

Protein sequencing

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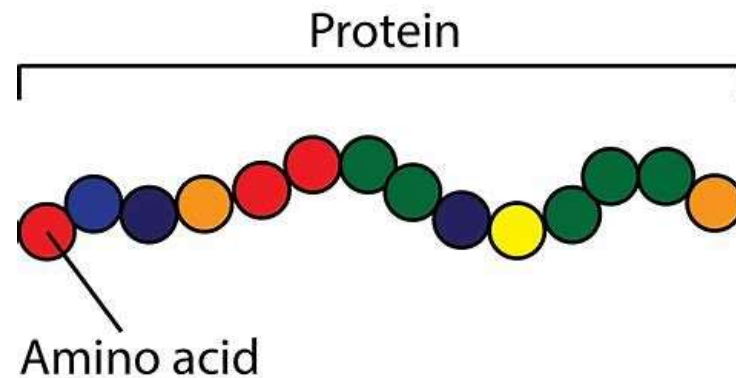
Protein sequencing

- Determining amino acid sequence all or part of protein
- Partial sequencing of protein gives sufficient information
- Major way of protein sequencing

N-terminal, C-terminal amino acid analysis

Edman degradation

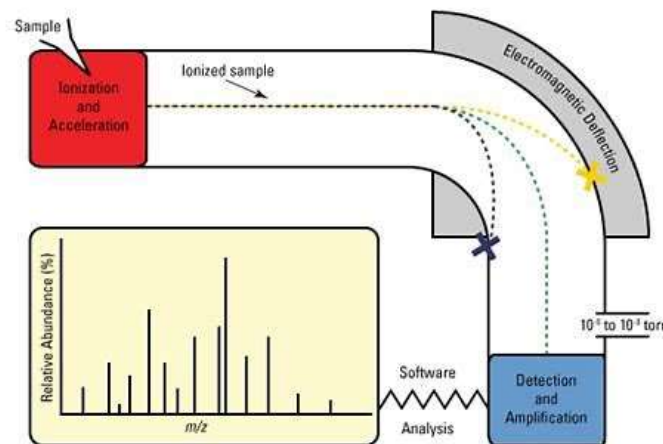
mass spectrometry



Holehouse, A.S., Garai, K., Lyle, N., Vitalis, A., and Pappu, R.V. (2015)

Mass spectrometry

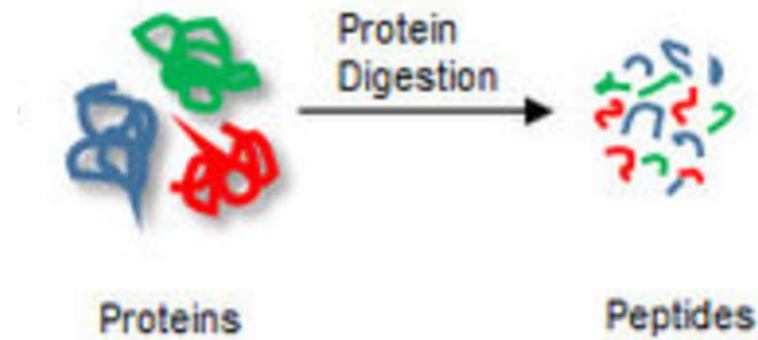
- Mass of single or multiple protein determination and characterization
- Basic principle is ionization, separation by magnetic or electric field, detection, analysis



<https://www.thermofisher.com/jp/ja/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-mass-spectrometry.html#/legacy=www.piercenet.com>

Method1: digestion

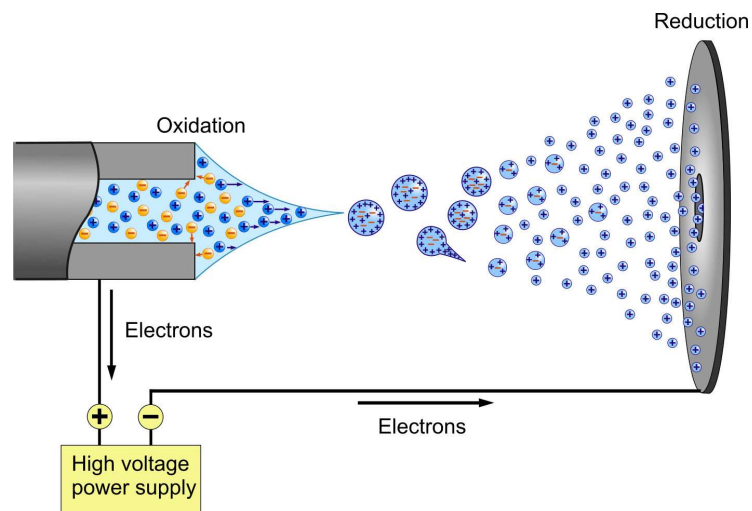
- Digest protein to peptides with trypsin (digestion readily available, specific), ideal size for analysis



