6th YSS EBF

Determination of thorium-conjugated antibodies from plasma by different LC-MS technologies

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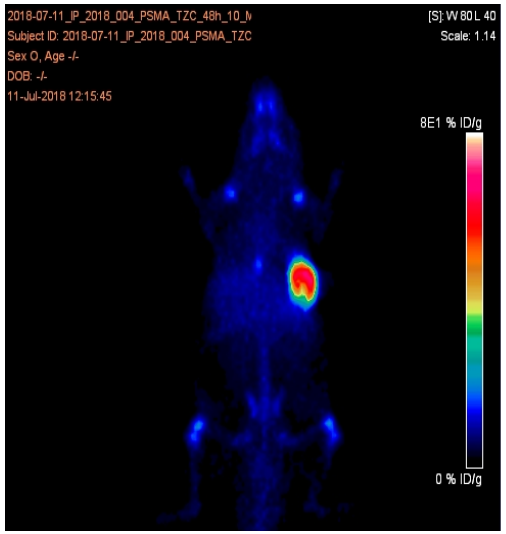
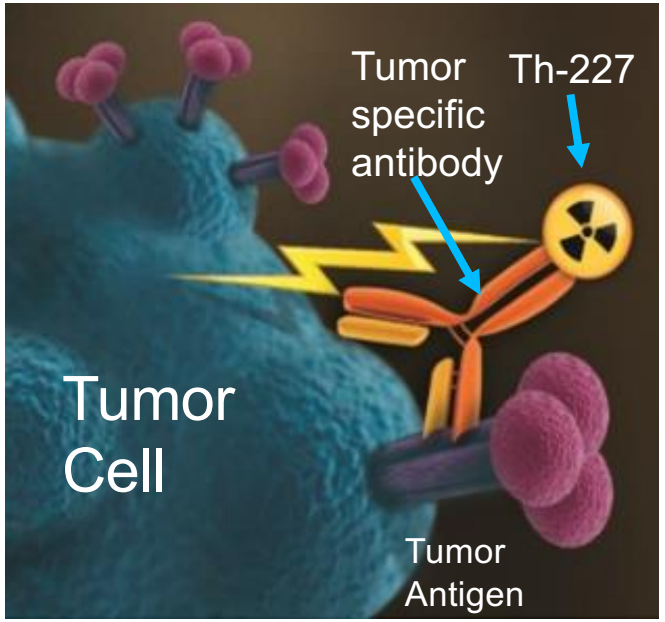
Introduction to thorium-conjugated antibody

Principle of Targeted Thorium-227 Conjugate (TTC‘s)

- Combination of an antibody and warhead like Antibody Drug Conjugate (ADC)
- TTC‘s: Antibody covalently linked to a chelator which contains an alpha emitting nuclide
- The released alpha particle is believed to induce DNA breaks in cancer cells and may lead to cell death
- New treatment option for cancer patients





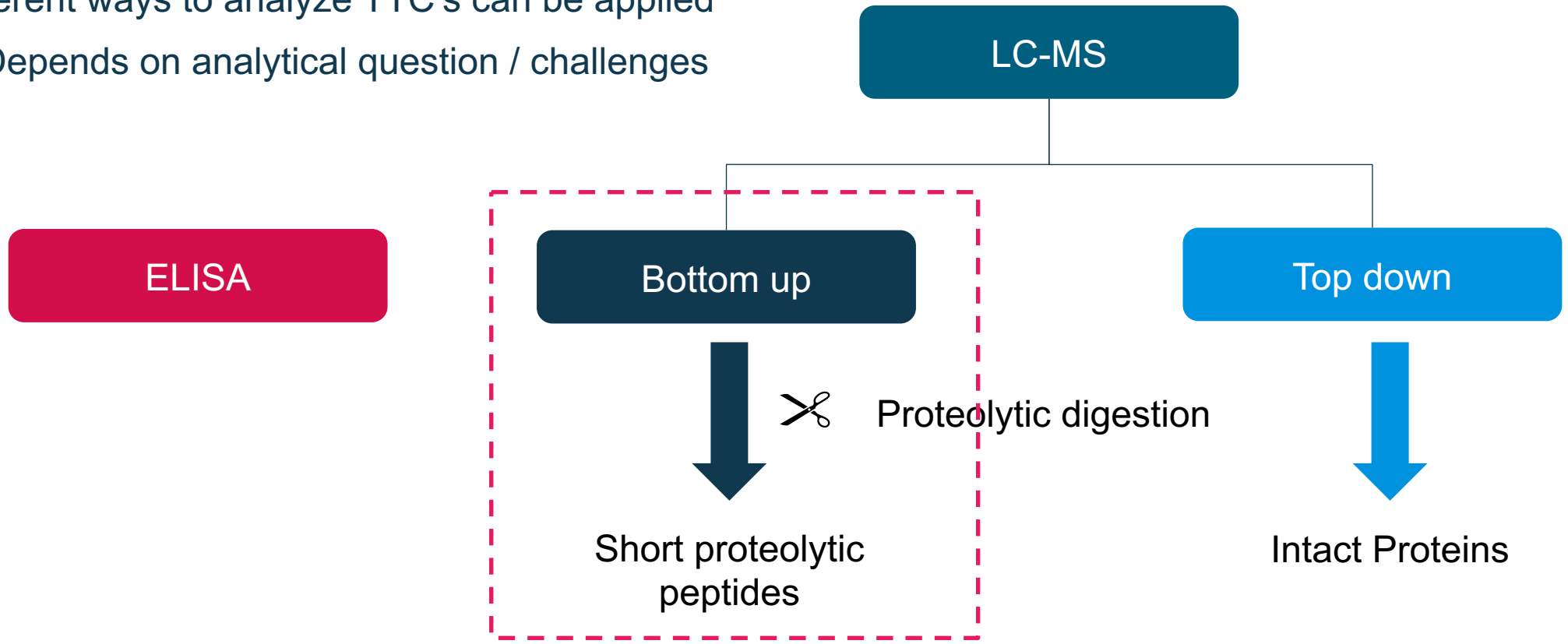
Which analytical methodologies can be applied for the analysis?

Analytical strategy

Ways to analyze antibodies

// Different ways to analyze TTC's can be applied

// Depends on analytical question / challenges

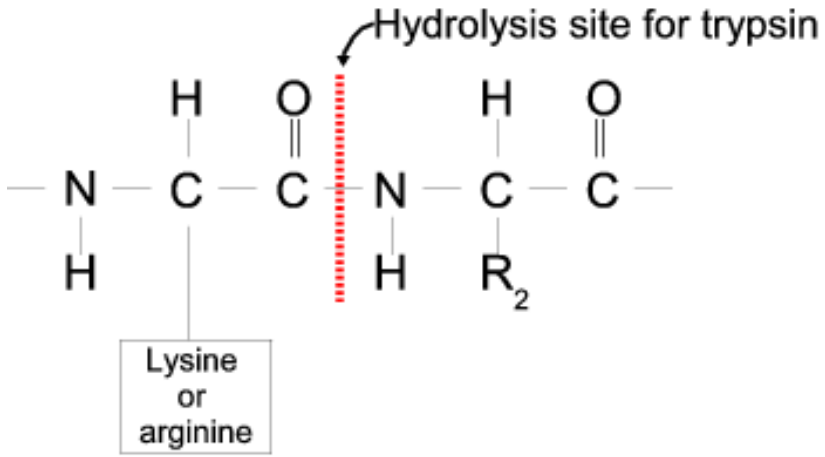


Different case studies: Comparison of ELISA and LC-MS results – gain of additional information by MS?

