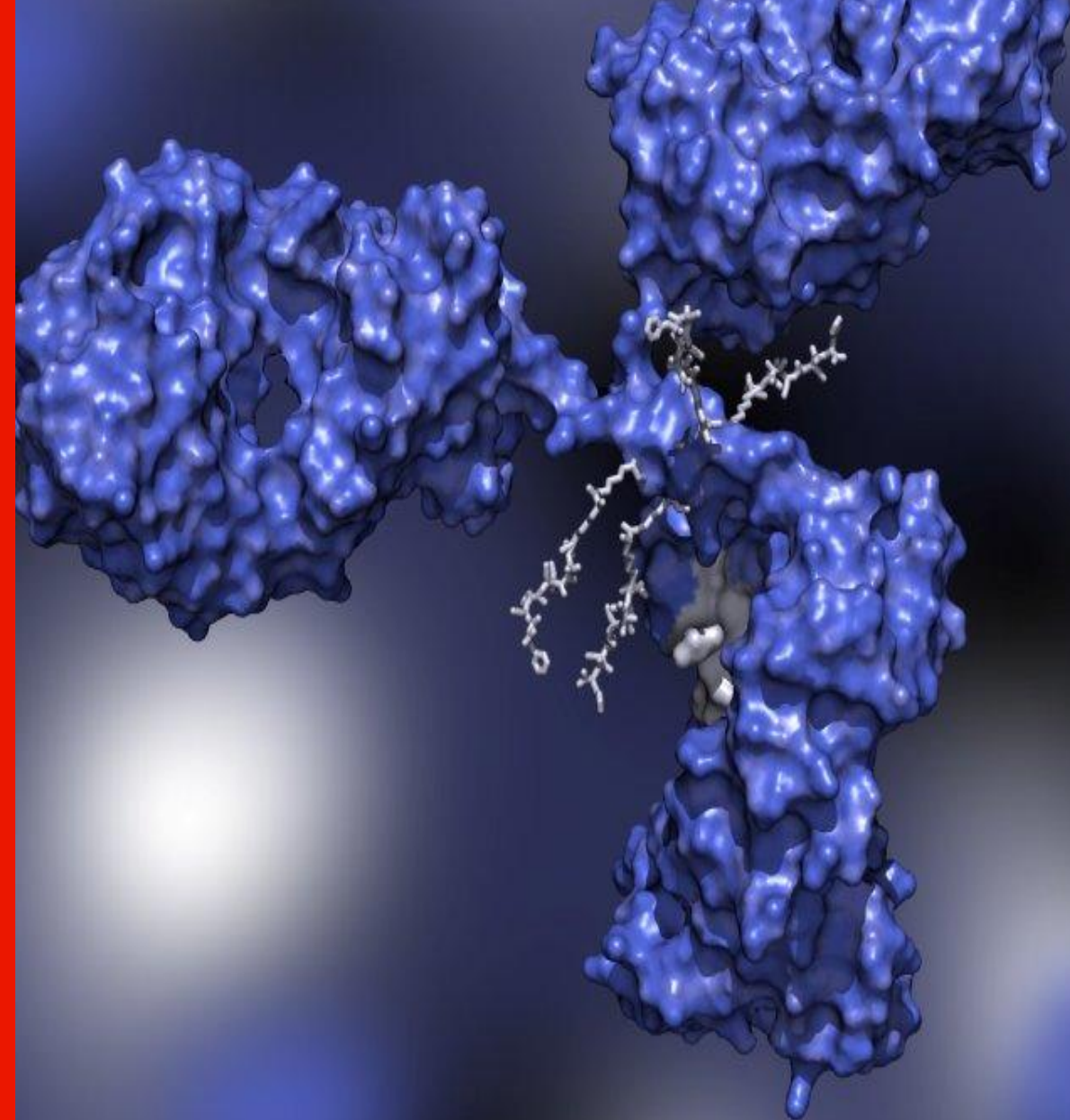


Purification-Free Mass Spectrometry: Characterizing Biotherapeutics and Spent Media

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Cell Engineering & Analytical Sciences

J&J Innovative Medicine



The Philosophy of High Throughput MS

Striking the right balance of sample preparation and data acquisition speed

Speed of Data Acquisition

High throughput enables high numbers of samples. However, does this mean fast turnaround?

Speed of Decision

Decision = **Sample Preparation** + Acquisition

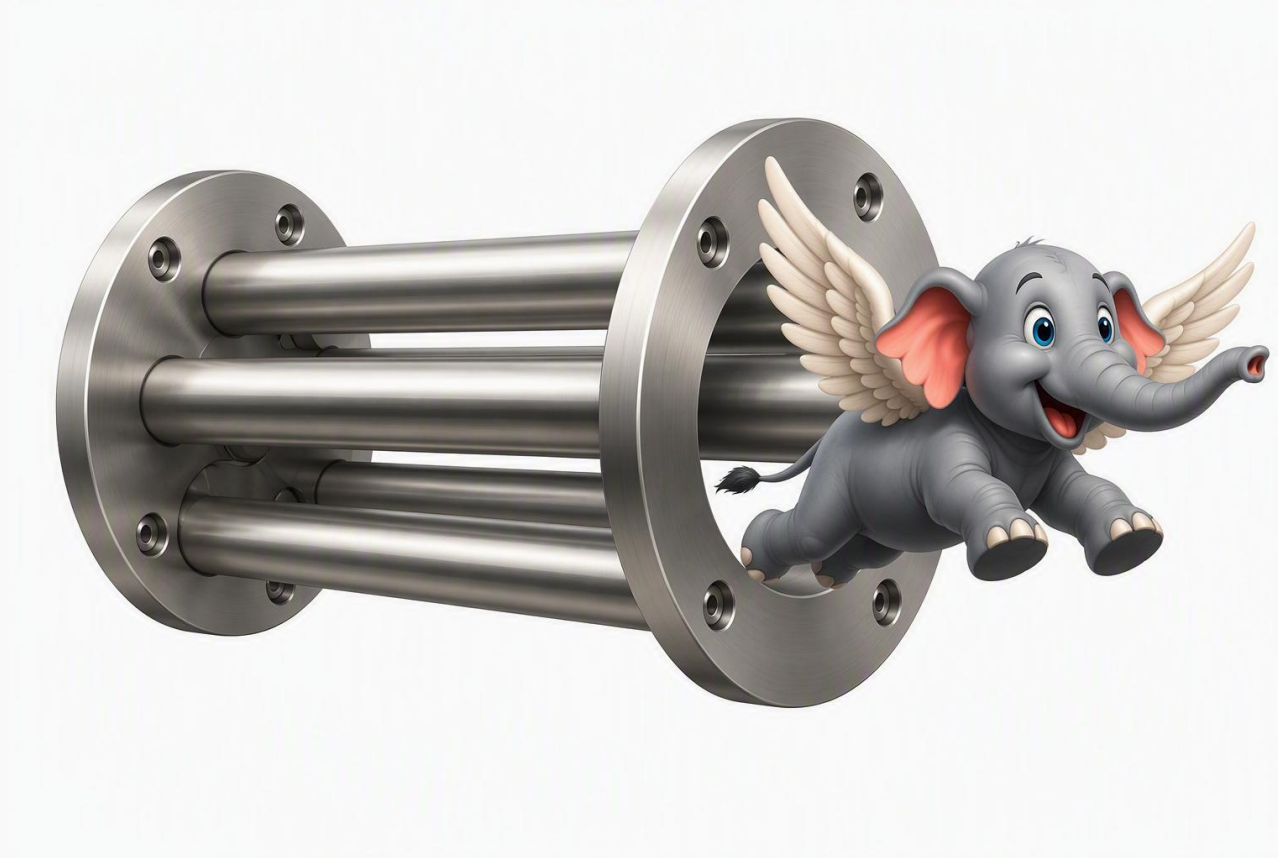
How do we move towards real time measurements?

Balancing the two

What is the balance between fast turnaround and high numbers of samples?

