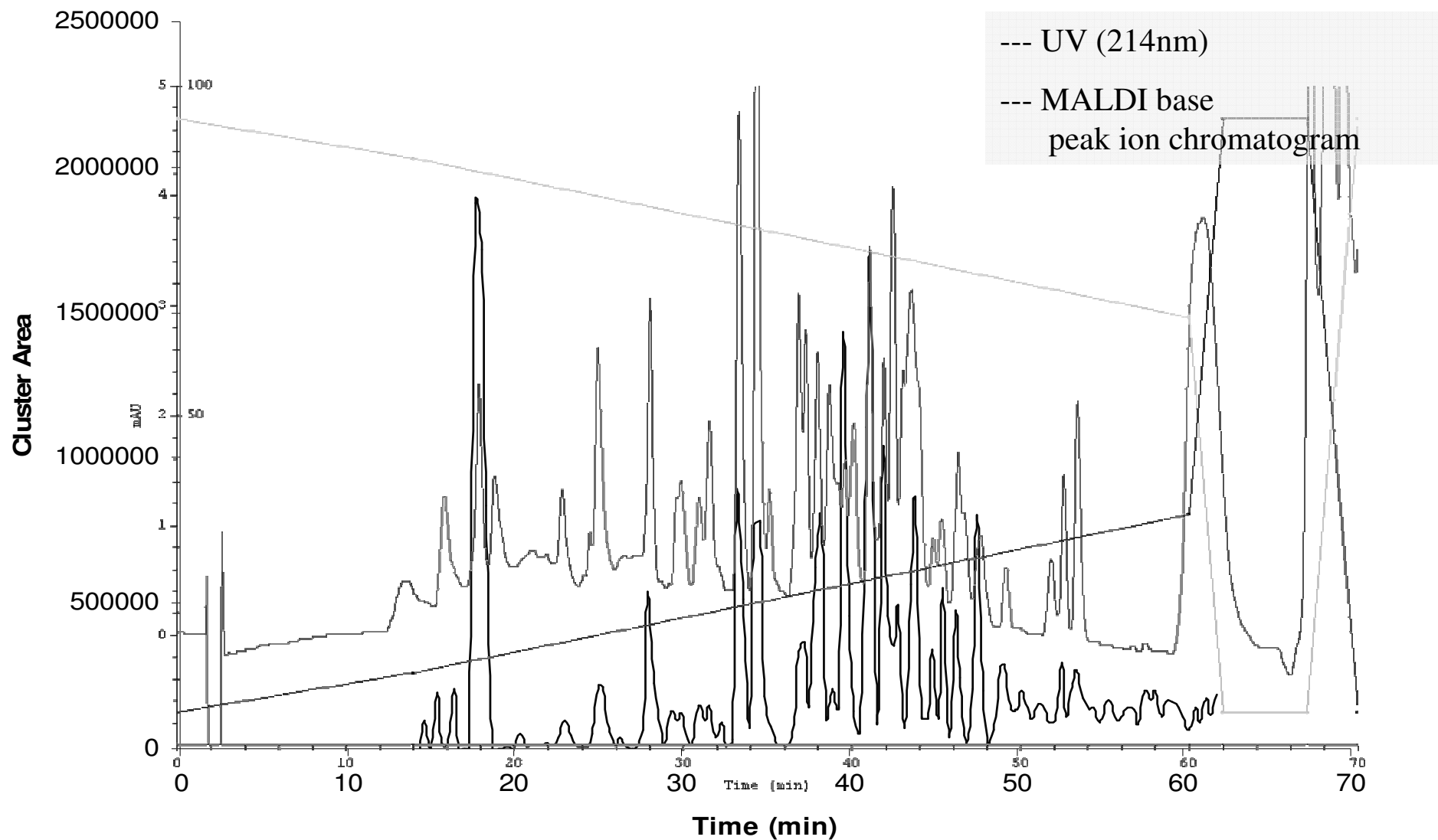


LC/MALDI - MS/MS



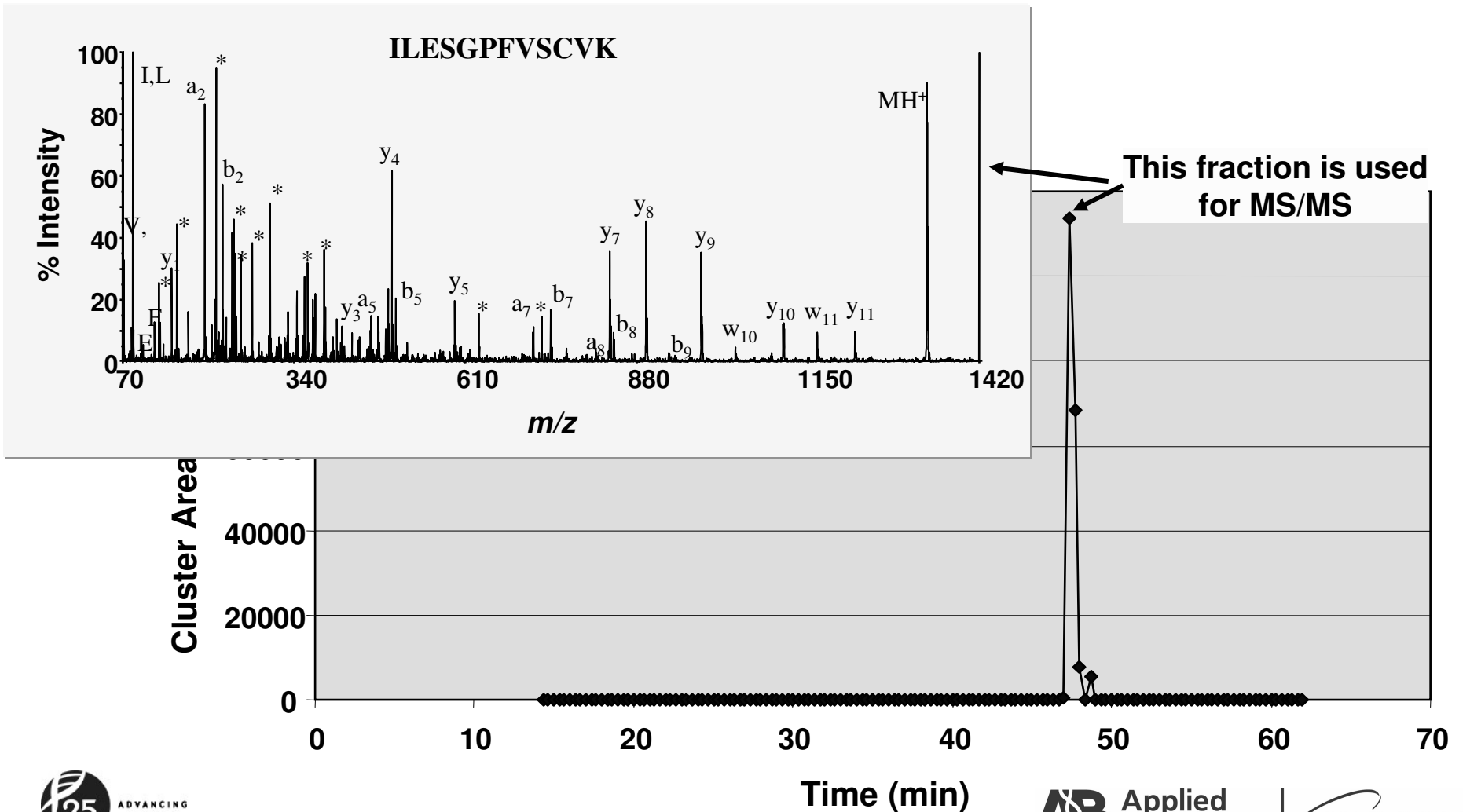


Transferrin 1 hr gradient: Match Resolution of LC and MS





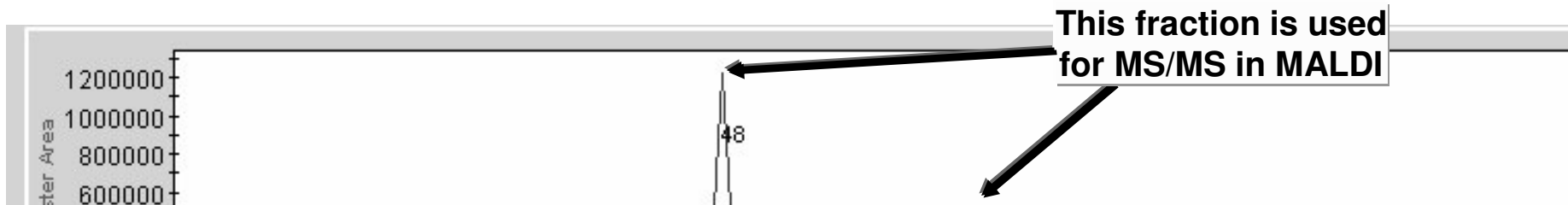
Dynamic Exclusion: Most Intense Signal Chosen