Analytical strategy

Bottom-Up – proteolytic digestion

- Trypsin is the most commonly used proteases
- Cleaves at arginine (R) and lysine (K)
- Example for one letter code peptide sequence



…IYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR…

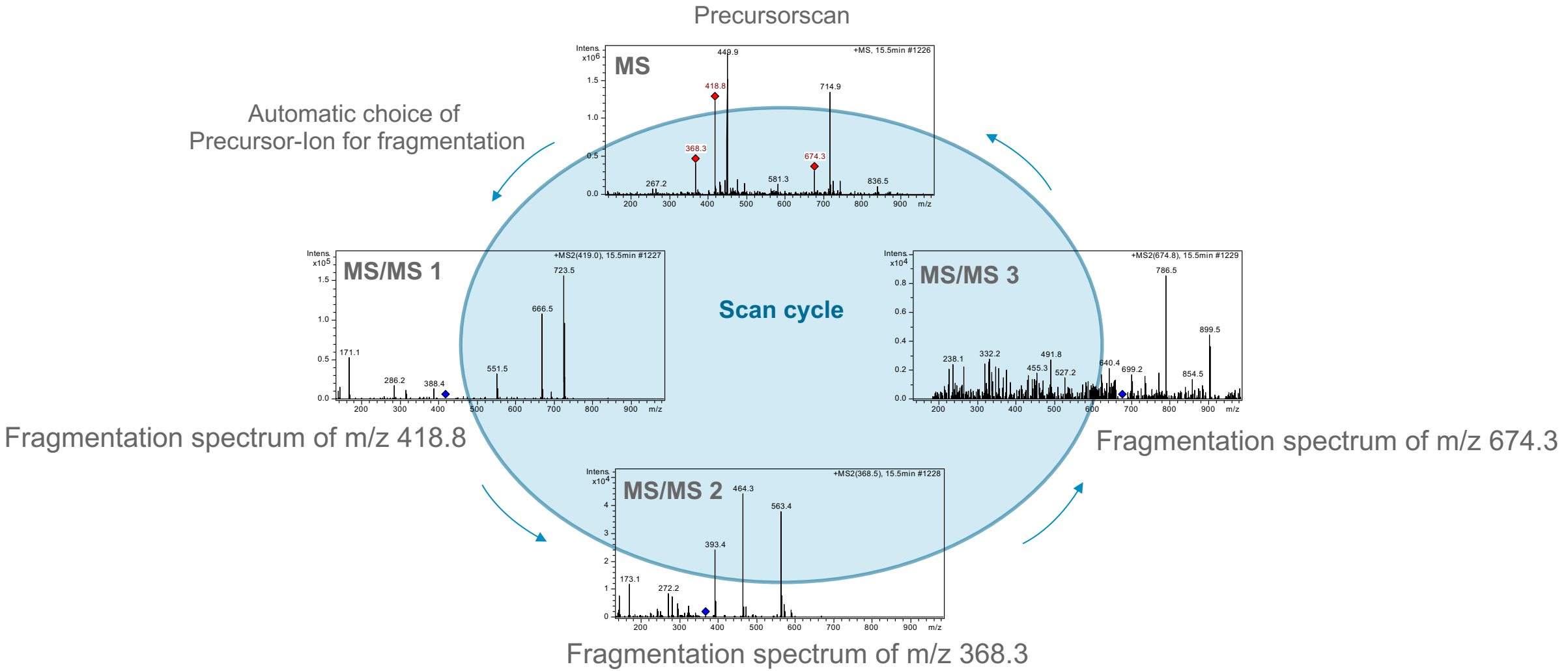
IYPTNGYTR YADSVKGR FTISADTSK NTAYLQMNSLR AEDTAVYYCSR

- When analyzing peptides by LC-MS
  - In silico digestion via e.g. Skyline
  - Information dependent acquisition (IDA)

http://resources.qiagenbioinformatics.com/manuals/clcgenomicsworkbench/650/BE_Proteolytic_cleavage.html

Analytical strategy

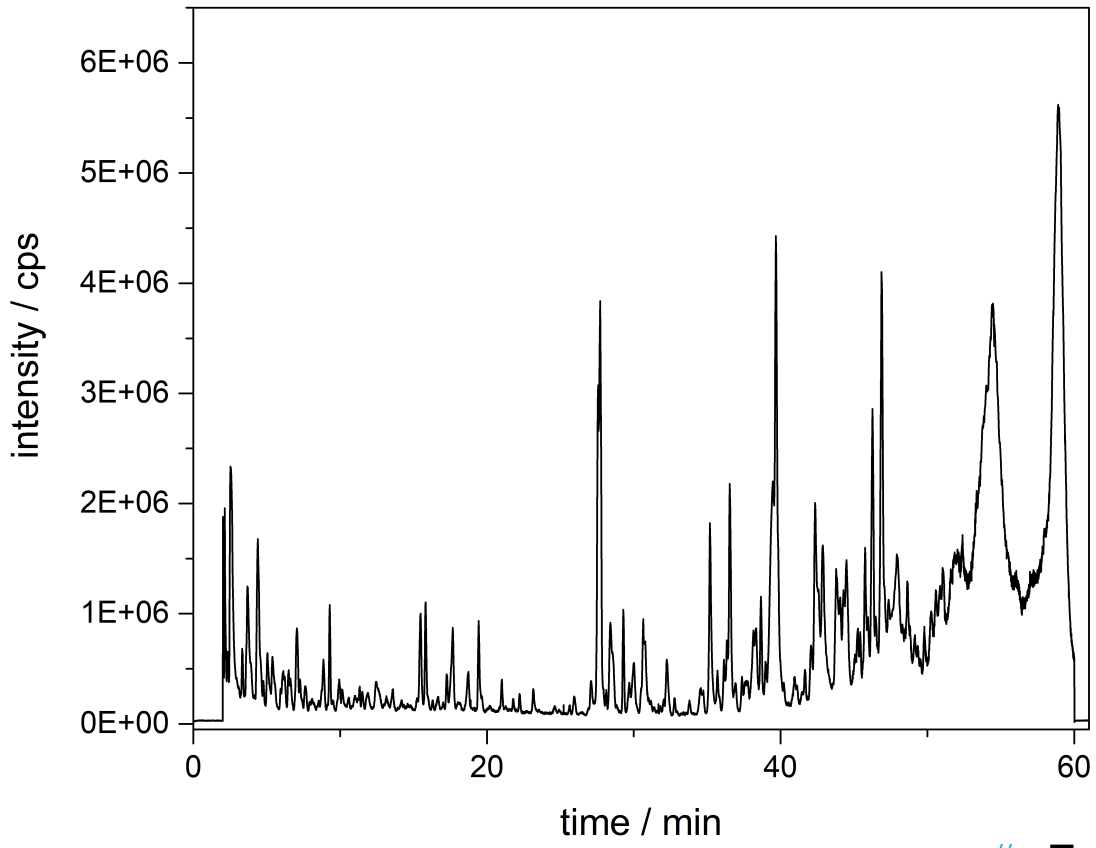
Principle of Information Dependant Acquisition (IDA)