Electrospray wings for molecular elephants

-John Fenn



Electrospray wings for molecular elephants

...and zebras and ants and trees and PEG. – John B. Fenn (probably)



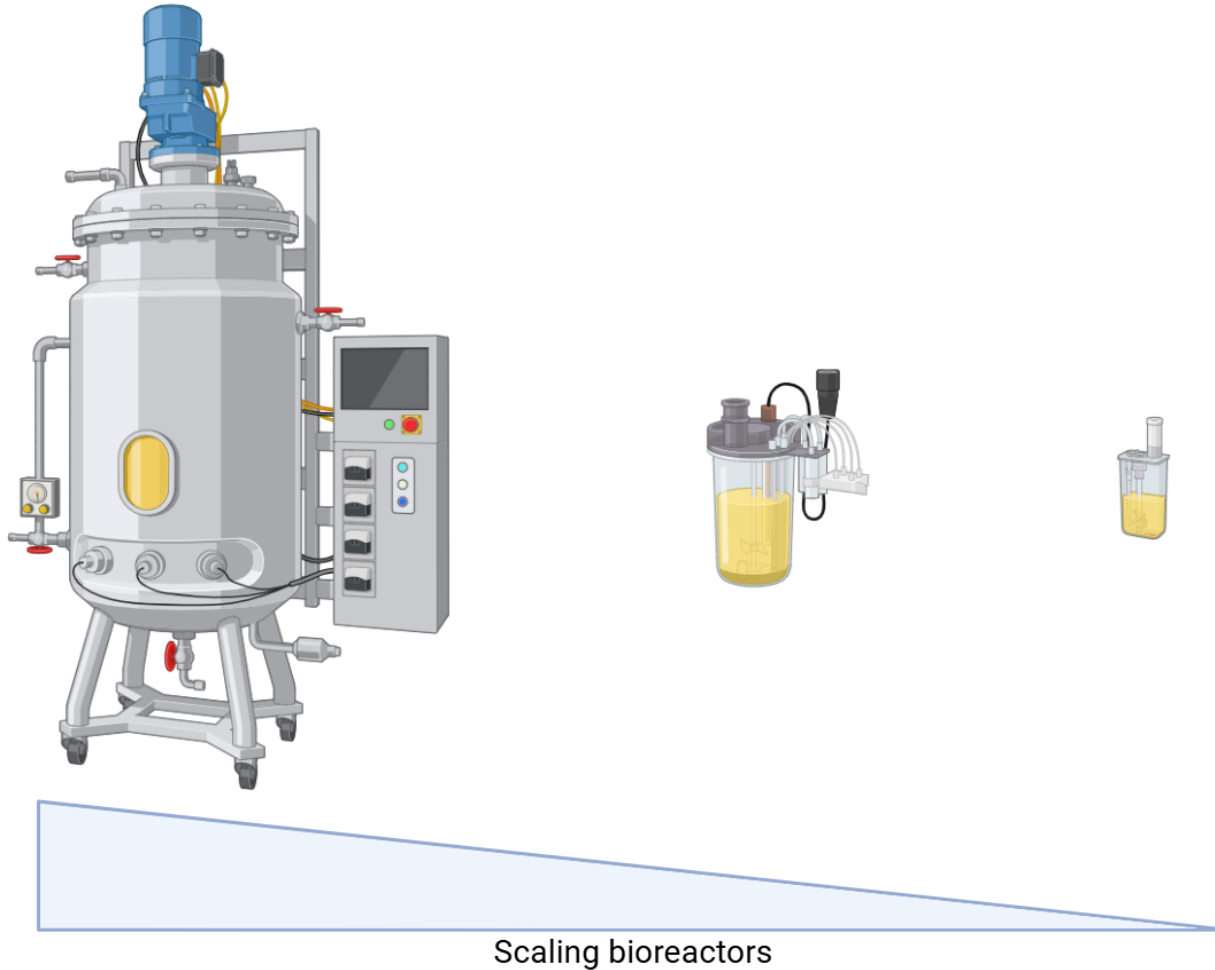
MS offers incredible
sensitivity

MS research revolves
around isolating analytes of
interest

***How do we target analytes in complex
matrices without sample preparation?***

The Duality of Biopharmaceuticals Development

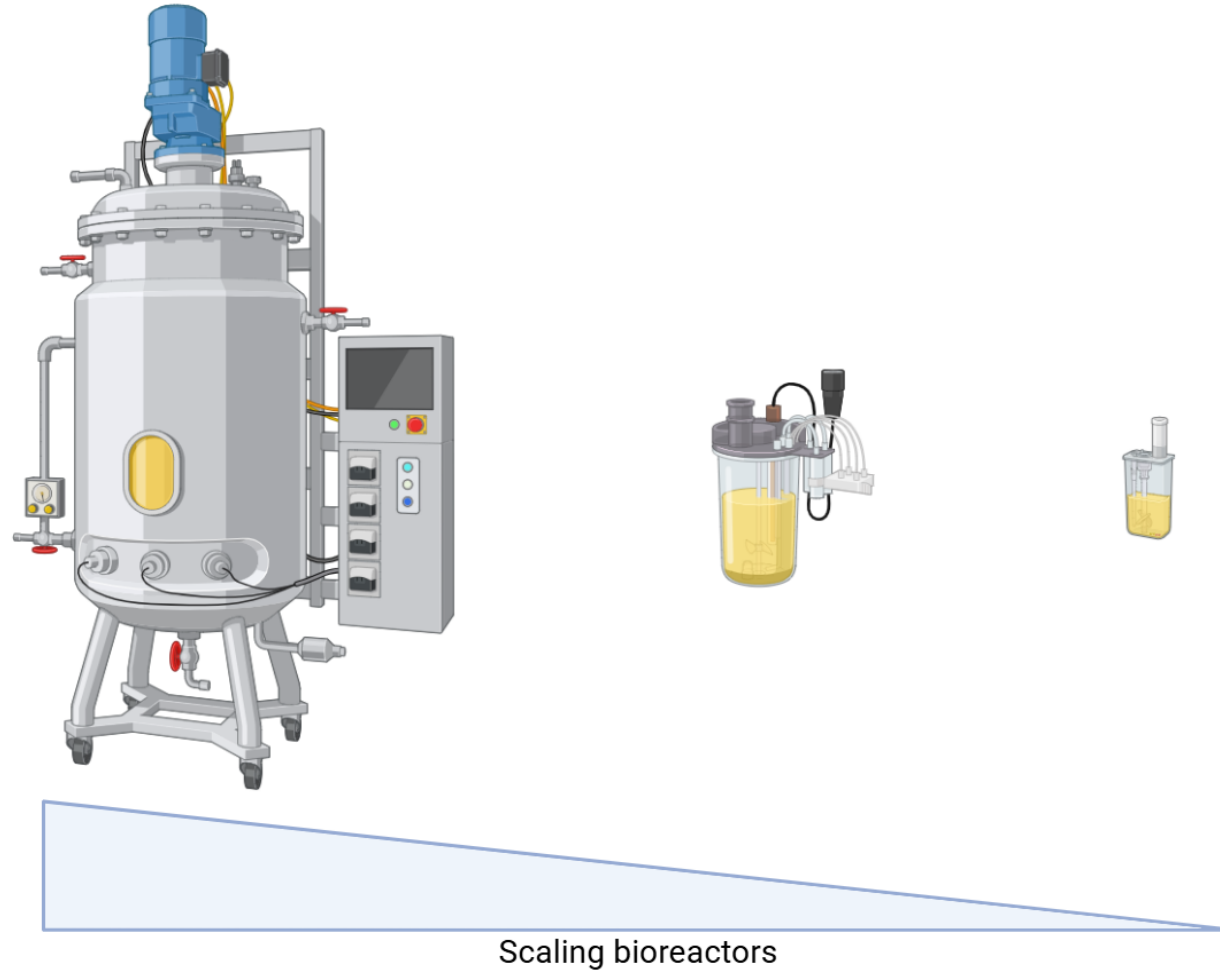
We want to understand not just the product, but also the process.



Process	Viable cell density
	Glucose, lactate, etc.
	Amino acid analysis
Product	Biophysical characterization
	Peptide mapping
	Intact mass analysis

The Duality of Biopharmaceuticals Development

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Strategies for Purification-Free Mass Spectrometry

Two approaches for process and product analytical

Amino Acid
Analysis

**Acoustic Ejection Mass
Spectrometry**

Excellent for high concentration
samples

Ultrafast (up to 1 Hz) data
acquisition

Heavily diluted enables complex
matrices

Can leverage MRMs for multi-
analyte analysis

Intact Mass
Analysis

**Tangential flow filtration and
micro-dialysis**

Removes low mass interferences
for analytes in complex matrices

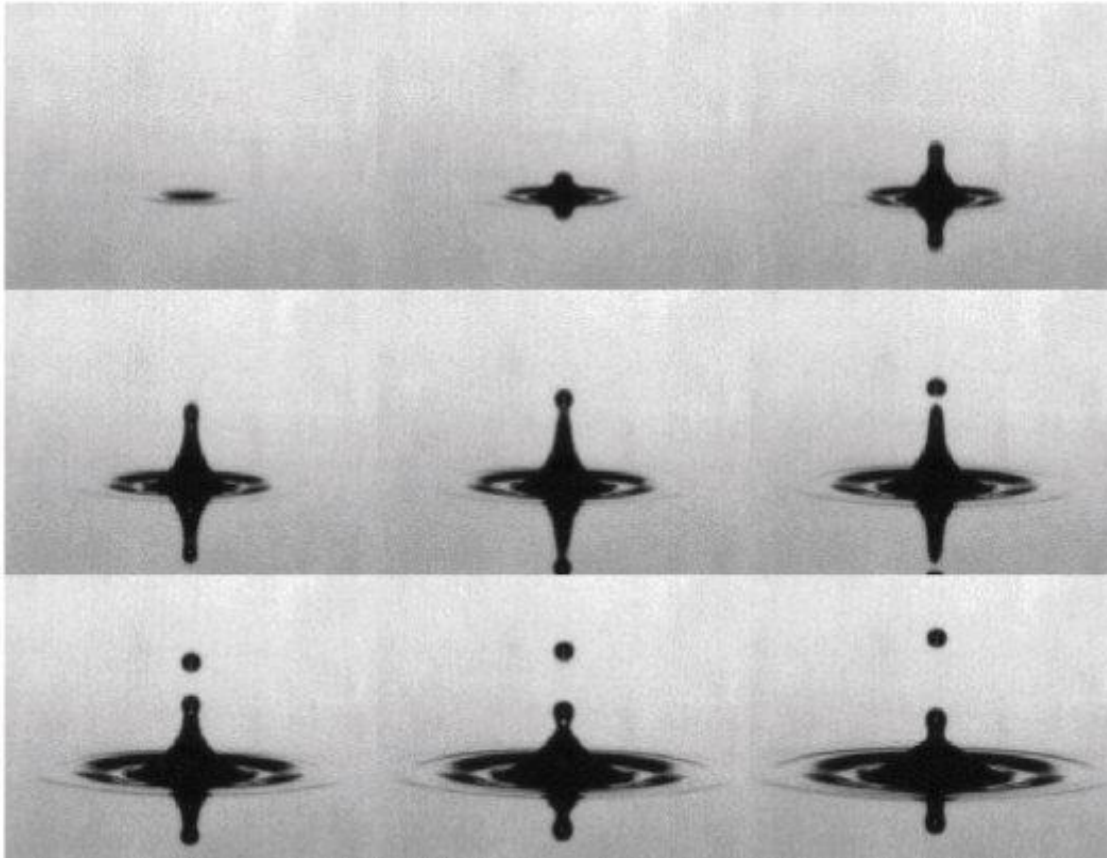
Excellent for large molecule
analysis (i.e. biotherapeutics)

