- How can we determine the amino acid **composition** of a polypeptide?

AGKLYMFPARGVA

- How can we determine its amino acid sequence?

Homework I:

Consider digestion with trypsin: will this be enough?

What else could you combine it with?

Homework Problem II

Determine the amino acid sequence of a peptide given the following information and
and write down the sequence using 1-letter amino acid code:

a) Complete hydrolysis by 6 M HCl at 110 °C followed by amino acid analysis indicated the presence of Gly, Leu, Phe and Tyr in a 2:1:1:1 molar ratio.

b) Treatment of the peptide with 1-fluoro-2,4-dinitrobenzene followed by complete hydrolysis and chromatography indicated the presence of the 2,4-dinitrophenyl derivative of tyrosine.
No free tyrosine could be found.

c) Complete digestion of the peptide with pepsin* followed by chromatography yielded a dipeptide containing Phe and Leu, plus a tripeptide containing Tyr and Gly in a 1:2 ratio.

* Note that pepsin cleaves polypeptide sequences at the N-termini of aromatic residues

Homework Problem III

1. Determine the amino acid sequence of a peptide given the following information:

a) Treatment of the peptide with 1-fluoro-2,4-dinitrobenzene followed by complete hydrolysis and chromatography indicated the presence of the 2,4-dinitrophenyl derivative of glycine.

b) Treatment of the peptide with trypsin* yielded the following three fragments:

YLDR
GSAK
WGSM

2. Would digestion with pepsin instead of the treatment with 1-fluoro-2,4-dinitrobenzene allow determination of the sequence of the peptide?

* Note that trypsin cleaves polypeptide sequences at the C-termini of K and R

Homework Problem IV

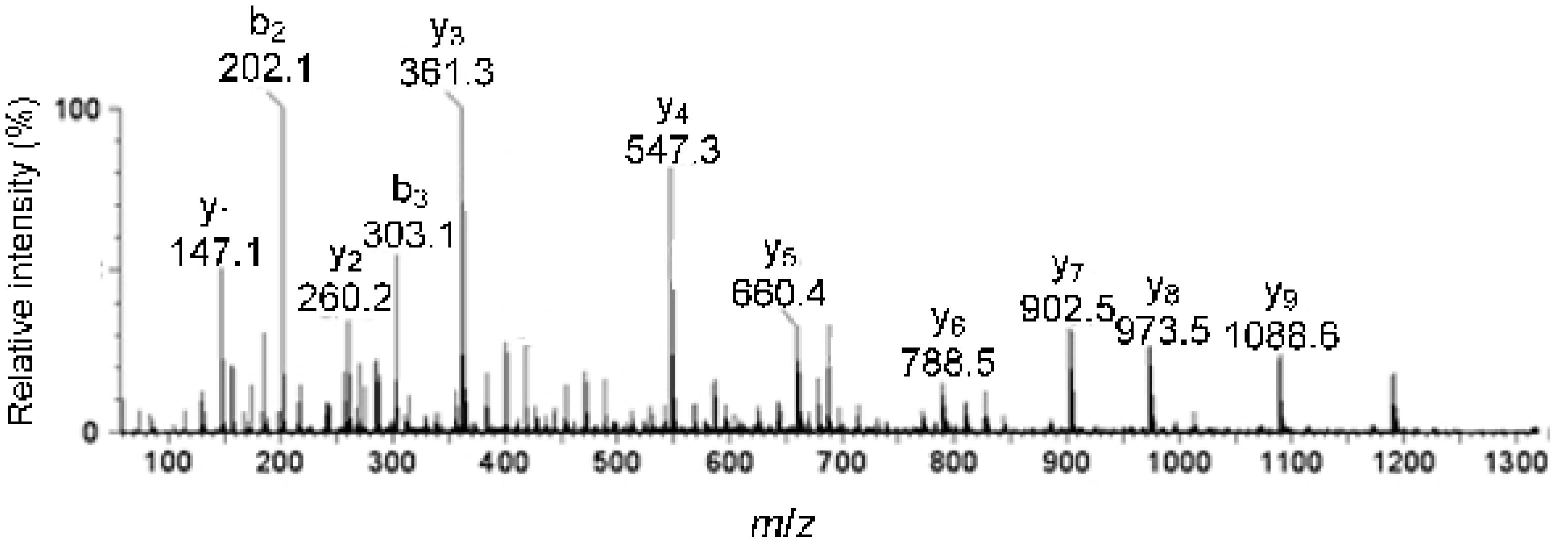
The Electrospray Ionization Mass Spectrometry (ESI-MS) of a protein gives a spectrum with the following sequence of outstanding peaks:

m/z: 888.1 977.0 1085.5 1221.1 1395.2 1627.7

Calculate the molecular mass of the protein using 3-4 pairs of peaks? How different are the calculated masses? Average the obtained results to get a final molecular mass.

Homework Problem V

The figure below is a mass spectrum from an experiment in which a peptide was sequenced by **tandem MS**. The *m/z* values for several "b" and "y" ions are indicated on the spectrum. Use the *m/z* values given on the spectrum, and the amino acid residue masses in the Table on next slide, to determine the amino acid sequence of the peptide.



Problem V (cont.)

a.a.	Mass (Da)
Gly	57.1
Ala	71.1
Ser	87.1
Pro	97.1
Val	99.1
Thr	101.1
Cys	103.1
Ile	113.2
Leu	113.2
Asn	114.1
Asp	115.1
Gln	128.1
Lys	128.2
Glu	129.1
Met	131.2
His	137.1
Phe	147.2
Arg	156.2
Tyr	163.2
Trp	186.2