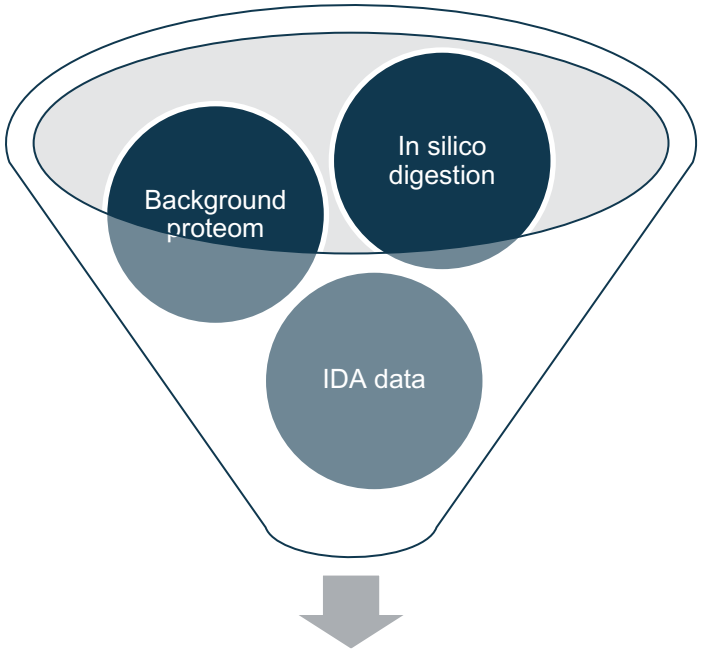


Analytical strategy

TIC from IDA run

// Tryptic digest from spiked monkey plasma



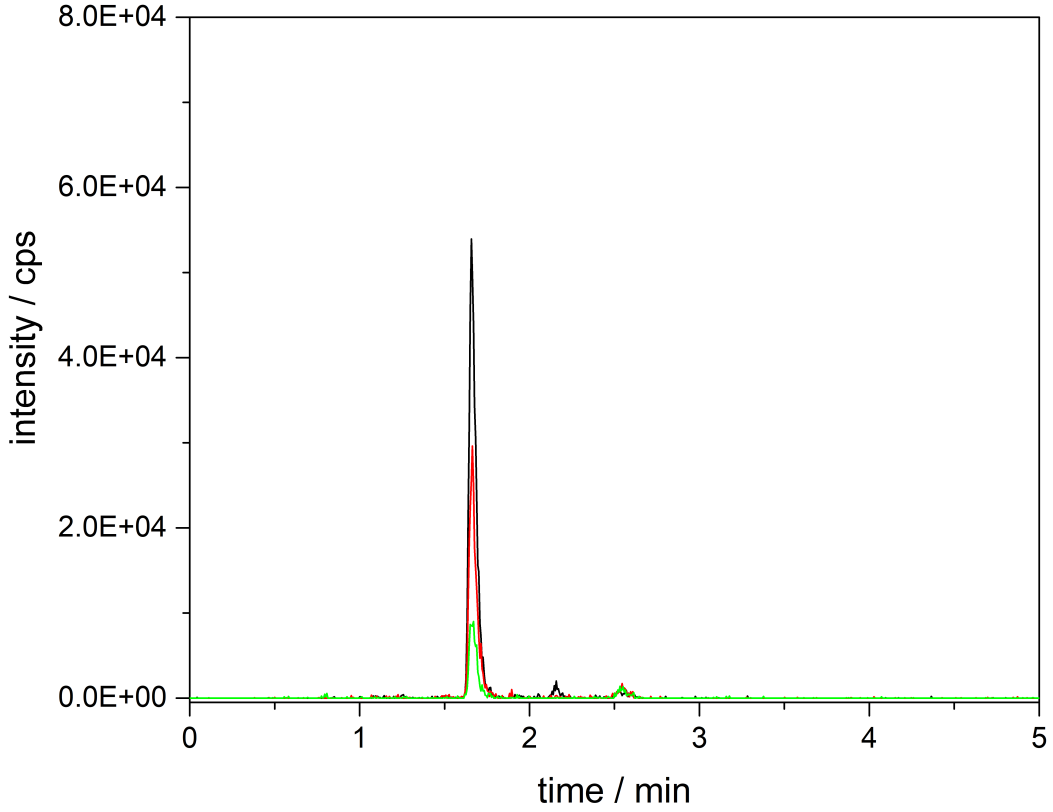


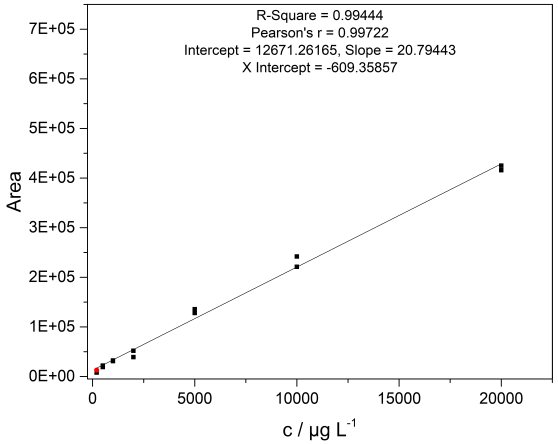
MRM candidates

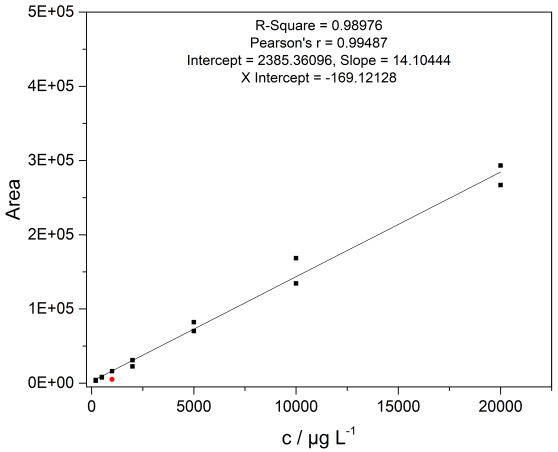
// Further selectivity increase by using immunoprecipitation

