

Vorschau

DeNovo

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1. Complementary fragmentation (15 Punkte)

Compare ETD and CID in their usage, working mechanisms and resulting spectra. Where and why does one benefit from complementary methods? Look for at least two other fragmentation methods in current research and report their preferred ion types.

2. (Extended) spectrum graph (20 Punkte)

Construct the spectrum graph as well as the extended spectrum graph for the following list of fragment ion m/z values.

No weighting of the nodes/edges is needed since no intensities are given. Do not forget to add the one letter AA codes to the parts where they belong.

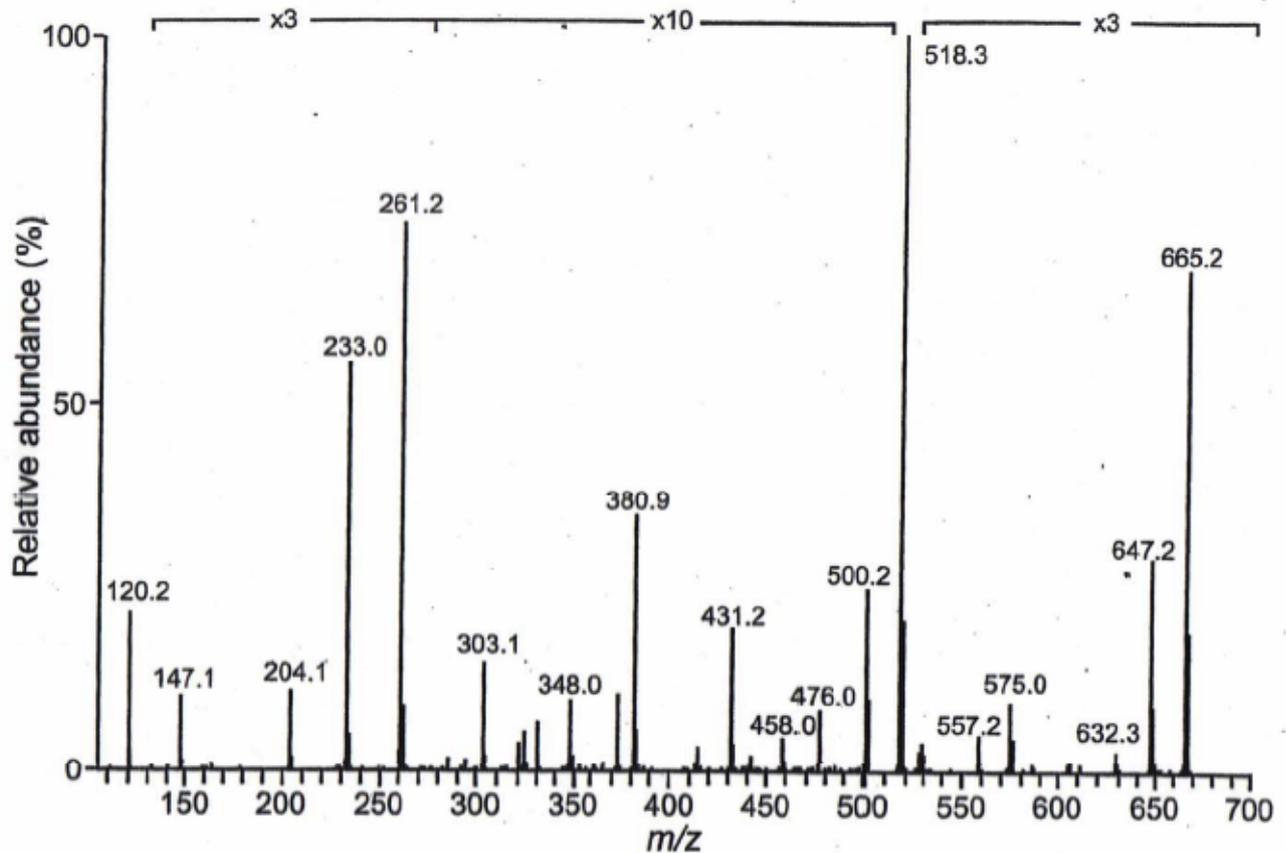
Spectrum: 106.05046, 114.09193, 177.08758, 245.13242, 308.12806, 316.16953, 403.20156, 421.21213.

Datei hochladen

Maximal erlaubte Upload-Größe: 550,0 MB

Erlaubte Dateierweiterungen: jpg,png,pdf,svg

3. Manual sequencing of MS2 spectrum (50 Punkte)



MS2 spectrum of a doubly charged precursor at 389.8 Th.

Sequence the spectrum shown above by hand. Besides LU8A/B you can use [this tutorial](#) as a second source of information. We will guide you through the procedure by splitting it into smaller questions. For sets of amino acids always report them in alphabetical order. Use X for I or L (I/L) and only consider annotated peaks.

Compute the protonated molecular weight for the peptide $[M+H]^+$:

How many amino acids are most likely part of its sequence (use average amino acid mass):

Look for immonium ions in the range of m/z 30 - 159. List all amino acids that correspond to your findings (one-letter-code, comma-separated). You do not have to look for modifications in this example.

Given an ion of one series, which (positive) mass window do you have to consider, when looking for the next one in the series (Hint: Consider the possible masses of amino acids):

From Da to Da.

How many peaks do you have to consider for the largest y-type ion (using the above window and the precursor mass):

What are the possible amino acids corresponding to the differences between the precursor and those peaks. List all in alphabetical order (one-letter-code, comma-separated):

The sample was digested with trypsin. Can you find hints for the terminal amino acids in the low mass region? What amino acid corresponds to the smallest y-ion?

Try to confirm your guess by looking for the largest b-ion. Where do you have to look for it (report m/z value)? Use the precursor mass and the mass of the terminal amino acid you suggested.

Try to find the second largest y-ion. Look for mass differences between peaks in the reasonable mass window for every peak that you found to be the largest y-ion. Keep in mind, that mass decreases of 17/18 Da often correspond to neutral losses and ion ladders including them become less likely. If you are unsure, just test all combinations.

You might have some possibilities for the two amino acids on the N-terminus now. What is the most likely terminus considering the immonium ions you have found and the evidence from the (b₂)-ion (add up their masses and look for a matching peak)? No commas and mind the ordering.

Continue going through the spectrum. Look in the mass window around the peaks already identified and try to validate with the complementary ion set. Bookkeep all assigned peaks so you don't assign them twice.

If you encounter the mass difference for the almost isobaric amino acids K and Q in the rest of the spectrum (internal part), which one is more likely given all the information provided up to here?

For the last amino acid you can double check by summing up masses. They have to add up to the precursor mass. Mind the termini and the proton.

In the end, give the complete sequence:

4. Precursor mass correction (15 Punkte)

You are given the following (ideal nominal) MS/MS spectrum for a doubly charged peptide: $S = \{227 (b_2), 276 (y_2), 330 (b_3), 375 (y_3), 490 (y_4), 544 (b_5)\}$.

Find the precursor mass with the algorithm in LU8A.