High sensitivity

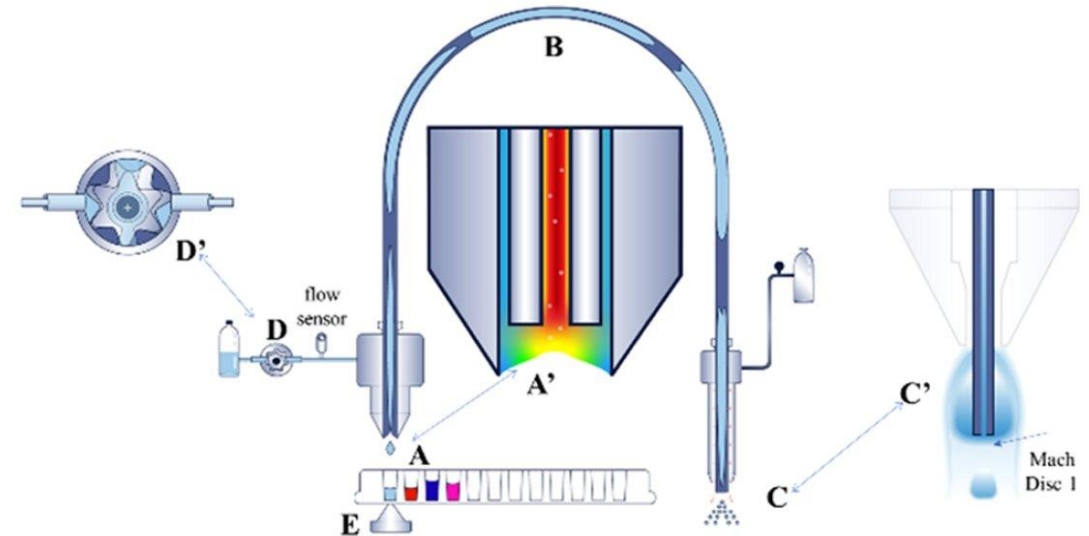
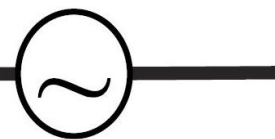
Retains native conditions for
intact mass analysis

What is Acoustic Ejection (AE)-MS?

Sciex Echo Plus MS

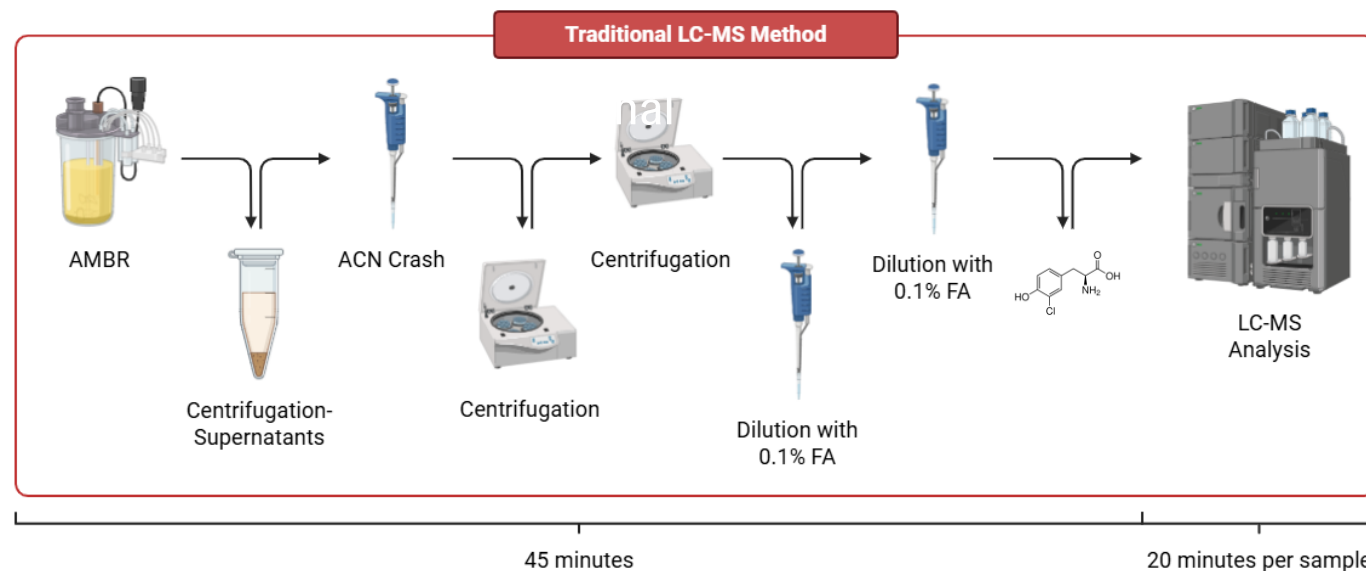


RF Power In



- A. OPI capture port oriented downward.
- A'. Drawing of the critical condition surface in "A".
- B. OPI with a 50 cm transport tube.
- C. OPI venturi pump/ESI nebulizer.
- C'. Sonic expansion creating pressure drop.
- D. Fluid delivery pump.
- E. Acoustic dispensing upward against gravity.

Spent Media Analysis: Comparing AE-MS to LC-MS?



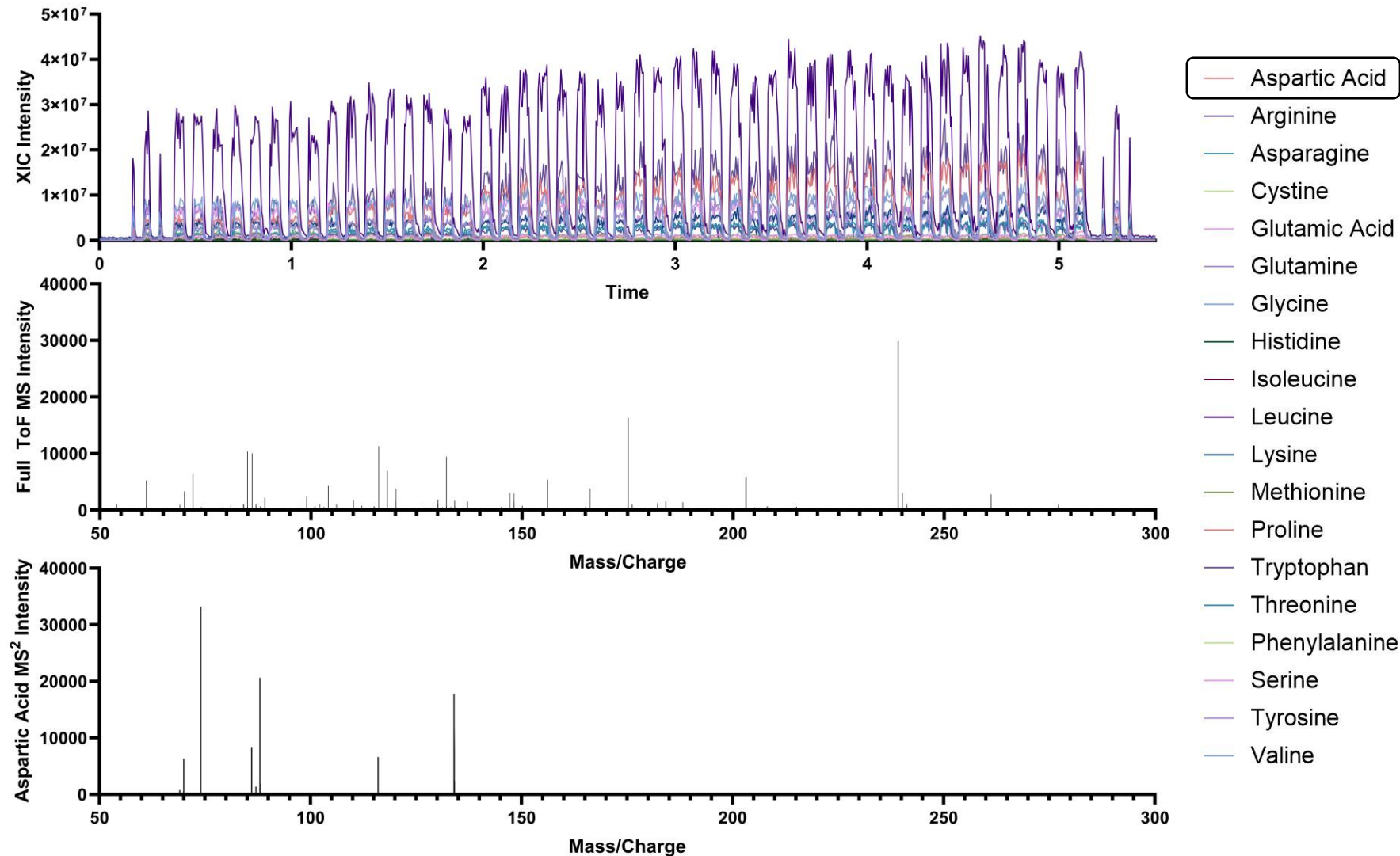
- 240 Samples
- **3.7 days**
- No replicates