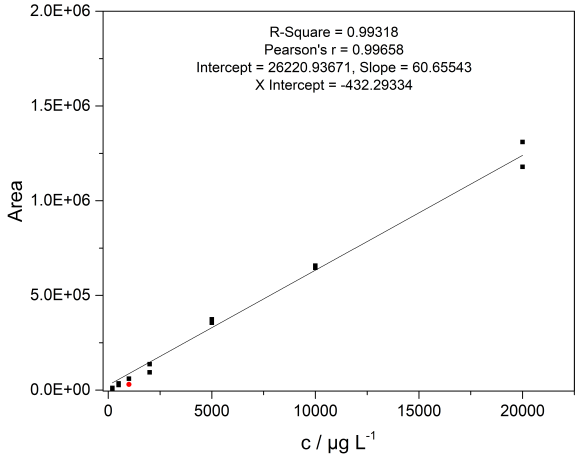
Analytical strategy

Resulting LC-MS/MS chromatogram









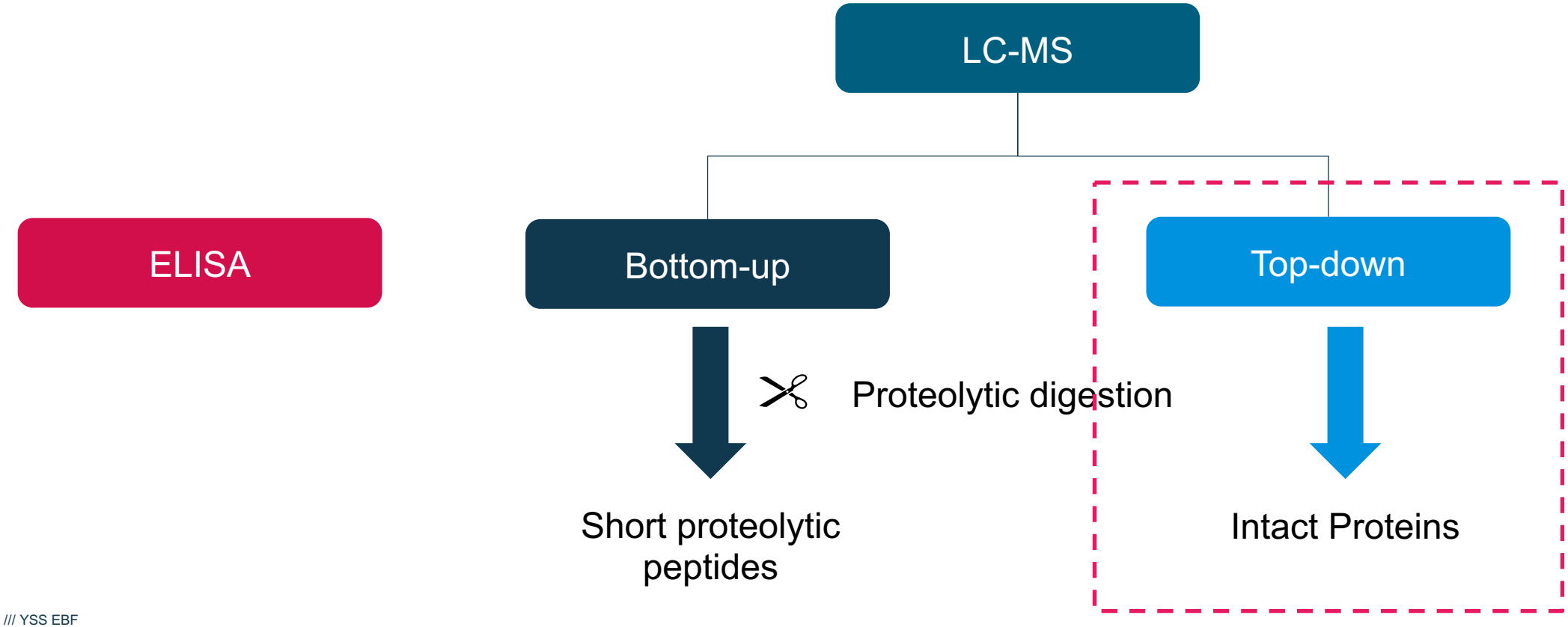
// Calibration using a working range of 200 – 20000 µg/L

// At least 2 different peptides with ≥ 2 mass transitions

Analytical strategy

// Different ways to analyze TTC's can be applied

// Depends on analytical question / challenges



Analytical strategy

Analysis of intact biomolecules

Challenges

// Secondary interactions between the analyte and stationary phase when using conventional phase material

// Broad charge state distribution (up to more than 50 charges)

Possible solutions

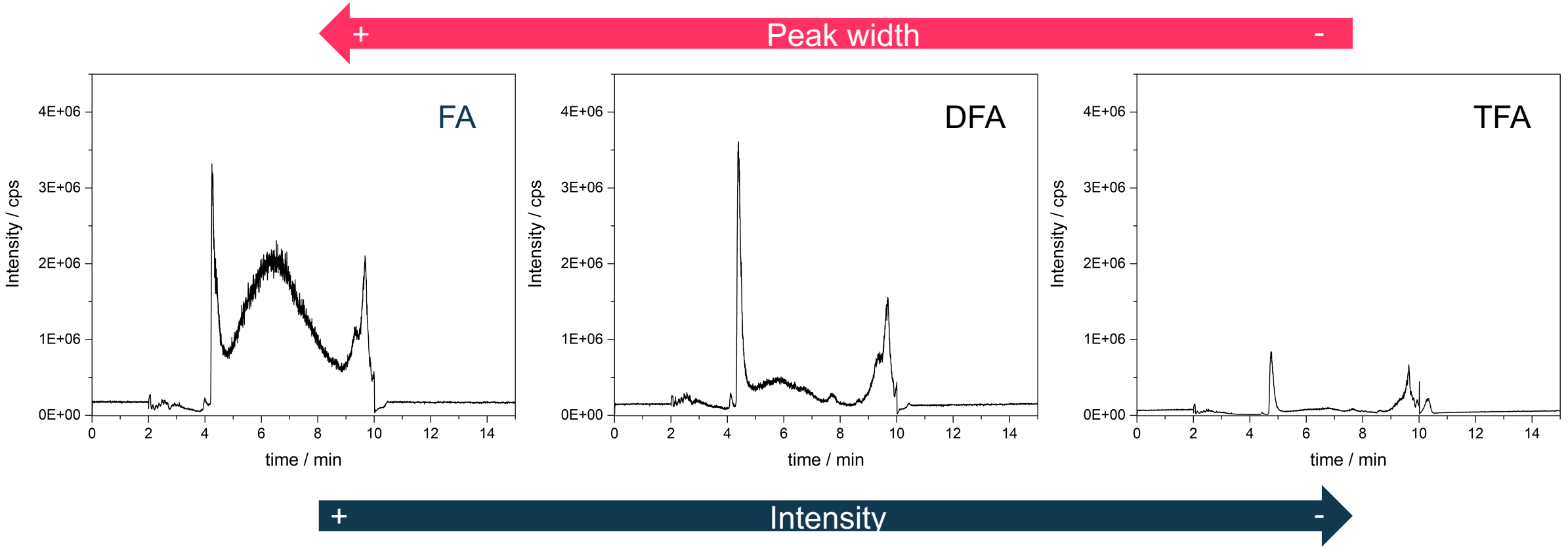
// State-of-the-art biocompatible chromatographic columns