Method2: ionization

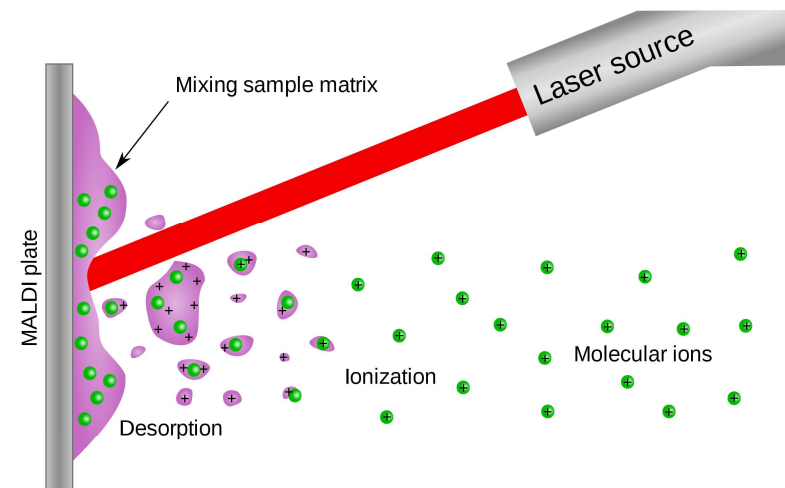
- Ionize and dissociate by electric or magnetic effect

electrospray ionization



<https://commons.wikimedia.org/wiki/File:Maldi.svg>

matrix assisted ionization



<https://commons.wikimedia.org/wiki/File:Maldi.svg>

Method3: mass separation

Time of flight



Ions are accelerated by electric field and the velocity of ion depends on the mass-charged ratio

Method3: mass separation

Fourier-transform ion cyclotron resonance



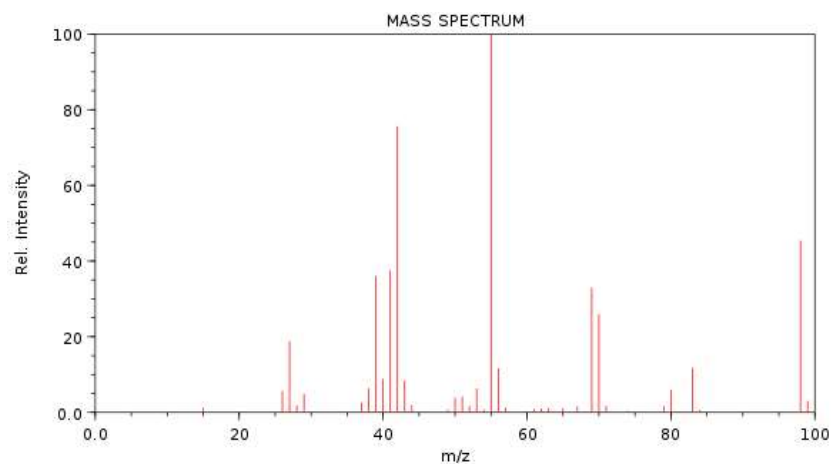
$$f = \frac{qB}{2\pi m}$$

Separation mass-to-charge ratio (m/z) of ions based on the cyclotron frequency of the ions in a fixed magnetic field

<https://commons.wikimedia.org/wiki/File:LQ-FTICR.jpg>

Method4: analysis

- Mass spectrum
- Collate with data base from the result of mass spectrometry



スライド 8

0名1 先生作成のため出典不要
OCW 名古屋大学, 12/13/2019

Advantages

- Useful for identification of protein when used in conjunction with other techniques such as NMR and IR spectroscopy
- Effective for localize protein to the various organelles

Draw backs

- Can not distinguish leucine and isoleucine because of same mass
- Impossible to identify between isomers of a compound having the same charge-to-mass (m/z) ratio