- 240 Samples
- **75 min in triplicate**
- **200x faster per sample**

- Direct analysis
- 50 nL sample volumes
- MRM MS method

Amino Acid Detection leveraging 20 MRM Transitions



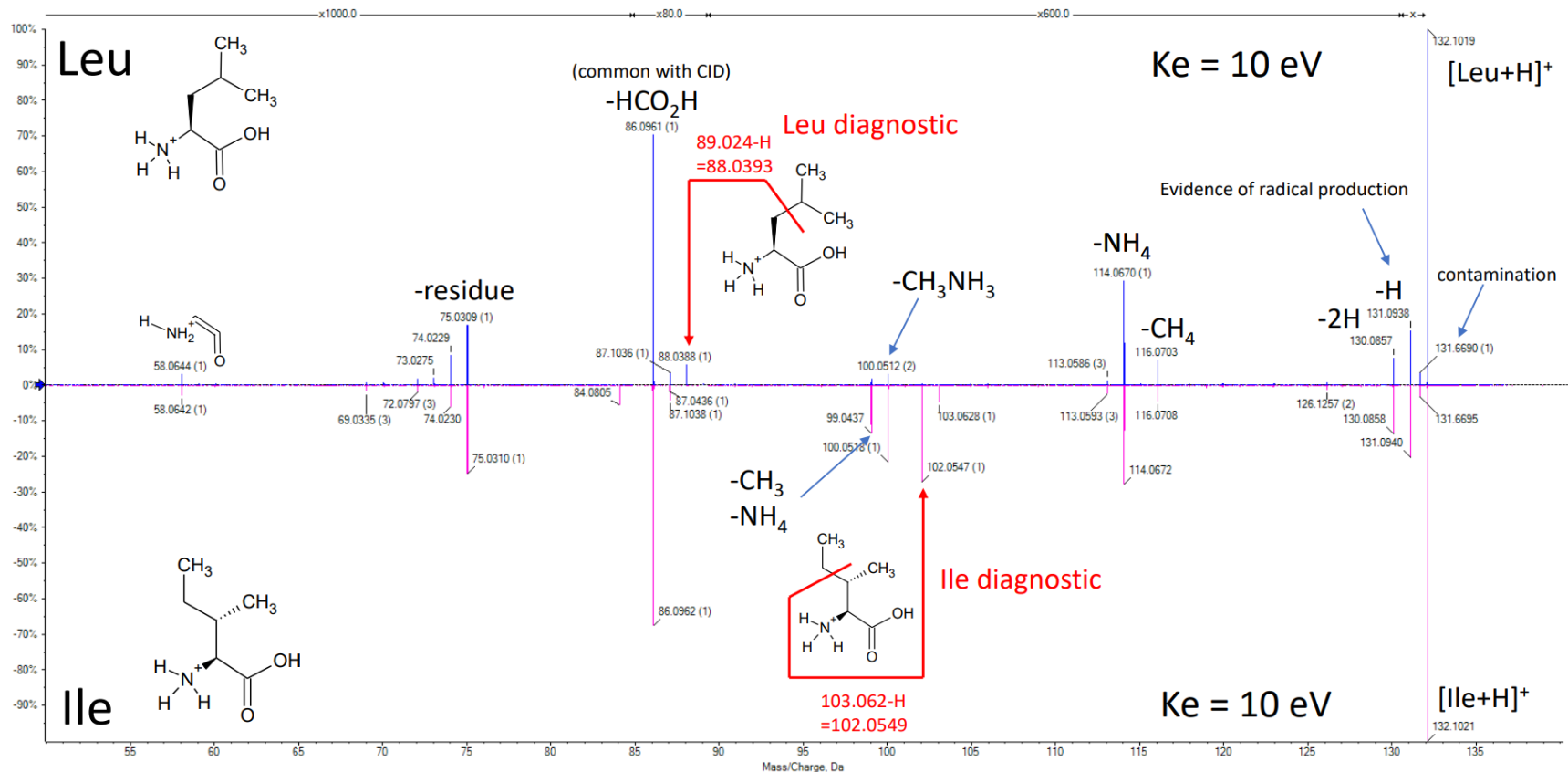
Acquisition Parameters

50 nL injection

6 seconds

20 MRM transitions

Electron Activated Dissociation (EAD) Enables Elucidation of Isoleucine vs. Leucine



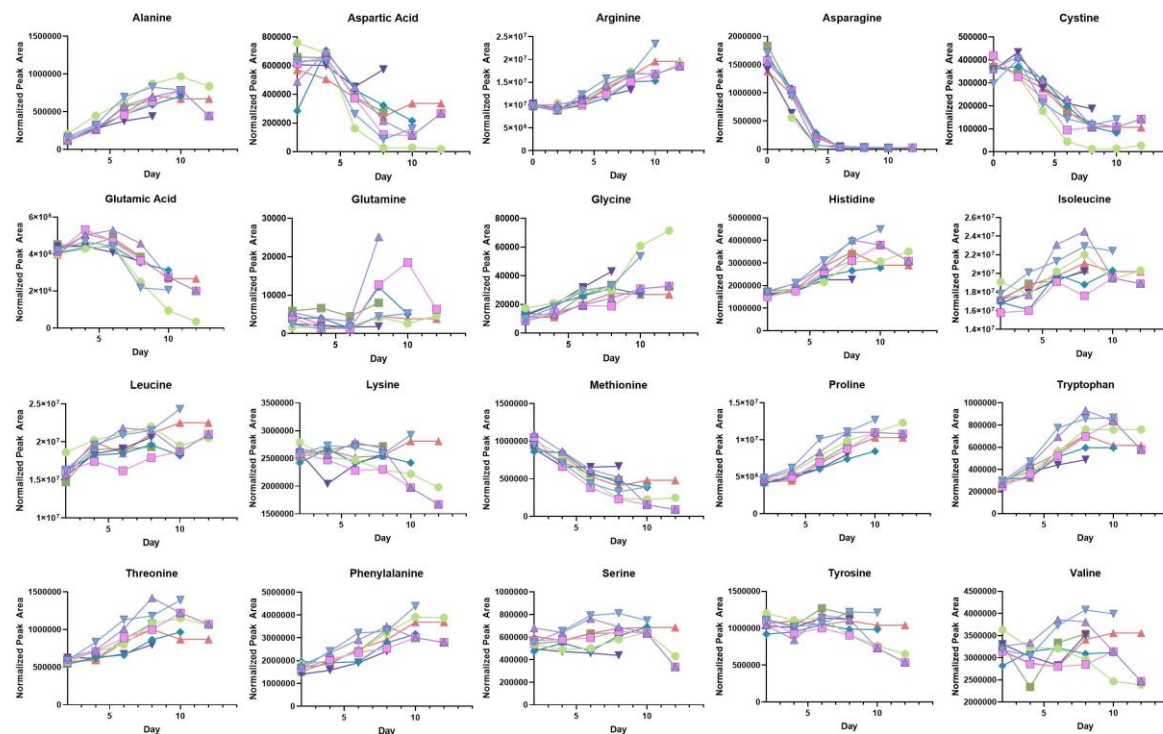
EAD allows for the differentiation of isobaric analytes