// Use of trifluoroacetic acid (TFA)

// Elevated column temperatures up to 90°C

Analytical strategy

Separation on a C4 column

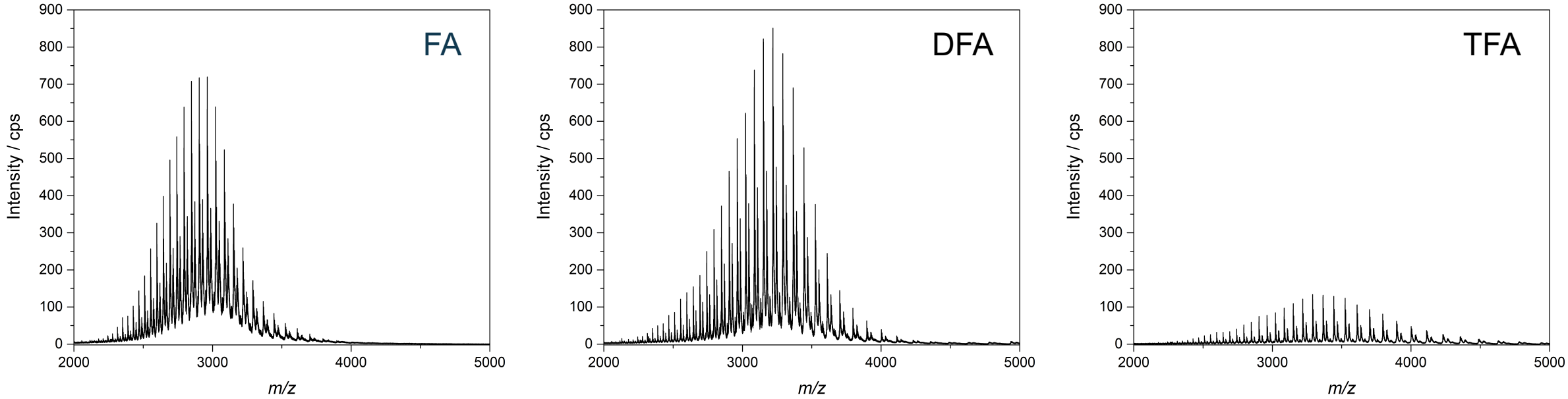


DFA = difluoroacetic acid

Analytical strategy

Influence on charge state distribution

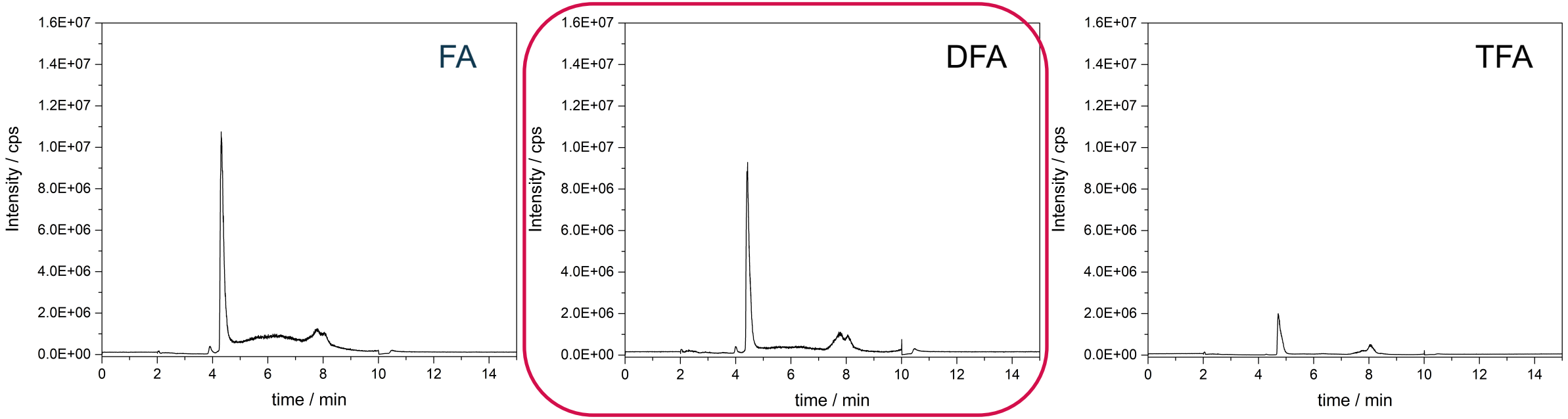
- Broader charge state distribution +



- Maximum Intensity shifts to higher masses +