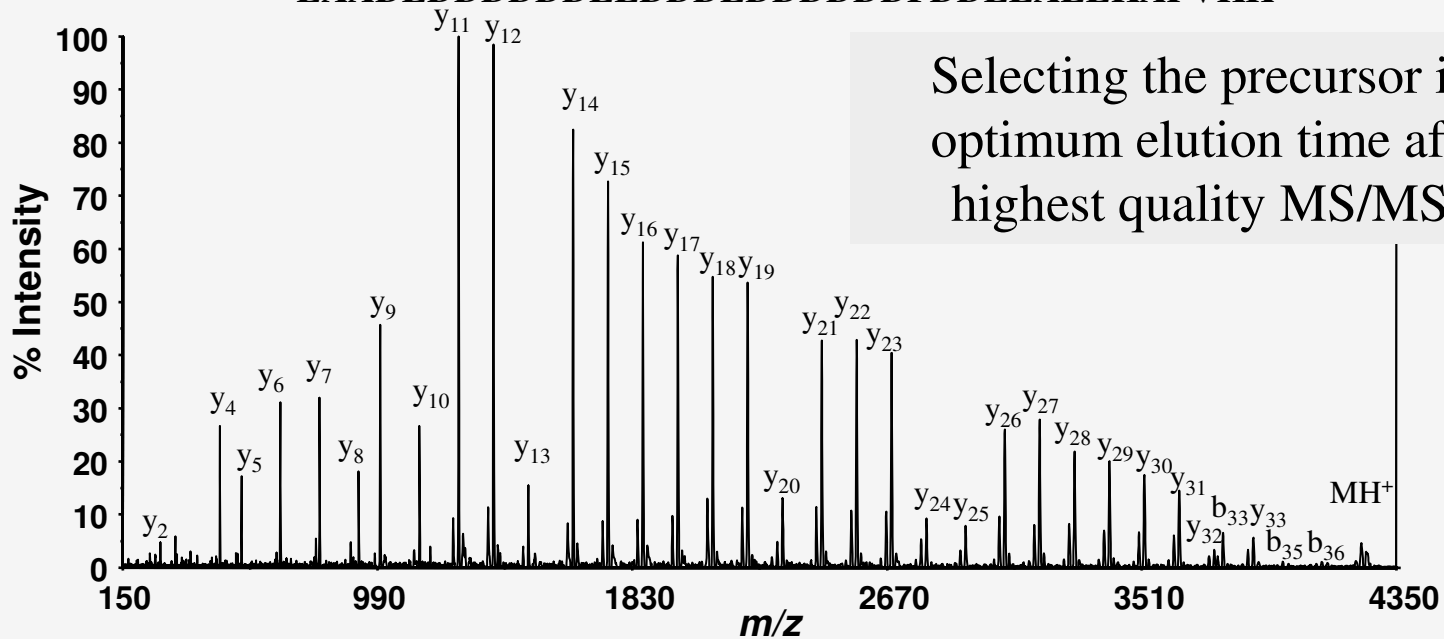


LC/MALDI result dependent experiment: Optimized selection of precursors for MS/MS

XIC for m/z 4246.5



LAADEDDEDDDEEDDDDDDFDDEEAEEKAPVKK

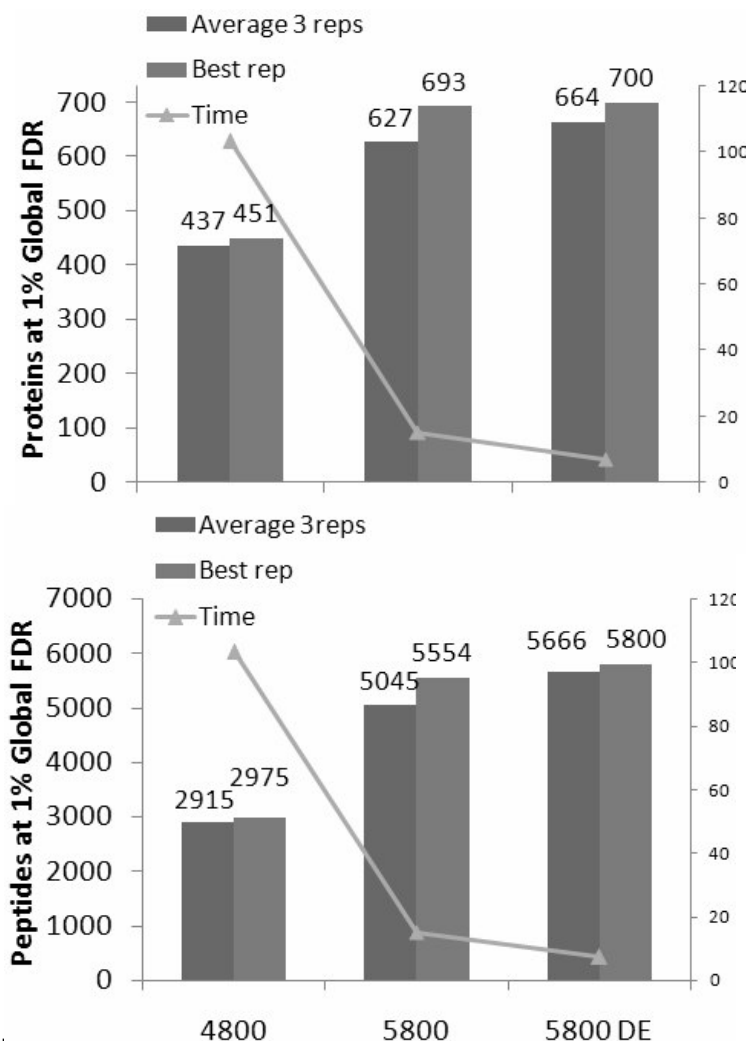


Selecting the precursor ion at the optimum elution time affords the highest quality MS/MS spectra



Summary of Protein Identification Results

- The numbers of proteins identified is 55% greater on the 5800 over the 4800
- The numbers of peptides identified is 100% greater on the 5800 over the 4800
- A steady increase in the number of peptides is observed as the additional acquisition features are utilized
 - Faster laser repetition rate
 - Faster laser repetition rate + DynamicExit™ Algorithm





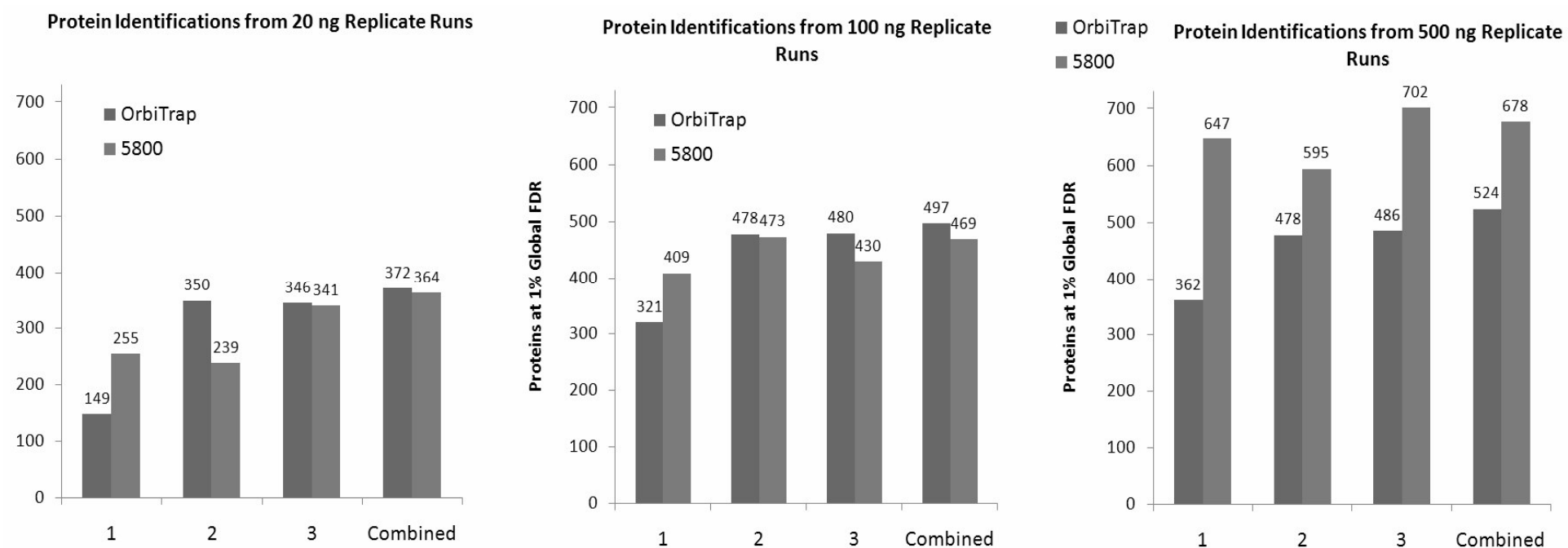
Assessing the Complementarity of ESI and MALDI