- Integrated EAD-based MRM transitions into the spent media method

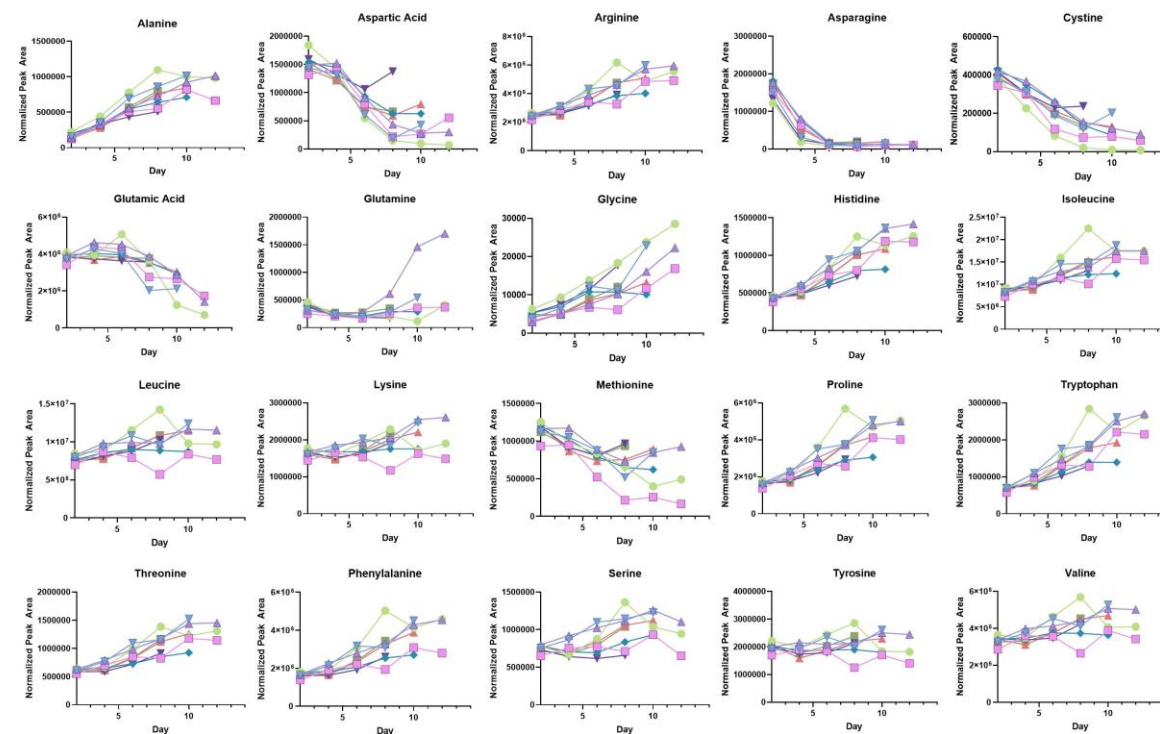
Amino Acid Quantitation Directly from Bioreactors

AE-MS offers statistically equivalent analyses compared with LC-MS

AE-MS



LC-MS

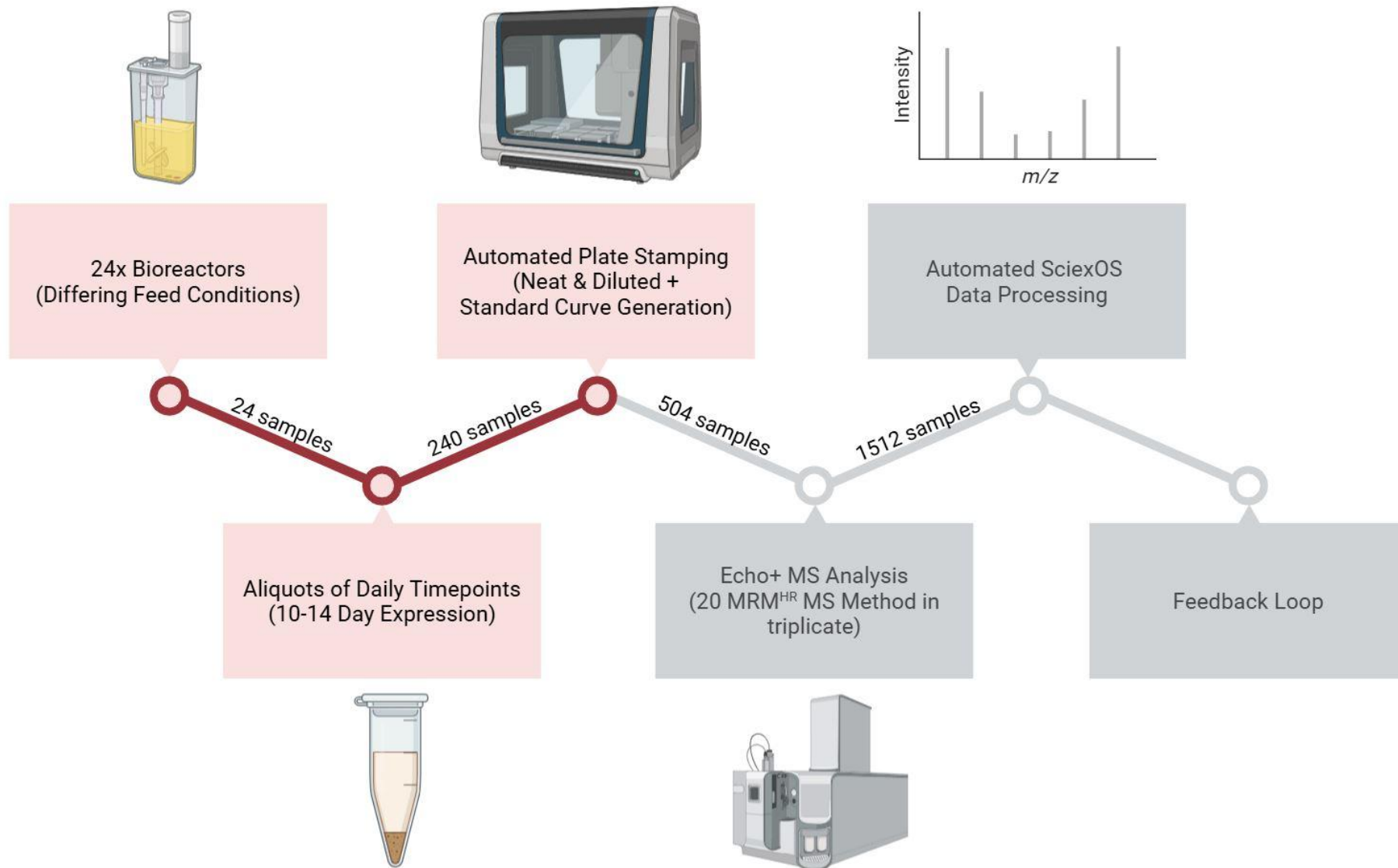


Mean %CV of 4.0

Median %CV of 3.1

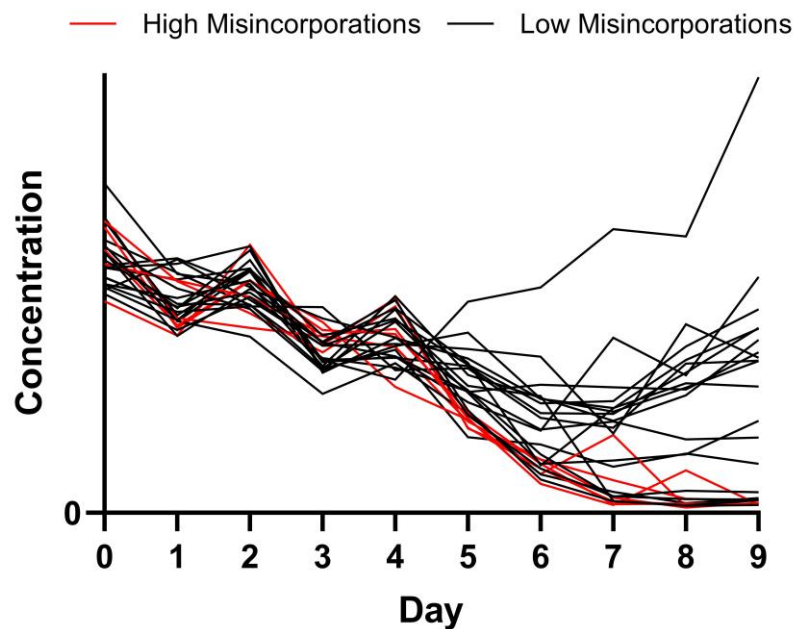
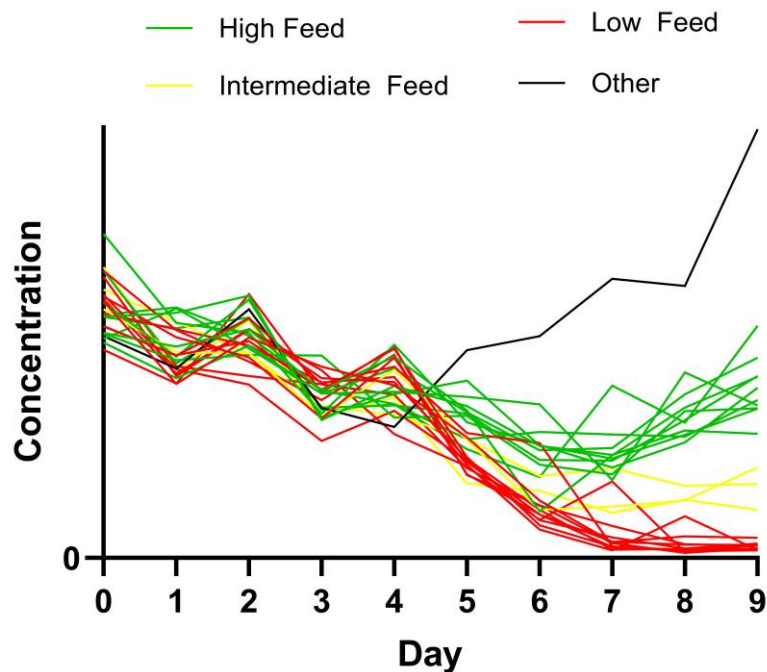
Spent Media Analysis for Sequence Variant Analysis