Analytical strategy

Separation on a Polyphenyl column



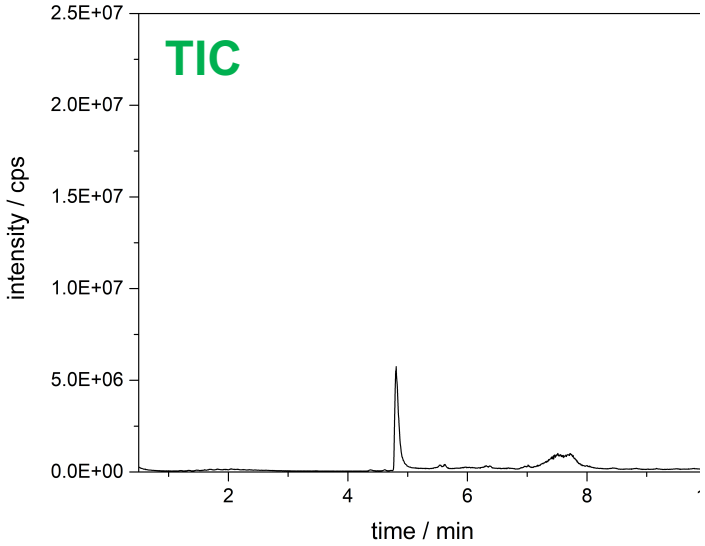
Analytical strategy

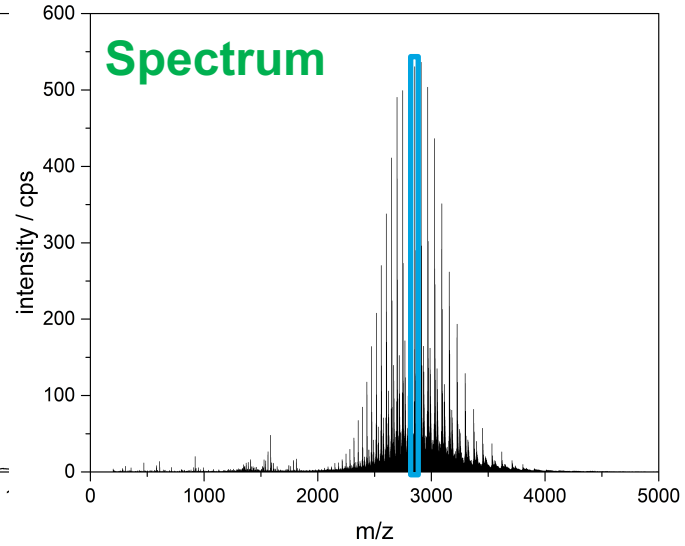
Top Down

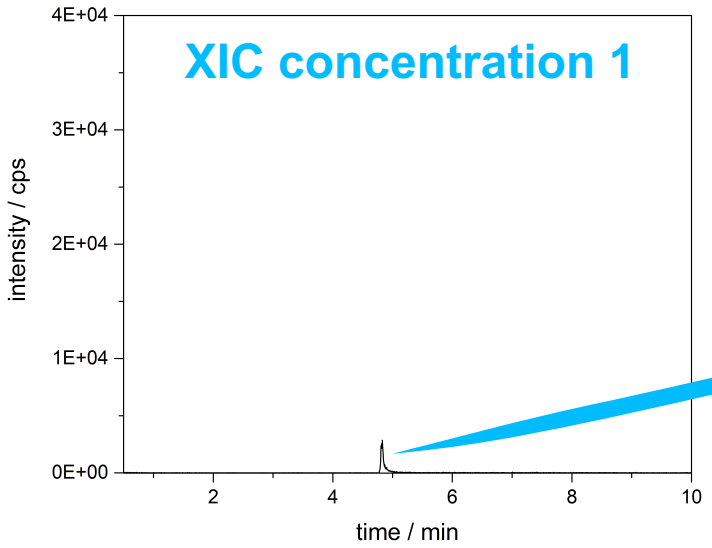
// Top-down quantification

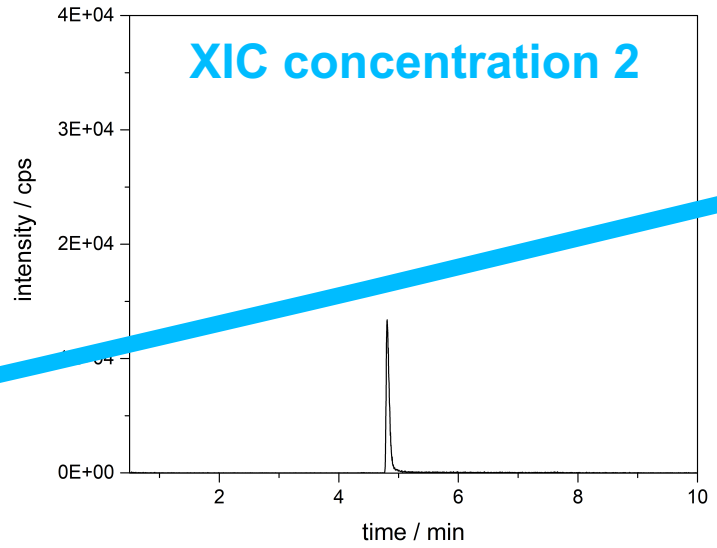
// Check spectrum for a suitable mass

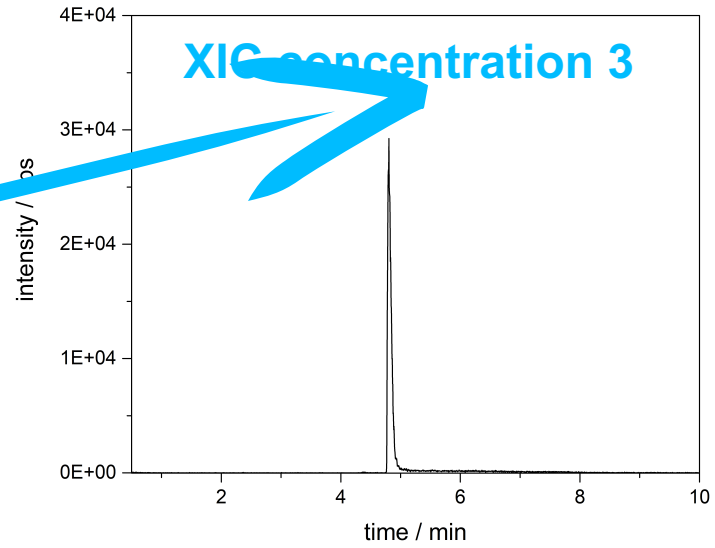
// Use of extracted ion chromatogram (XIC)