- Two dominant ‘soft’ ionization strategies
- LC MALDI has been more flexible due to decoupling of acquisition and LC, however, the technique has been speed limited.
- 10-15X increase in speed of the AB SCIEX TOF/TOF™ 5800 System means it is now interesting to re-visit the advantages of the different acquisition strategies
- Previous work has suggested a complementarities in information
- Here, we wanted to assess the complementarities vs. random acquisition variation of ESI vs MALDI



5800 vs OrbiTrap: *E. coli* Total Cell Lysate 500, 100, 20 ng

Extending the limits of protein discovery





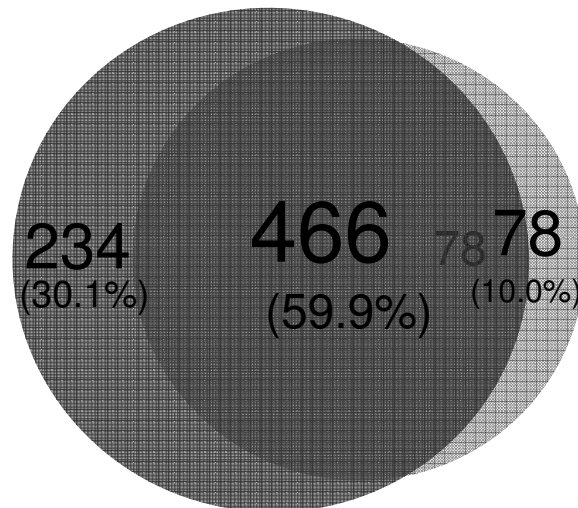
Protein / Peptide Intersection

500 ng Loading Level – Comparison of Single Best Replicates

- ESI with MALDI acquisitions appear complementary and suggest greater depth of coverage from combination than a signal technique alone.
- But how similar are replicates from the same instrument?

5800 system
700 proteins

Orbitrap
544 proteins



851
(15.3%)



Peptide Level Intersection of Replicates

- Average intersection at the peptide level between 500 ng replicates:
 - For 5800 system: **64%** (Sample 1-3)
 - For Orbitrap: **61%** (Samples 4-6)
 - Between instruments: **25%** (red boxes)

- This clearly shows that intra-instrument replicates are more similar than inter-instrument replicates

		Sample Index					
		1	2	3	4	5	6
5800 500ng R1	1	1					
5800 500ng R2	2	0.645	1				
5800 500ng R3	3	0.633	0.628	1			
Orbi 500 ng R1	4	0.24	0.251	0.248	1		
Orbi 500 ng R2	5	0.253	0.263	0.265	0.614	1	
Orbi 500 ng R3	6	0.25	0.257	0.261	0.59	0.616	1



Protein Level Intersection of Replicates

- Average intersection at the protein level between 500 ng replicates:
 - For 5800 system: **78%** (Sample 1-3)
 - For Orbitrap: **74%** (Samples 4-6)
 - Between instruments: **60%** (red boxes)

- Even at the protein level, differences are apparent

		Sample Index					
		1	2	3	4	5	6
5800 500ng R1	1	1					
5800 500ng R2	2	0.792	1				
5800 500ng R3	3	0.777	0.774	1			
Orbi 500 ng R1	4	0.6	0.622	0.613	1		
Orbi 500 ng R2	5	0.599	0.623	0.617	0.732	1	
Orbi 500 ng R3	6	0.604	0.611	0.609	0.753	0.738	1

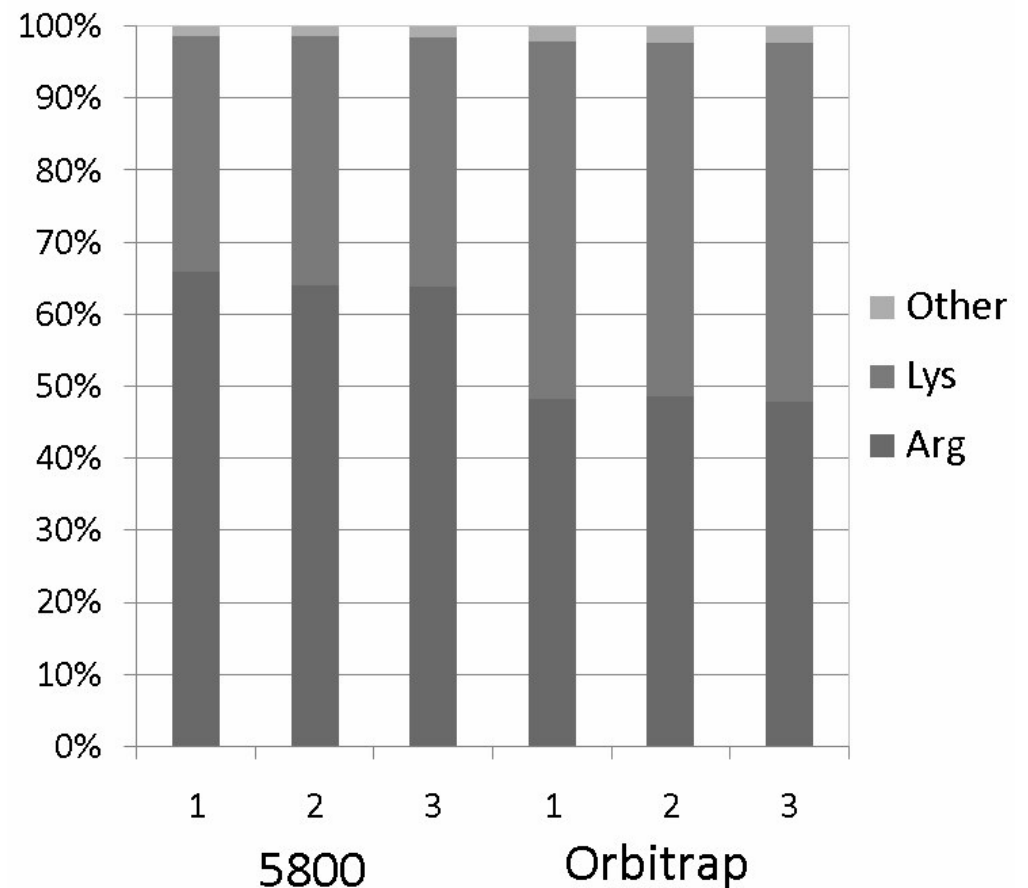


Assessing Peptide Level Differences

Peptide C-Termini

- Peptide level comparison was done at the 500ng sample loading level
- There is a slightly higher proportion of Arginine-terminating peptides detected in LC MALDI
- This difference is consistent across replicates

Termination Differences in Replicates



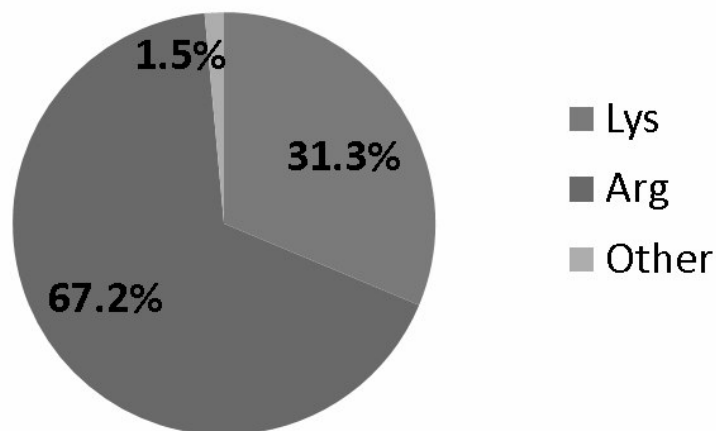


Assessing Peptide Level Differences

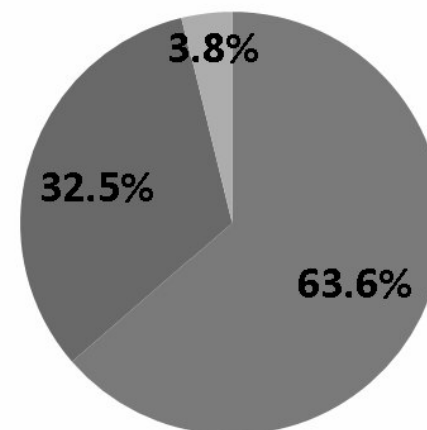
Peptide C-Termini

- Focusing on peptides UNIQUE to each instrument shows the effect clearly.
- The peptides only found by MALDI were Arg-terminating more often than Lys.
- The peptides only found by ESI were Lys-terminating more often than Arg.