Process Development



Threonine Trends with Feed Levels

Threonine levels correlate with addition of more threonine



Three Feed Conditions

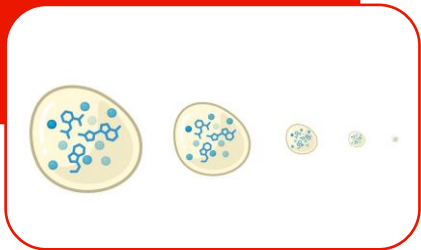
- Clear delineation of low, intermediate and high feed conditions
- Threonine depletion in spent media drive misincorporations
- Misincorporations are *process-dependent* and can be mitigated with higher levels of threonine feed

Spent media analysis enables ultra-fast process monitoring.

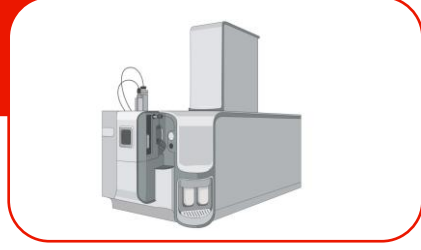
Ultrafast Extracellular Metabolomics

68 Metabolites Identified with 6 Second Ejections

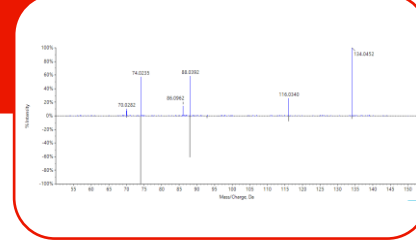
Spent Media



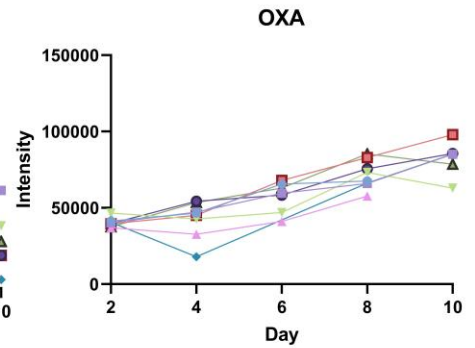
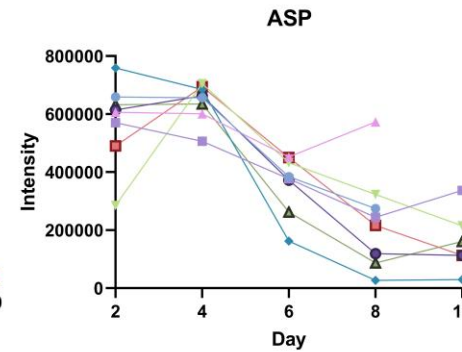
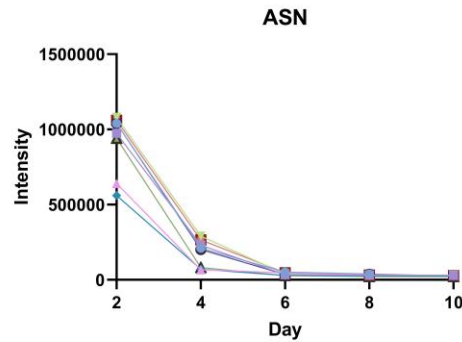
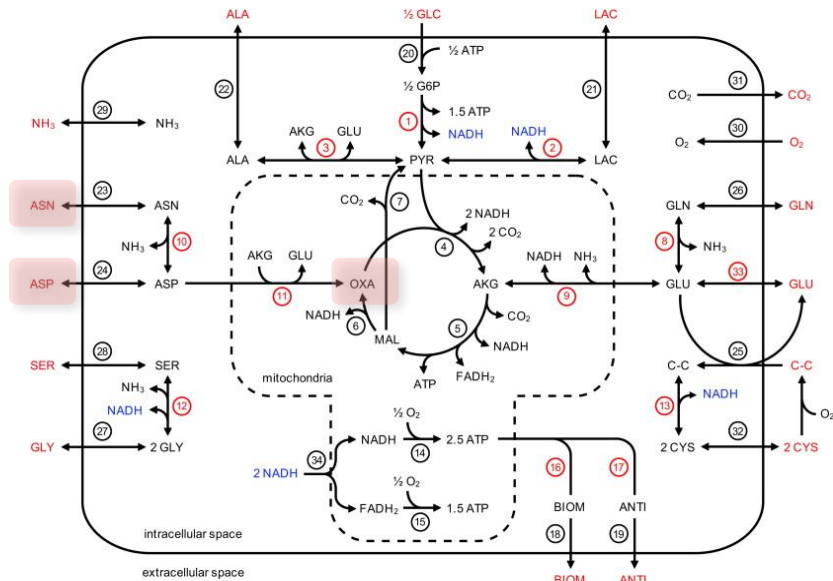
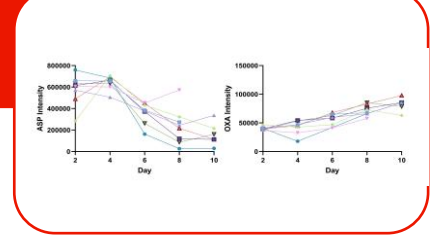
Untargeted MS



Library Search

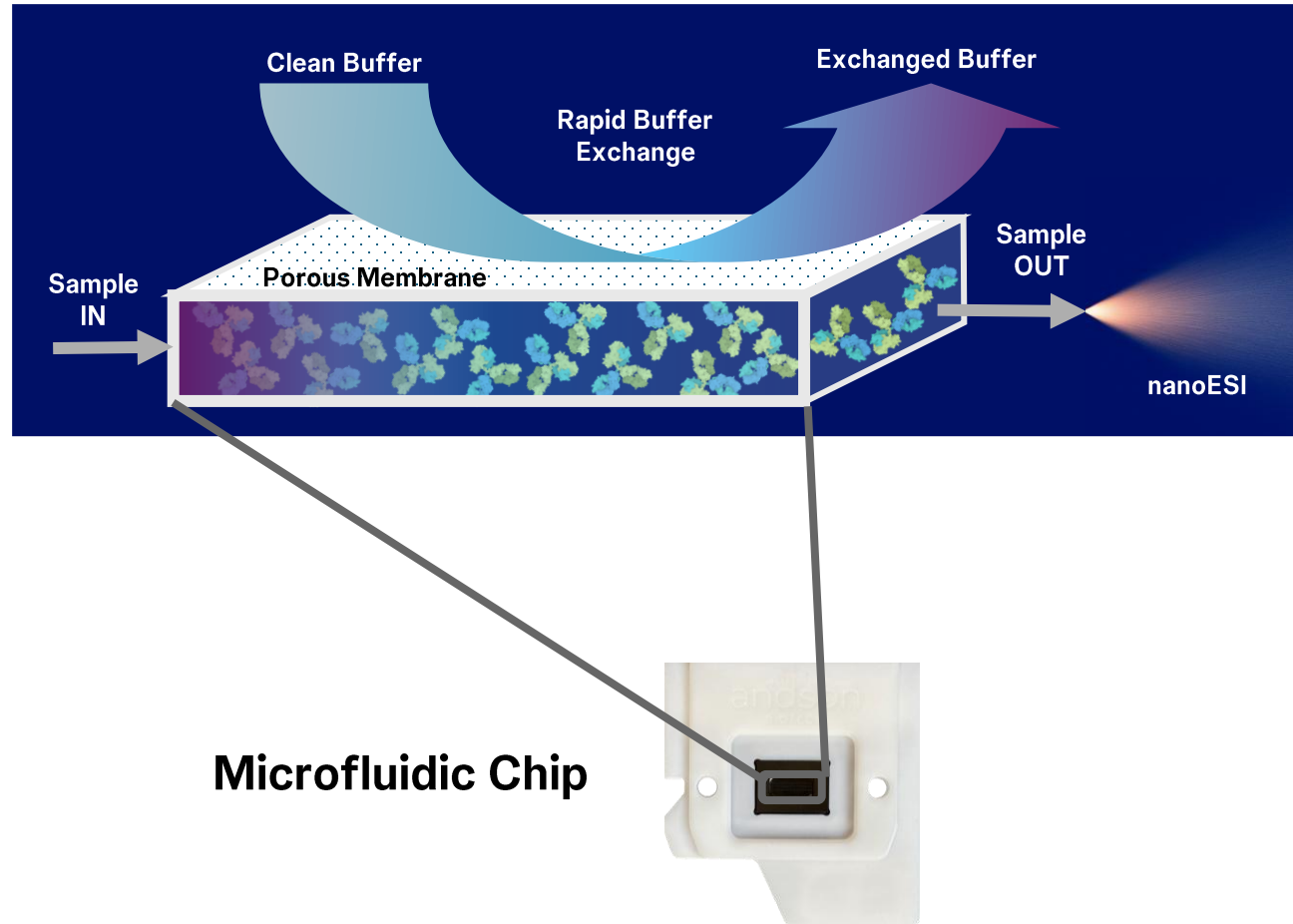


Quantitation



Tangential-Flow Filtration & Micro-Dialysis

Andson's Dynachip X1



Intact Mass Analysis

Rapid and efficient removal of low mass interferences for complex matrices

Excellent for complex samples (i.e. biotherapeutics)