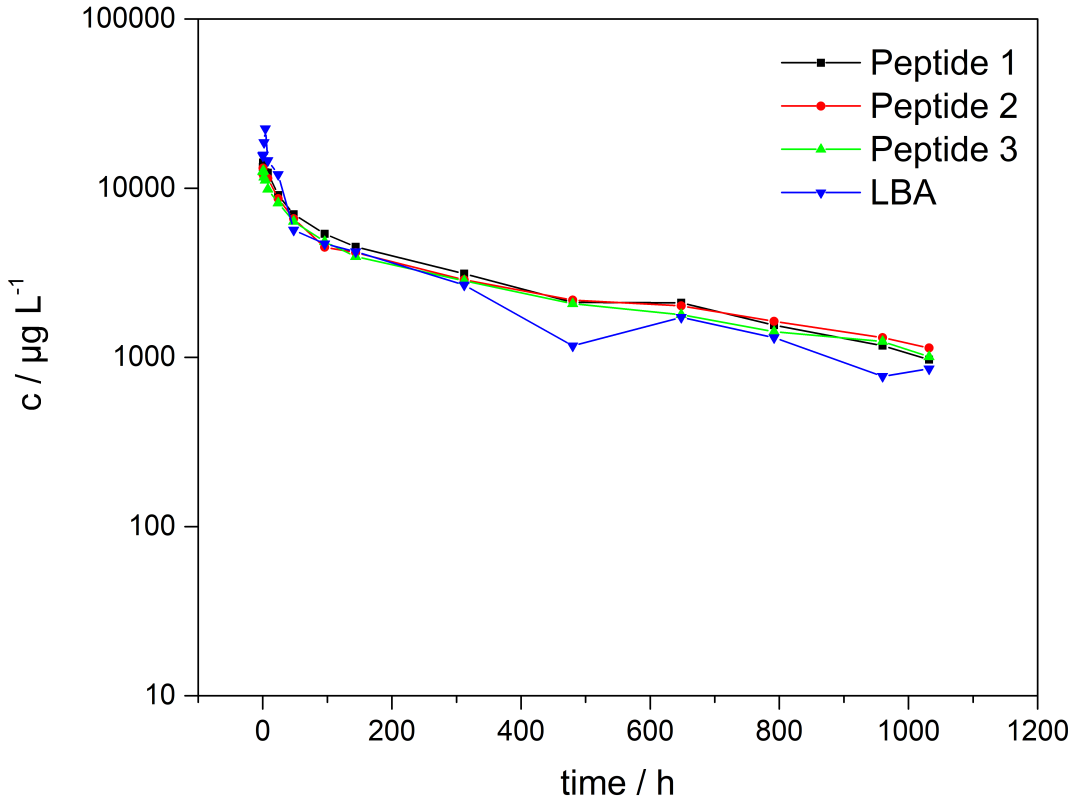


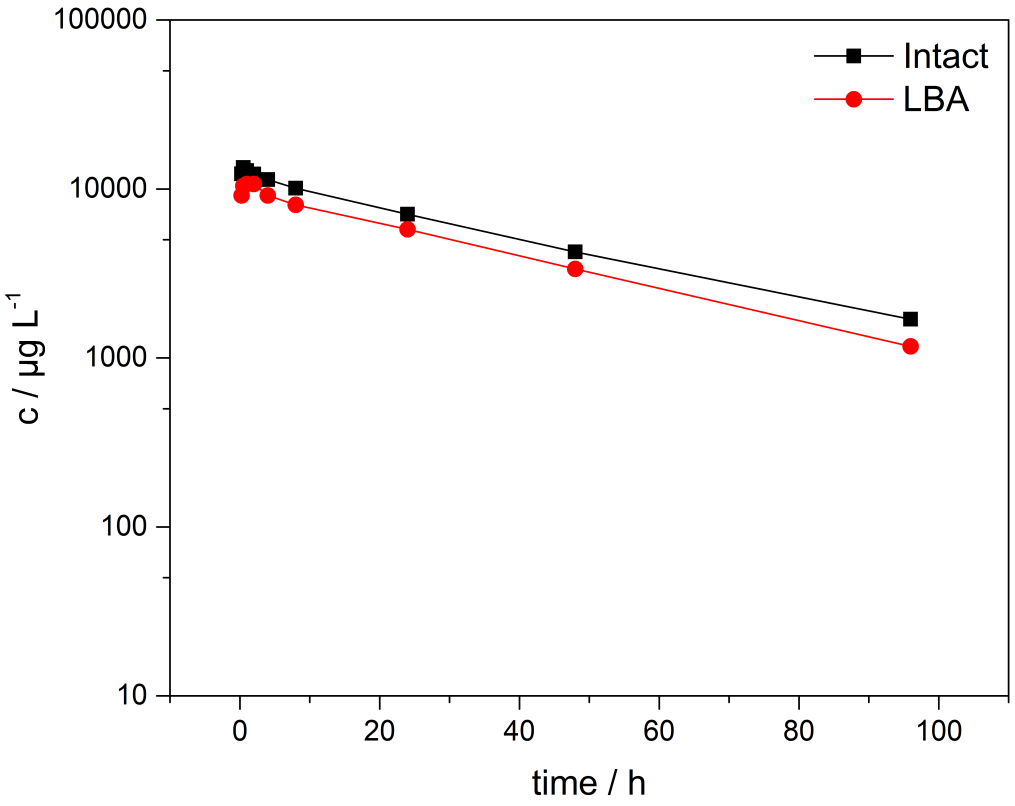




Analytical Strategy

Comparison ELISA, Bottom-Up and Top-Down approach



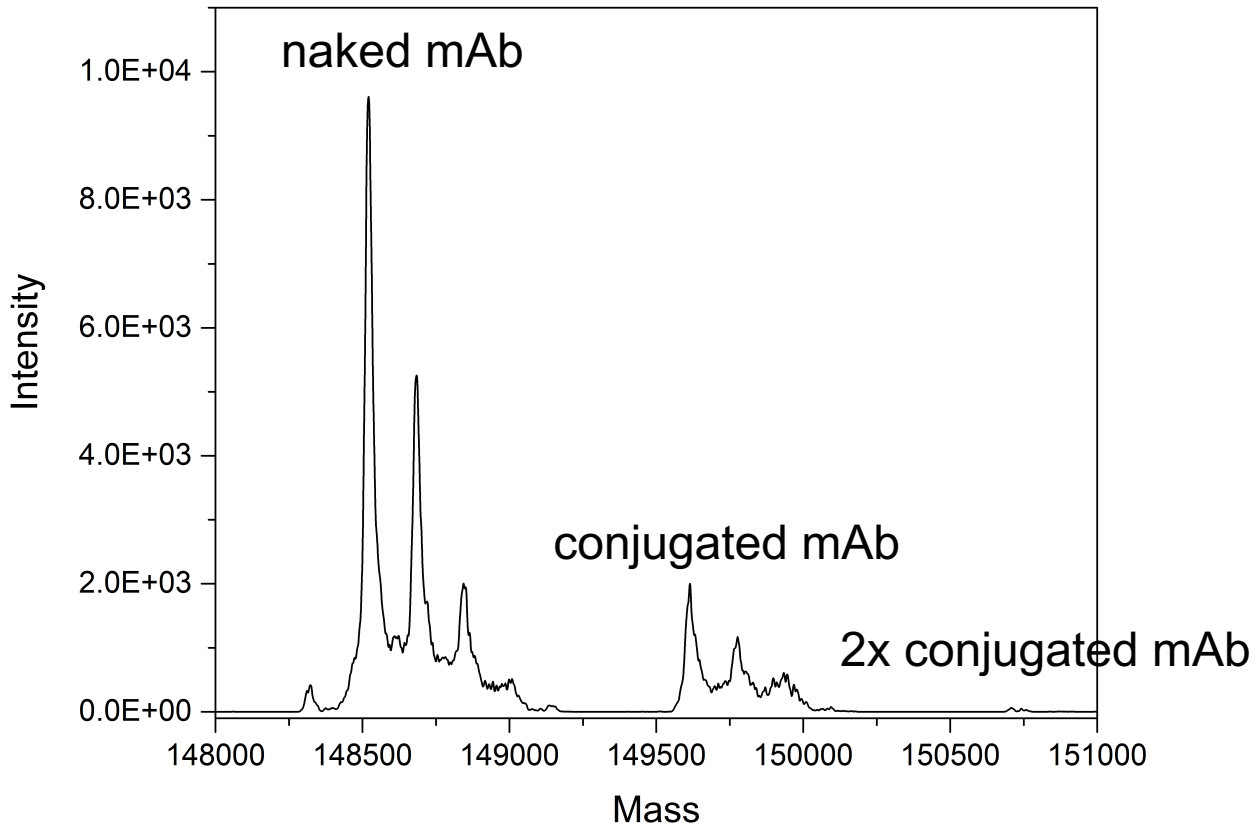


Analytical Strategy

Benefits of Top-down approach – Why using HRMS?