Termination of 5800-Specific Peptides



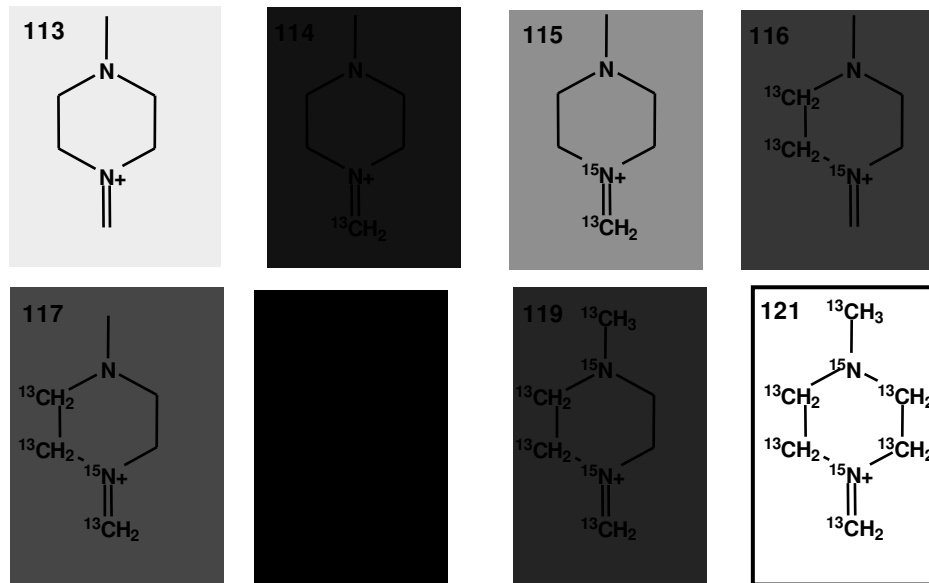
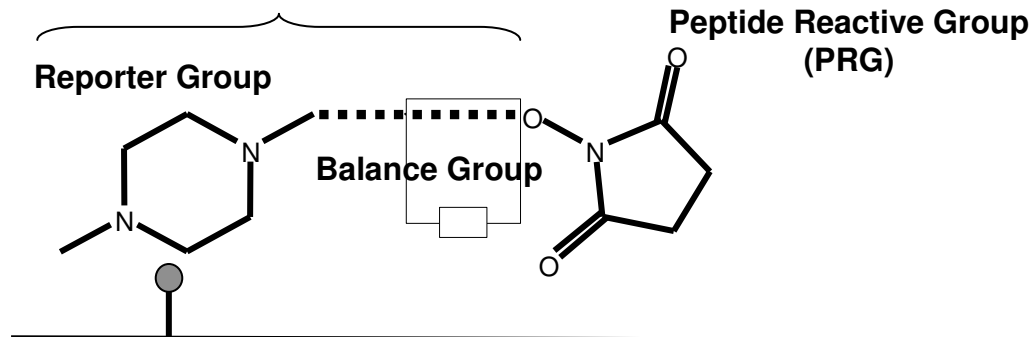
Termination of Orbi-Specific Peptides