Low sample volume requirements (0.1 µg)

Retains native conditions for intact mass analysis

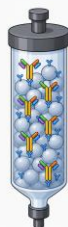
Vendor agnostic interface

Biotherapeutics Direct from Crude Cell Cultures

Speed to answer for *small sample sets*

~125 minutes for one sample

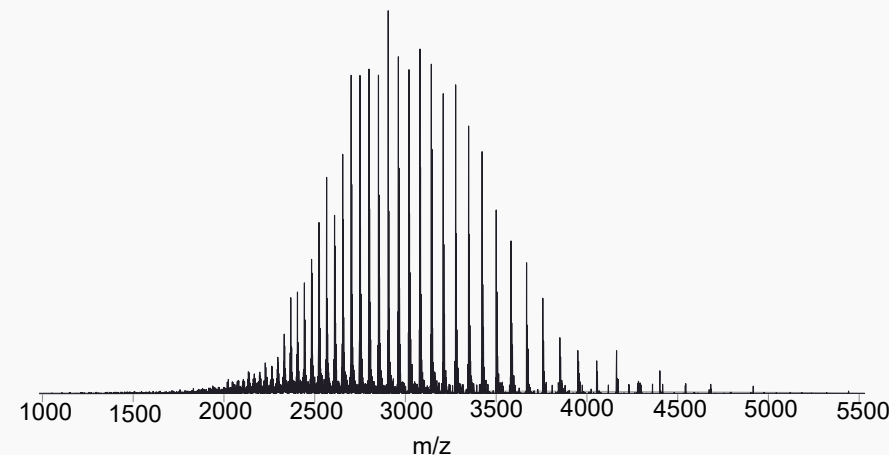
Traditional LC-MS



Protein A
Purification



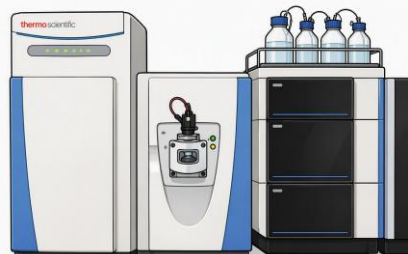
Reverse Phase MS
Analysis



AMBR15
Expression

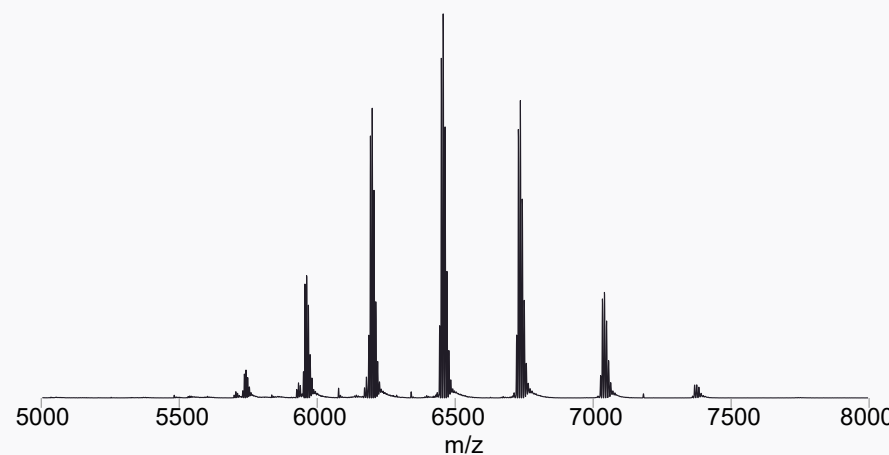


Andson Dynachip



Native MS
Analysis

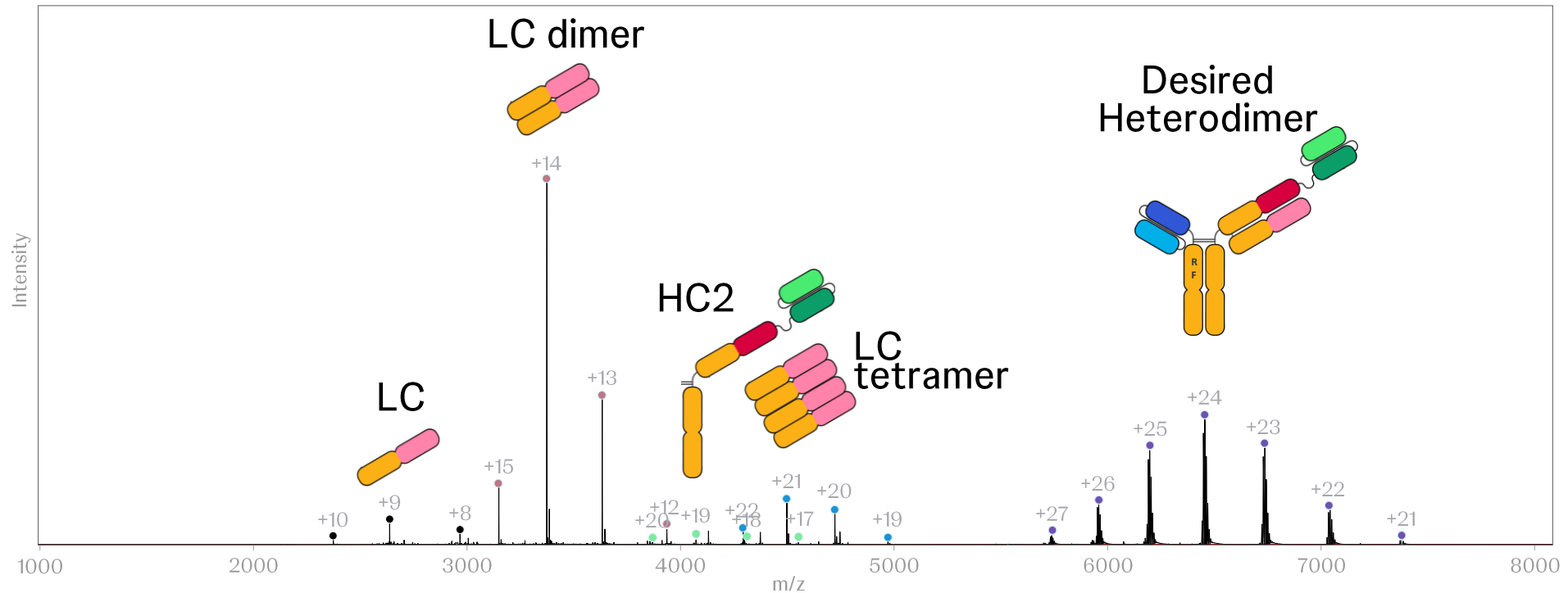
Purification-Free MS



15 minutes for one sample

No Protein A: What can we learn?

No excess chain removal

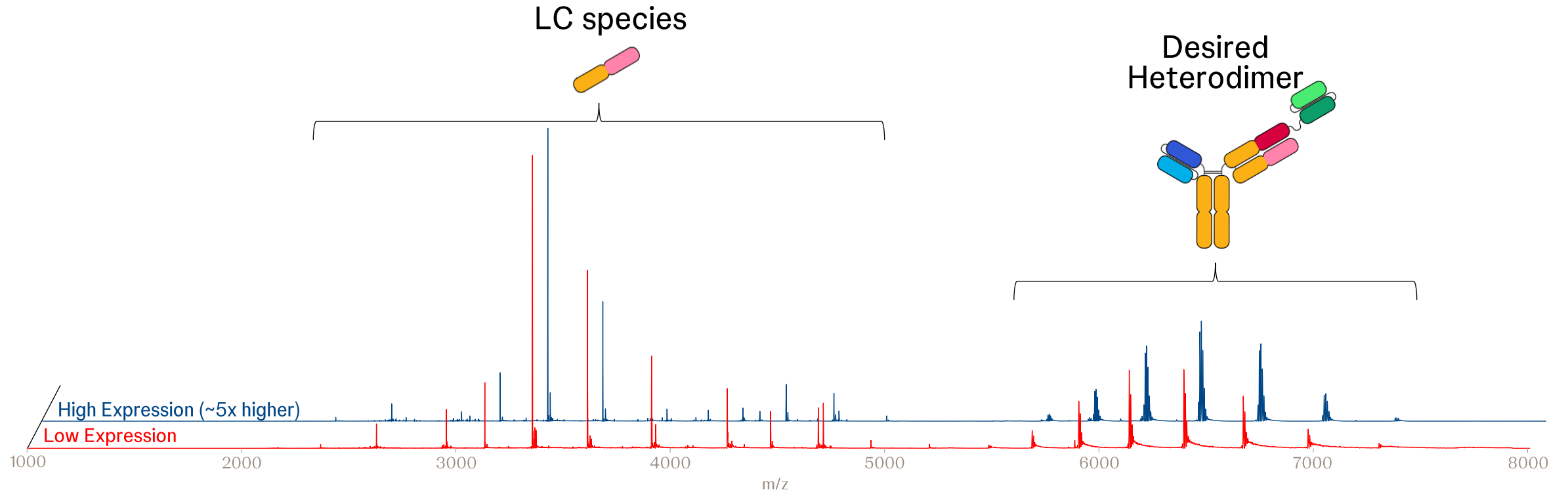


Observe expression profiles that would normally be purified away.