// Deconvoluted mass of „naked“ and „conjugated“ antibody

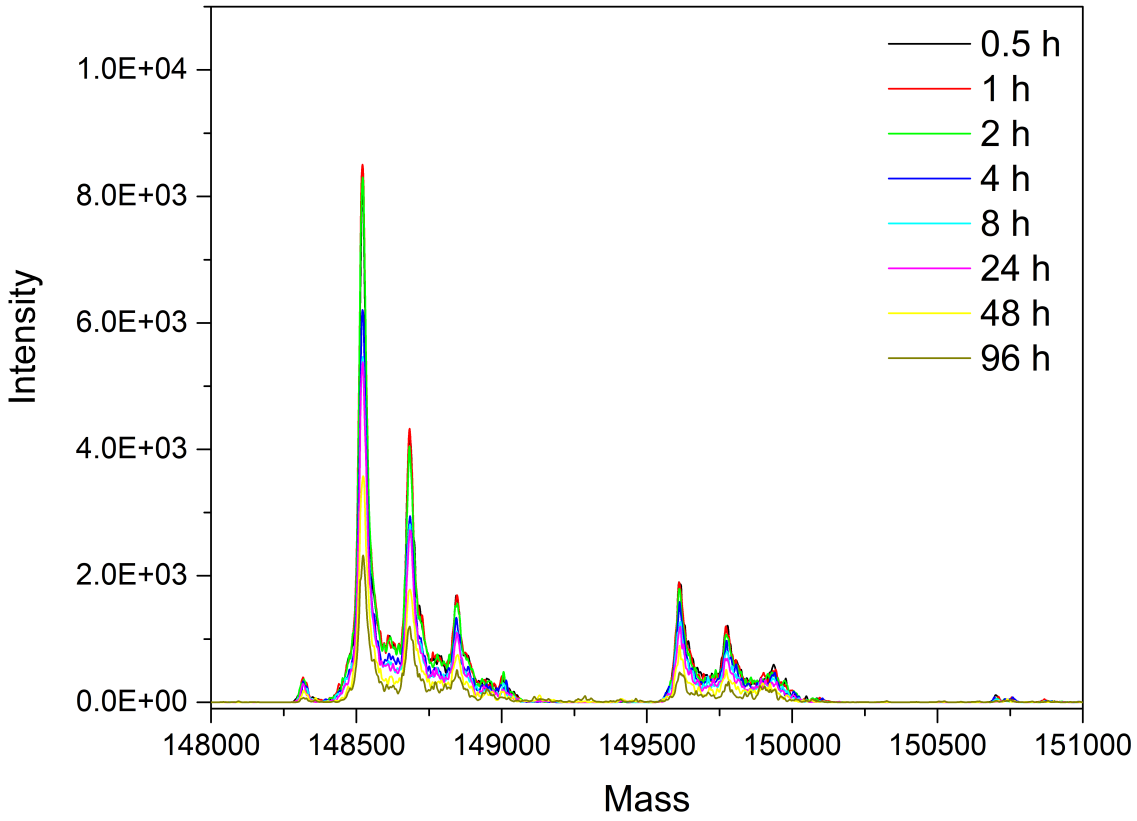
// Selectivity advantages

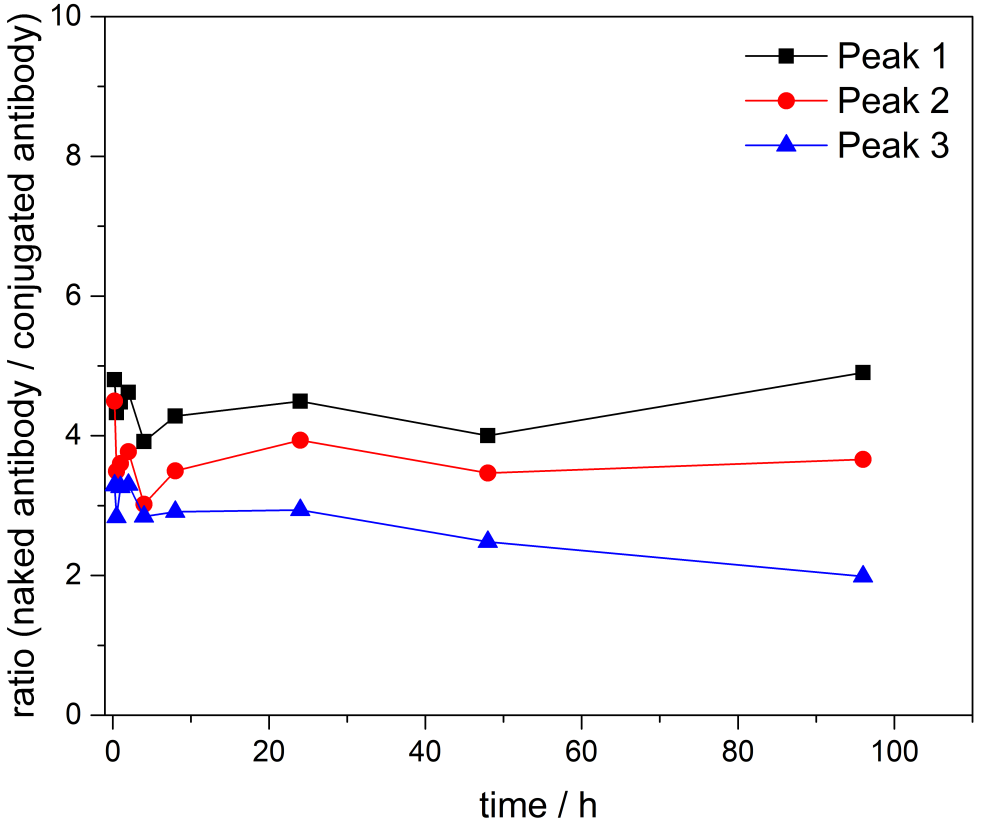


Analytical Strategy

Benefits of Top-down approach – Why using HRMS?

// Deconvoluted mass of naked and conjugated antibody over time (0.5h – 96h after dosing)





Conclusion

Results and future steps

- **Comparable results using all techniques!**
- Each technique has its advantages
- Depends on analytical question / strategy which technique should be used
- General advantages of mass spectrometry compared to LBA
  - Selectivity!
  - Mass information is available at any time

- Possible future optimization for Top-Down approach
  - Use of deconvoluted signals for data analysis instead of using extracted ion chromatogram (XIC)
  - Use of miniaturized separation technology like nano- and/or micro-LC
    - In-house evaluation is ongoing

Thank you!

Bye