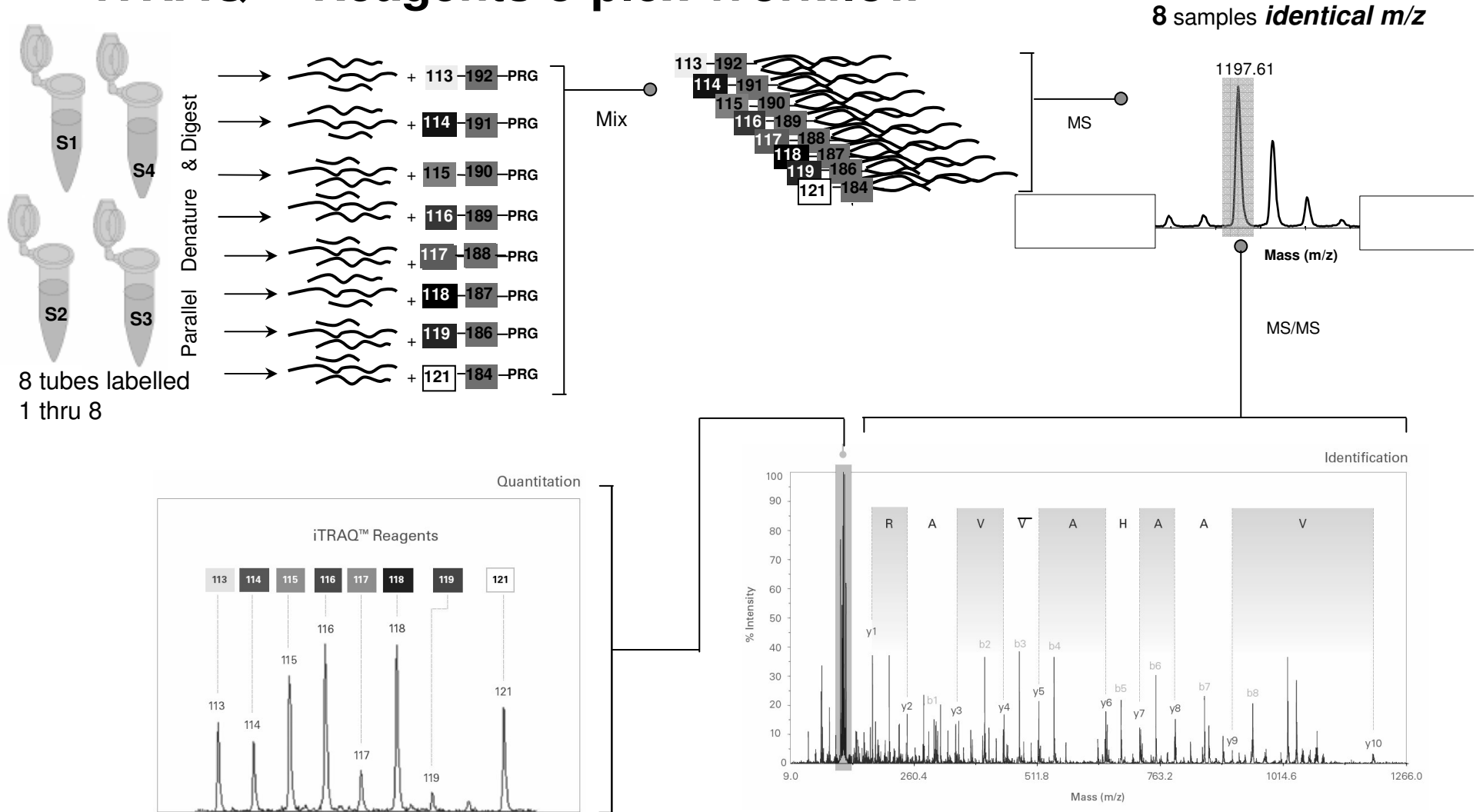
iTRAQ™ Reagents 8-plex Chemistry

Isobaric Tag: Total mass = 305



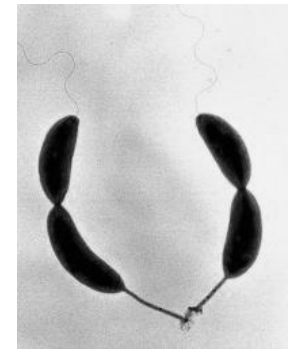
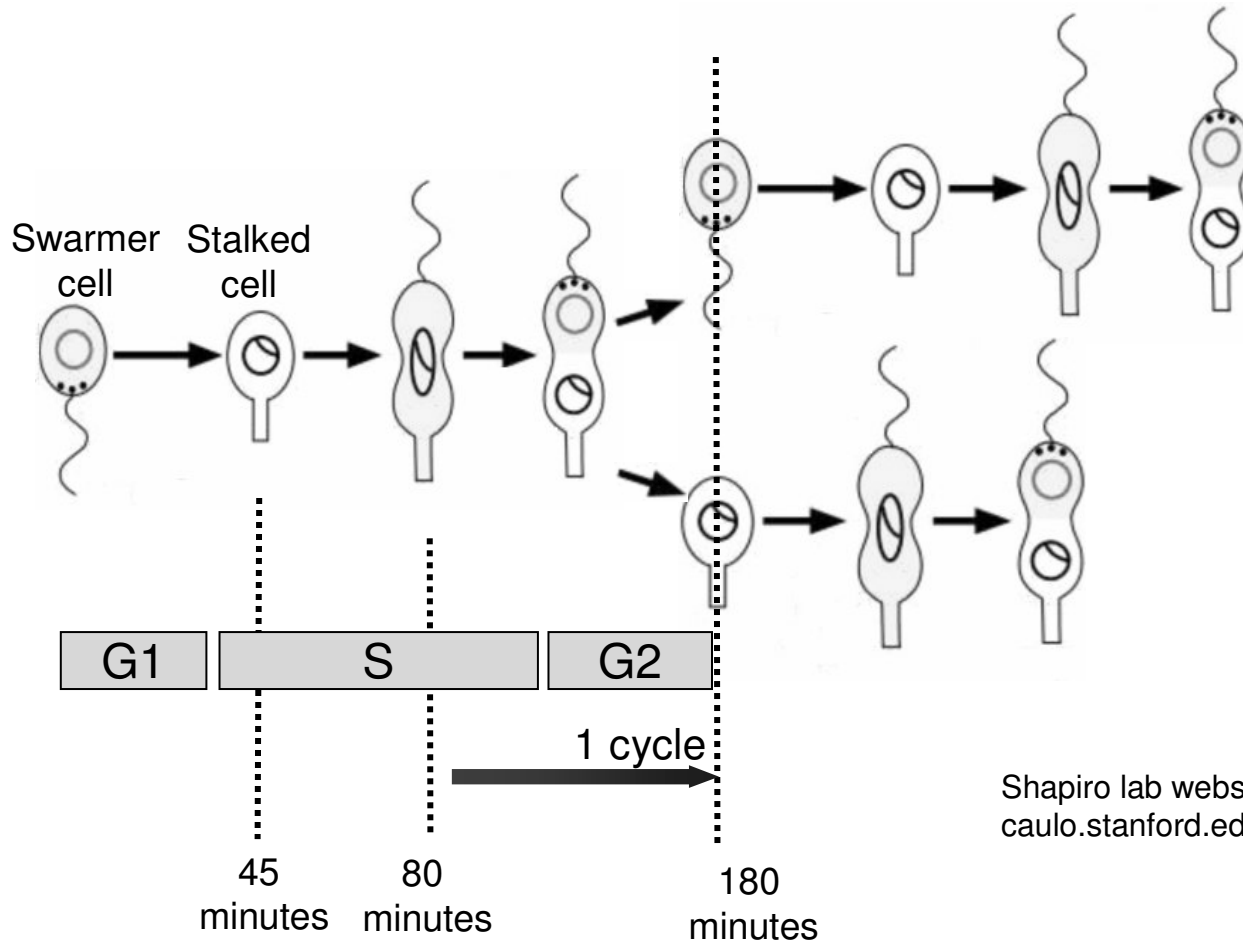


iTRAQ™ Reagents 8-plex Workflow





Caulobacter crescentus Cell Cycle

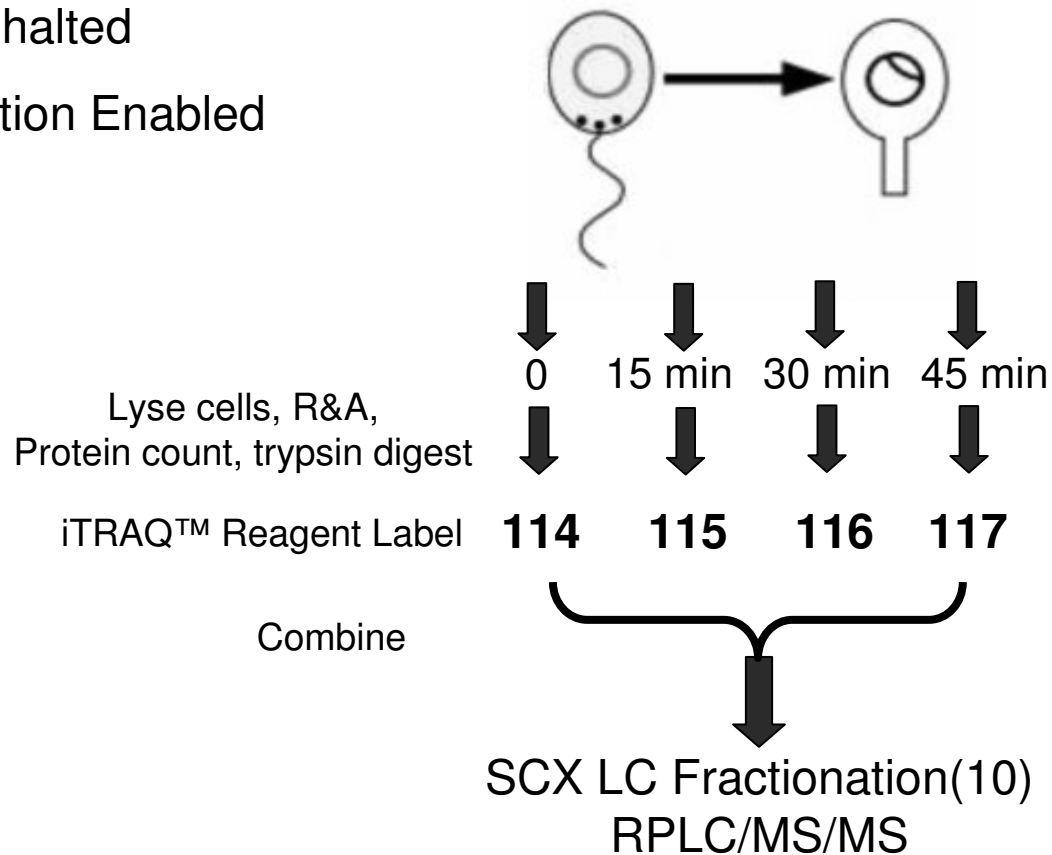


Shapiro lab website, Stanford U.
caulo.stanford.edu/caulo



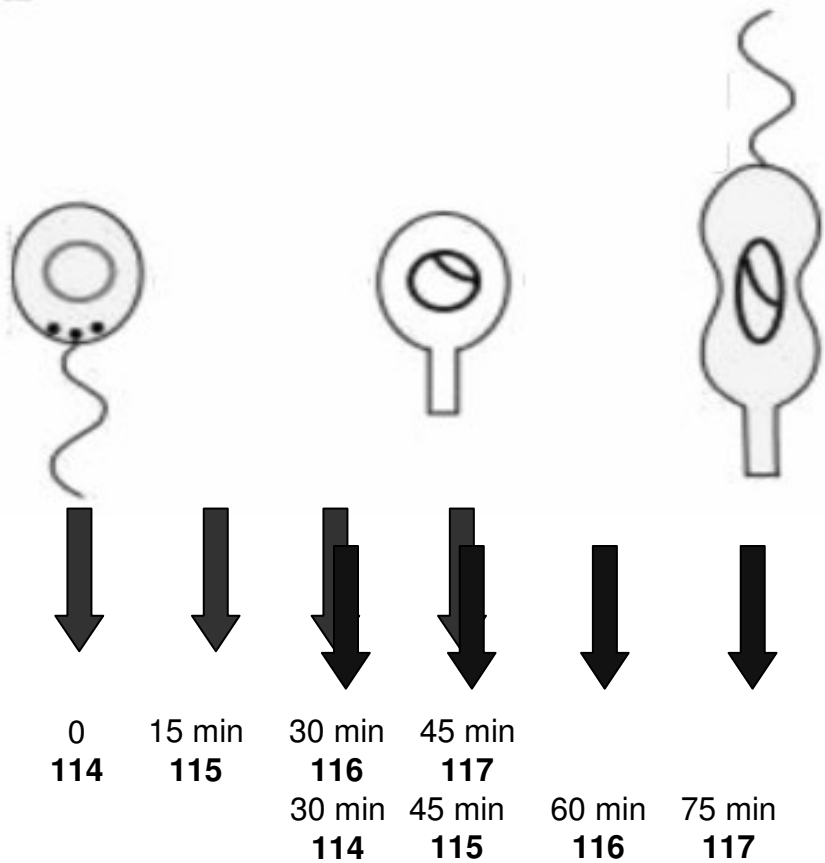
First Experiment – Cellular Differentiation

- Ejection of flagellum and pili
- Motility halted
- Replication Enabled





Second Experiment – Cellular Differentiation



Replicate time course experiments using 4 plex reagents

- Data Set A 0 – 45 minutes
- Data Set B 30 – 75 minutes

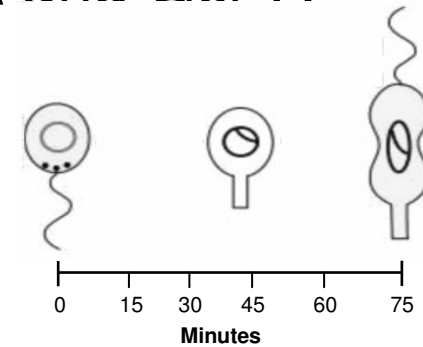
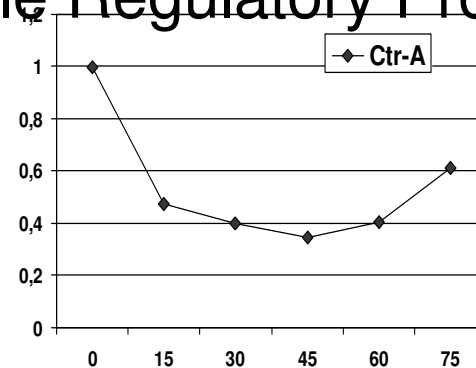
Phenotypic changes during this phase include:

- Ejection of flagellum, formation of stalk
- Loss of motility/chemotaxis
- Initiation of replication
- Observed 20% SD in each 4-plex experiment

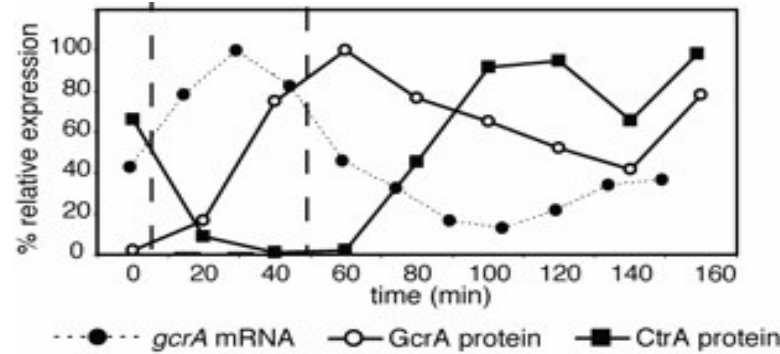


Global Cell Cycle Regulatory Proteins Ctr-A and Gcr-A

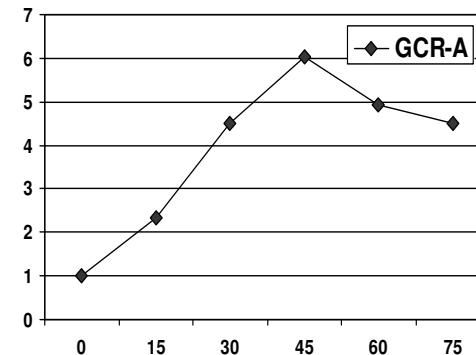
**iTRAQ Reagent
(4plex Data)**



Western Data



**iTRAQ Reagent
(4plex Data)**

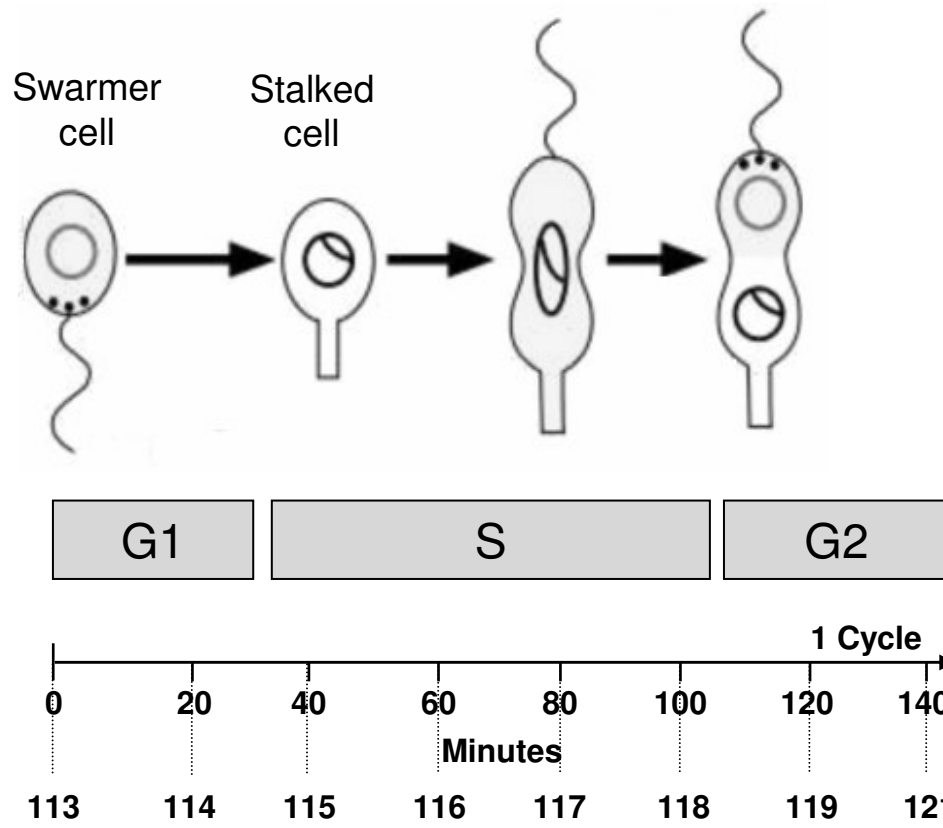


2. Holtzendorff J, Shapiro L
 .Science. 2004 May 14;304(5673):983-7



Caulobacter crescentus Cell Cycle

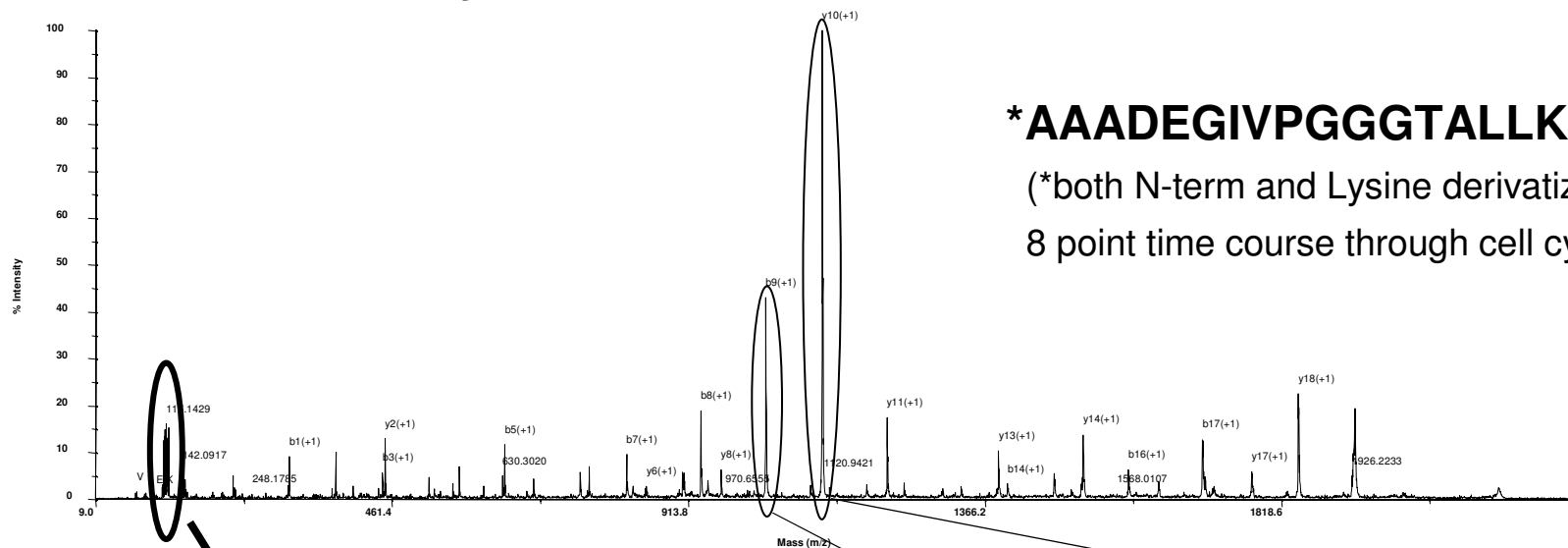
Can now expand the Time Course Study to 8 time points!



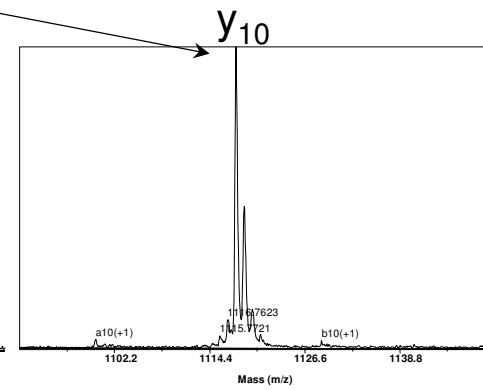
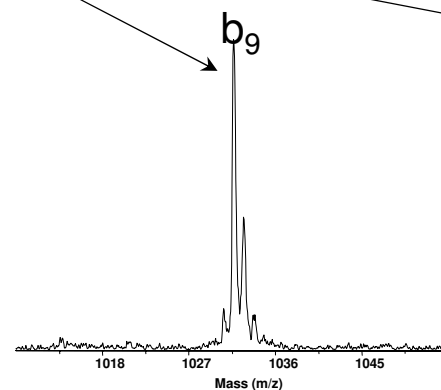
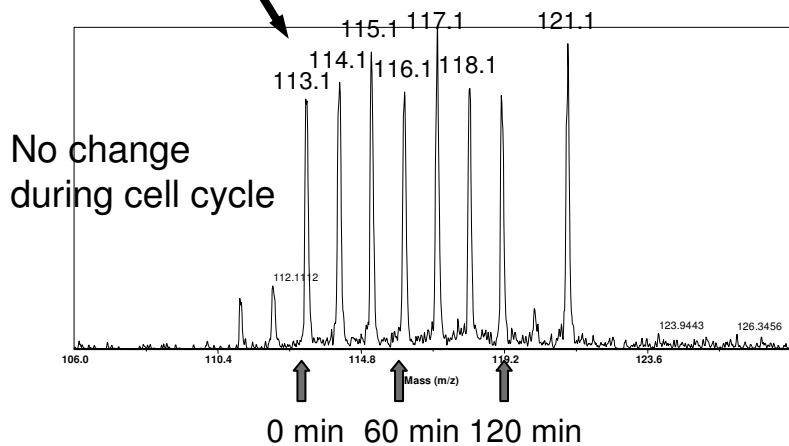