Abundant expression of excess free LC, LC dimer, and LC tetramer.
Low levels of excess HC2.

What is the impact of improper chain ratios?

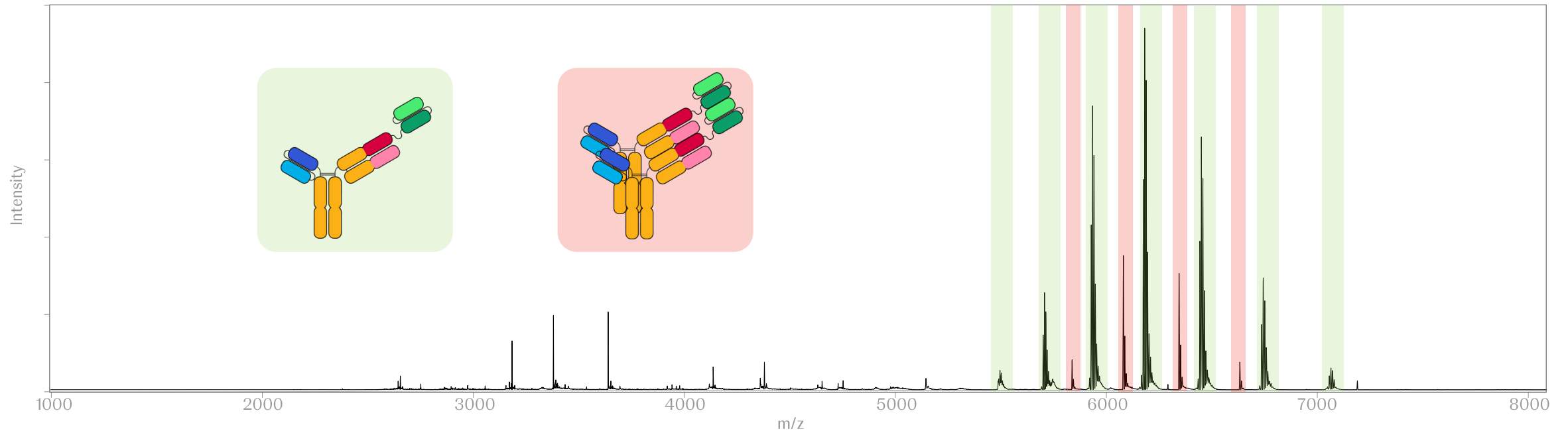
High expression clones have more balanced expression of chain ratios



Balanced expression of chains plays an important role in expression efficiency of desired product.

Detecting Aggregates

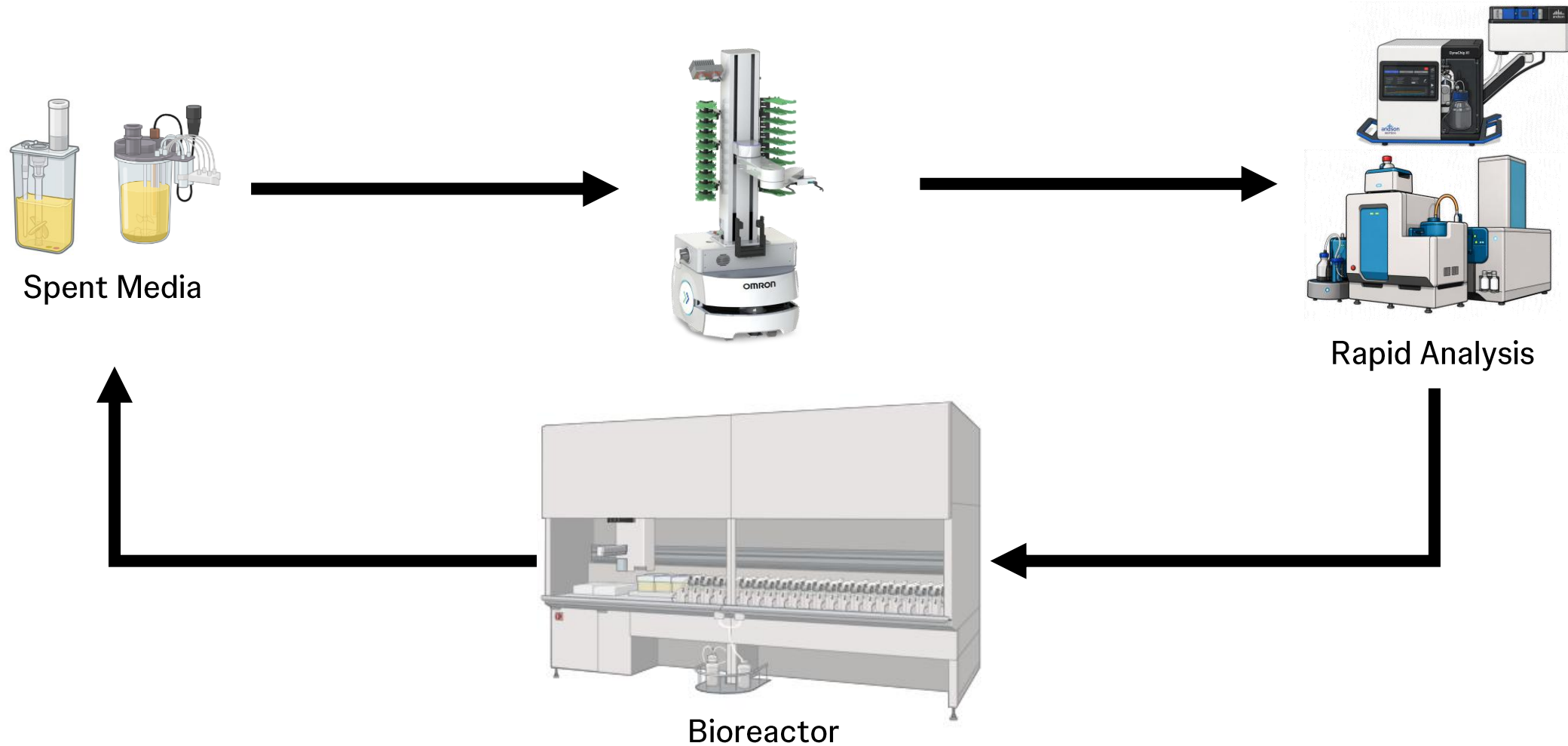
Aggregates can be quantified under native conditions



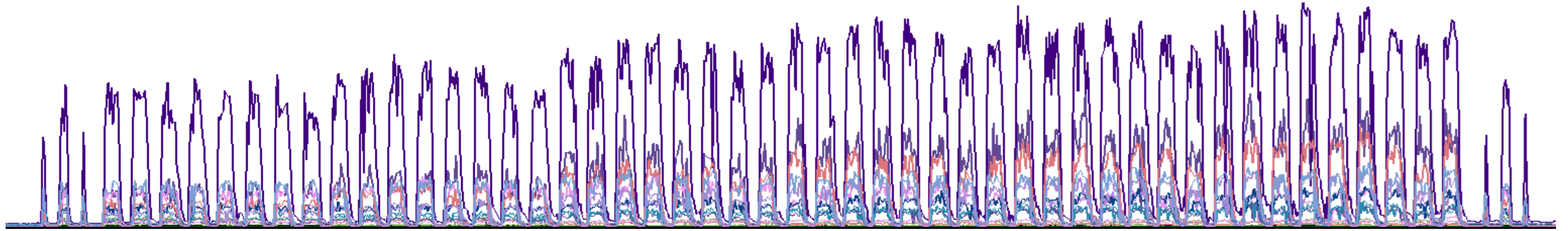
Non-covalent aggregates are easily detectable with high-resolution native mass spectrometry.

Future Looking: Real-Time Monitoring of Bioreactors

Feedback loop driven bioreactors



Conclusion



AE-MS offers improved speed with 200x faster data acquisition times for amino acid quantitation.

Absolute quantitation of amino acids directly from spent media enables unprecedented insight into process development.

Untargeted metabolomics can be performed on spent media.

Tangential flow filtration enables purification-free biotherapeutic analysis without compromise.

Dynachip X1 provides invaluable insight into unperturbed chain ratios and aggregate analysis.

Purification free mass spectrometry is possible and enables higher throughput and faster answers.

Acknowledgements

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