MS/MS ID of 60 kDa Chaperone B82334

C. Crescentus cell cycle time course



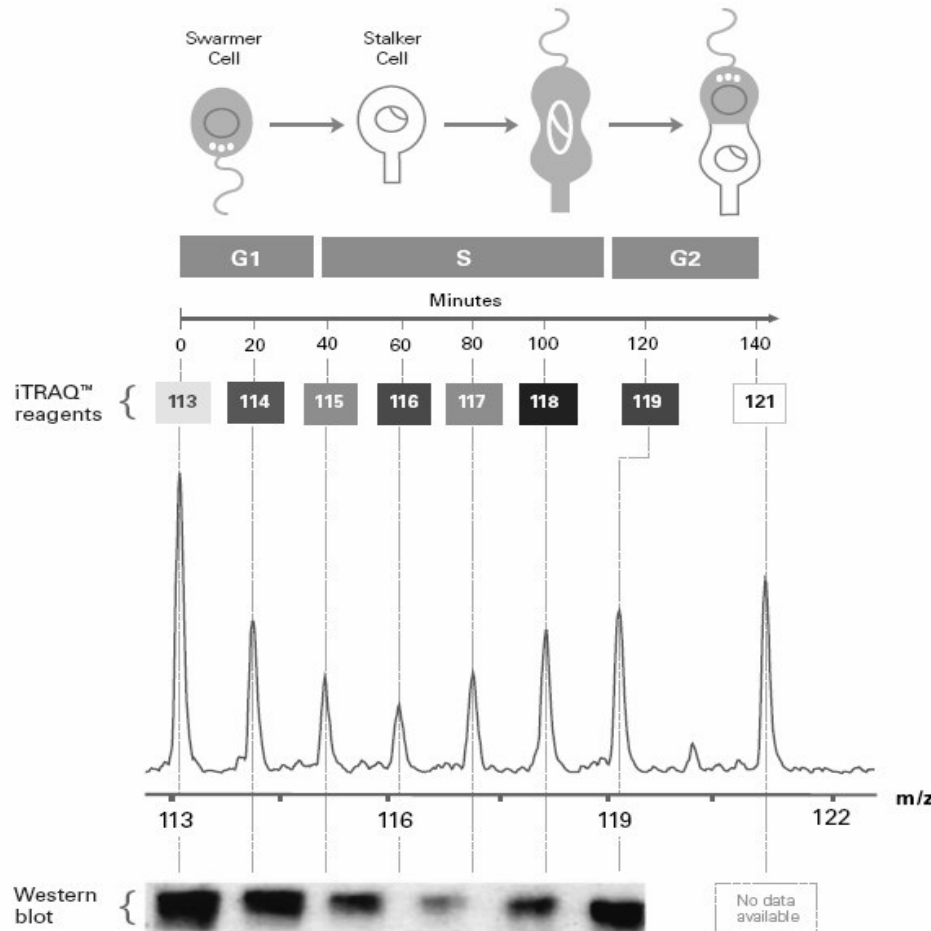
AAADEGIVPGGGTALLK
 (*both N-term and Lysine derivatized)
 8 point time course through cell cycle





Quantitation of Cell Division protein Fts-Z

8 Point Cell Cycle Time Course of *C. crescentus*





Selected 8-plex iTRAQ Applications

(submitted or in press)

- Evaluation of Fluoromethyl-2,2-difluoro-1-(trifluoromethyl)vinyl Ether ("compound A") Effects on Urine Protein Excretion in Rats
- Differential Protein Expression of Human Vitreous Fluids with Melanoma Disease Using iTRAQ[®] Reagents-8plex
- Comprehensive Quantitative Analyses on Protein Dynamics of the Human Pathogen *Staphylococcus aureus* by the Implementation of an 8-plex iTRAQ[™] Labeling
- Utilizing Isobaric Tagging Reagents to Screen Cerebral Spinal Fluid Samples for Potential Alzheimer's Biomarkers
- Quantitative Analysis of Mice Synaptic Membranes with 8-plex iTRAQ[™] reagents



4800 MALDI TOF/TOF™ Analyzer

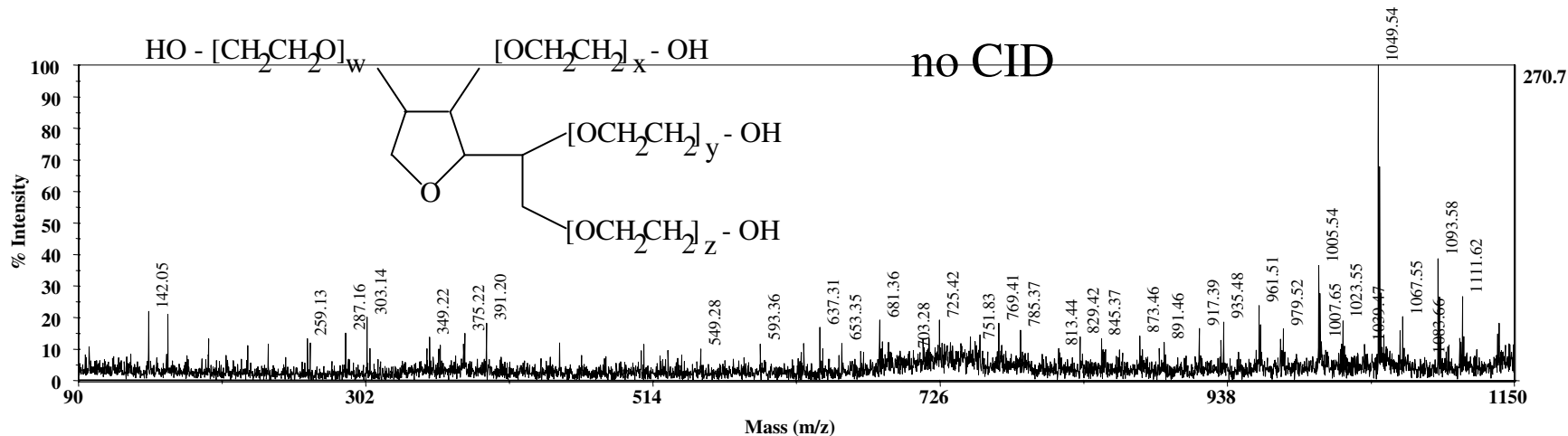
Other Unique Applications:

- Polymers
- Fatty Acids
- Carbohydrates
- Imaging

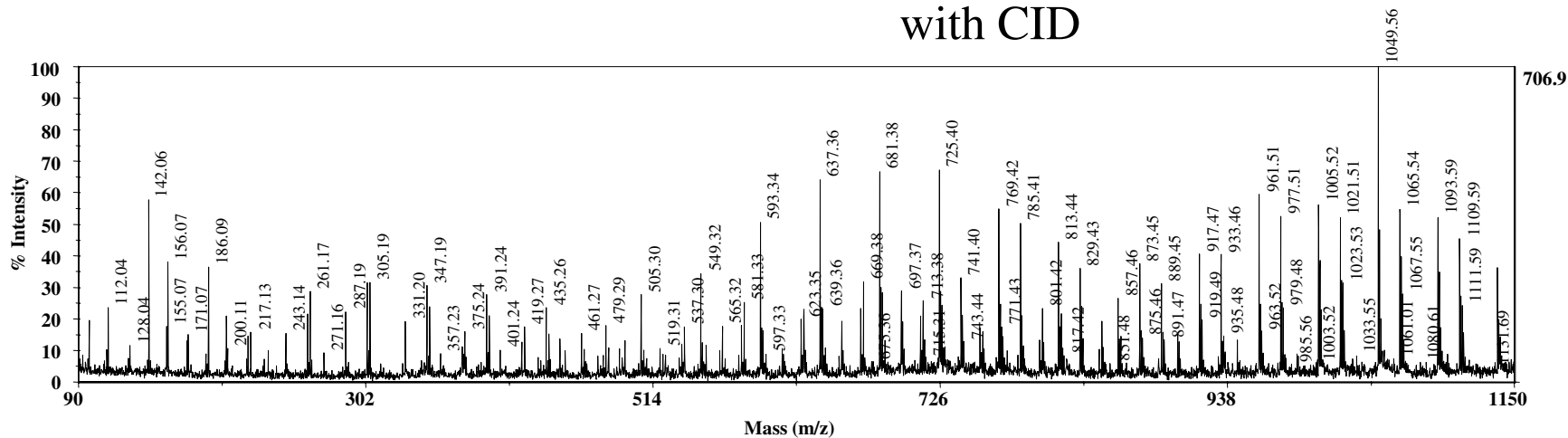




4800 TOF/TOF: Polymer Fragmentation

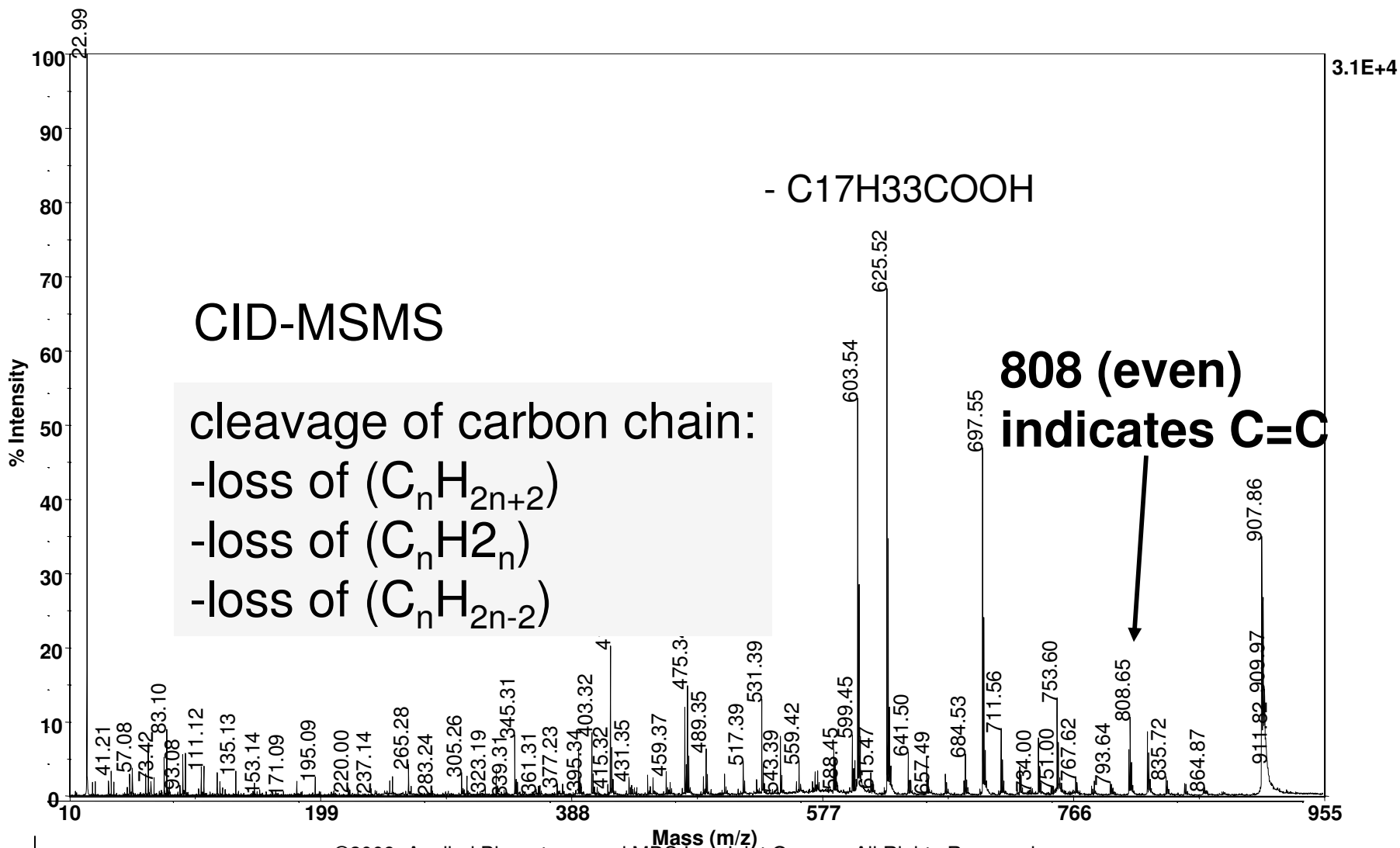


MS/MS of sorbitan polyethoxylate (Tweens component) MNa+ 1243.72 with CID



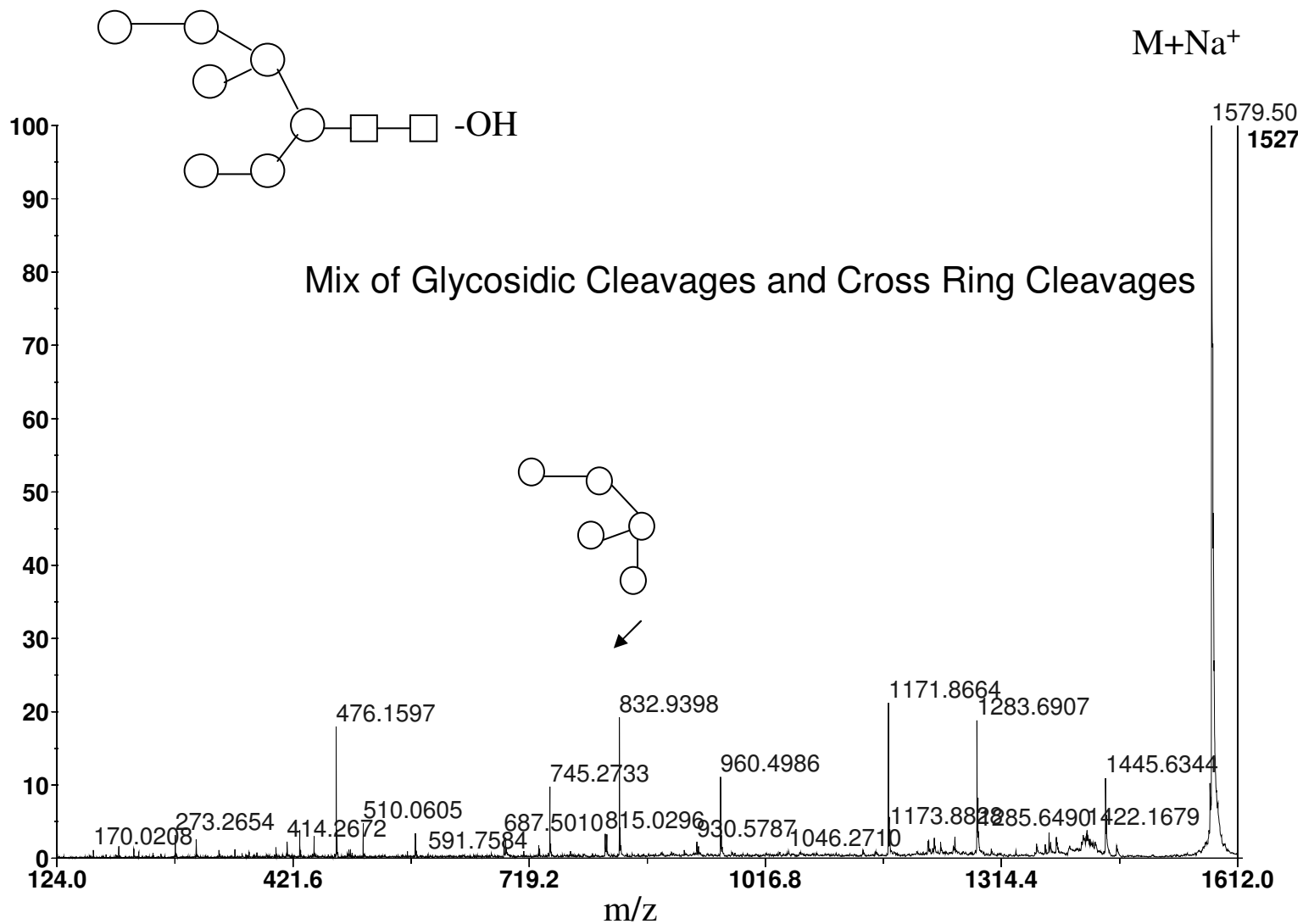


TOF/TOF: MS/MS of a Triglycerid





MSMS of (Man)₇(GlcNAc)₂(D3)





MALDI-MS/MS Summary

- Complementarity to ESI
- Time independent
 - sample „frozen“ on plate until spent
 - MS decoupled from LC
 - MS/MS decoupled from MS

MALDI and ESI necessary for
comprehensive proteomics laboratory



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