

Protein characterization

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The Nobel Prize in Chemistry 2020



埃玛纽埃尔·卡彭蒂耶
Emmanulle Charpentier

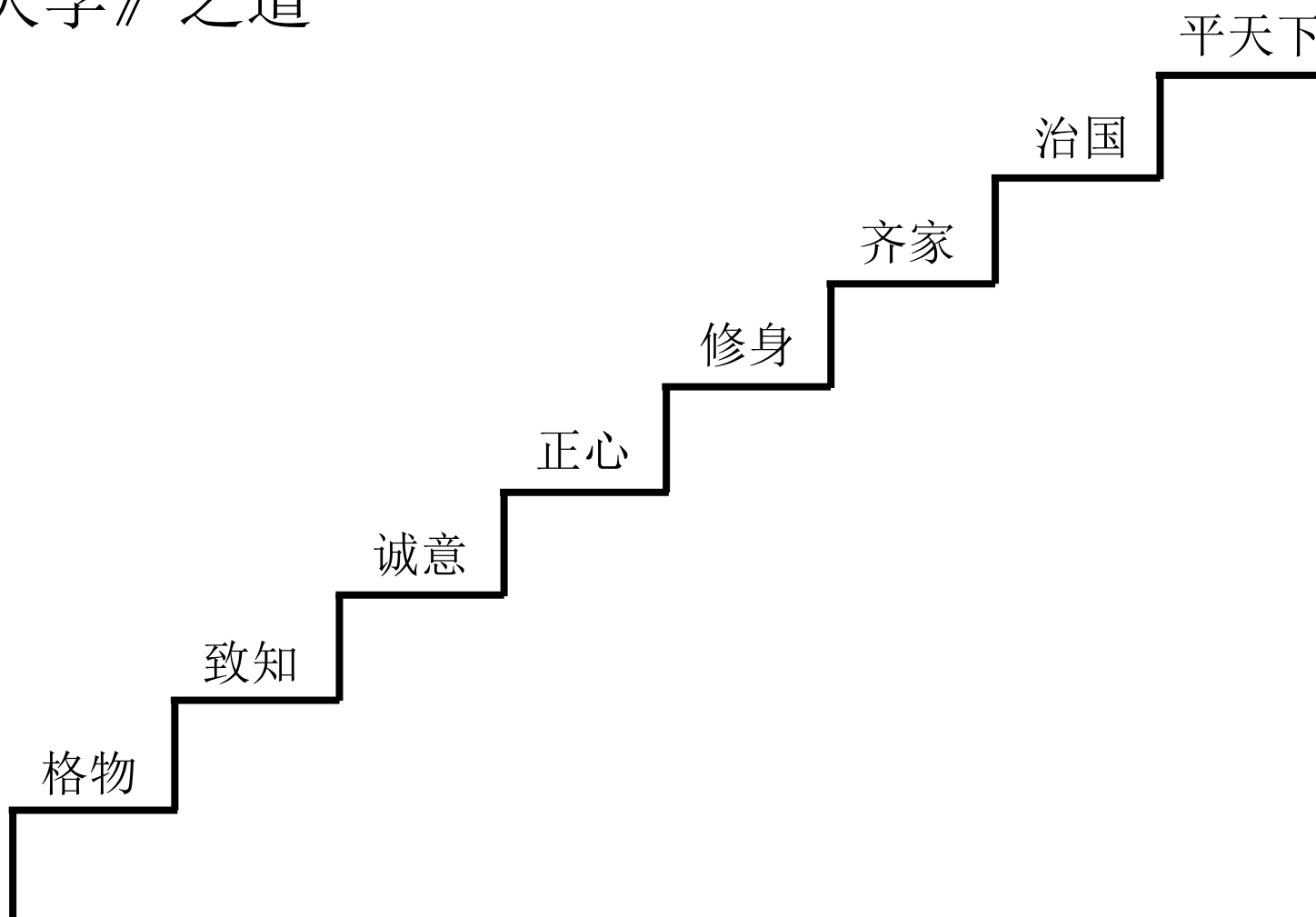


詹妮弗·杜德娜
Jennifer Doudna

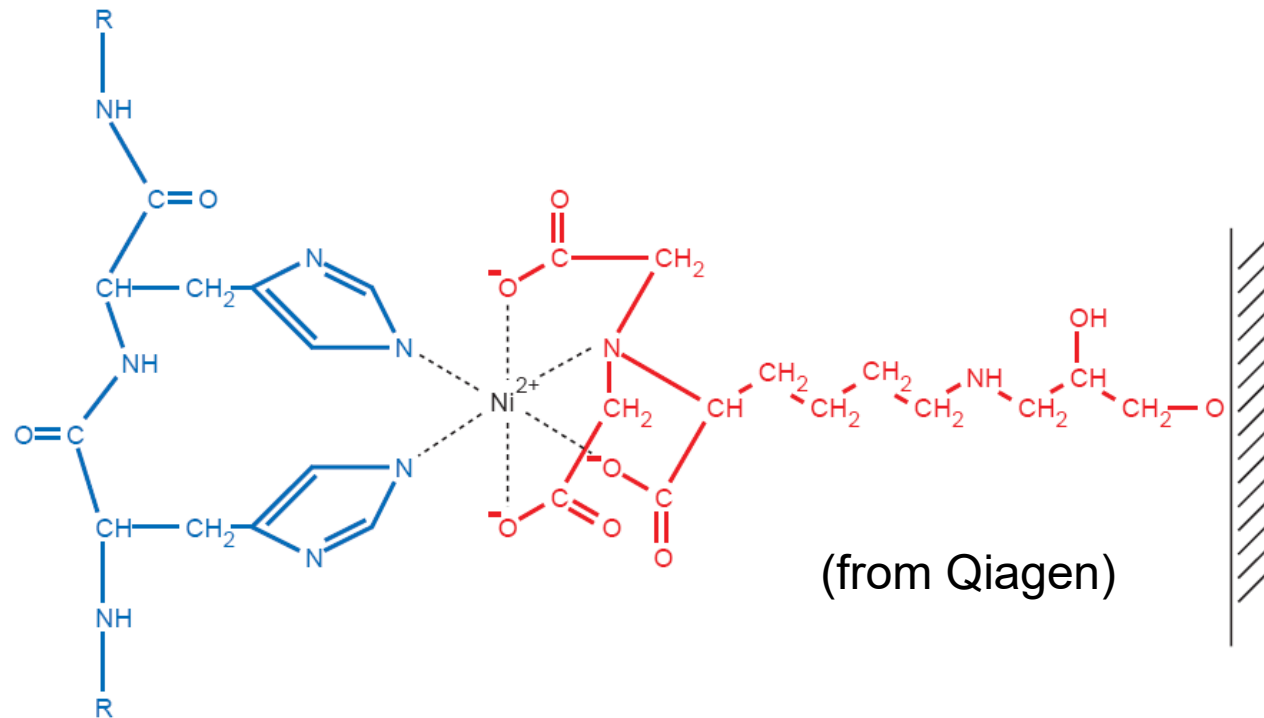
“for the development of a method for genome editing”



《大学》之道

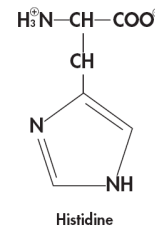
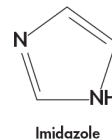


Immobilized metal chelating chromatography (IMAC) (金属螯合层析)



Popularly used for (His)₆-tagged proteins;

Imidazole to elute.



protein purification techniques

➤ Based on physical and chemical characteristics:

size gel filtration chromatography

charge ion exchange chromatography

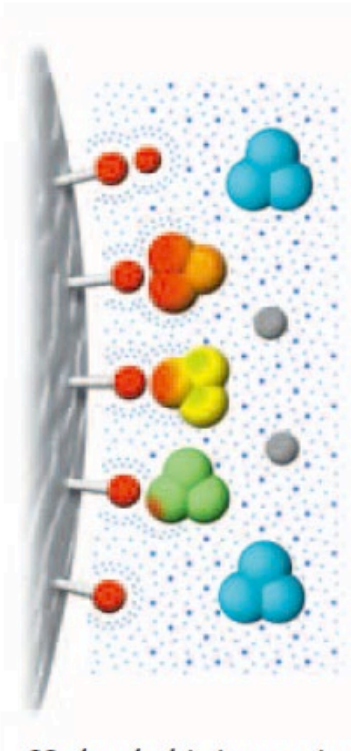
hydrophobicity hydrophobic interaction chromatography

➤ Based on biological characteristics:

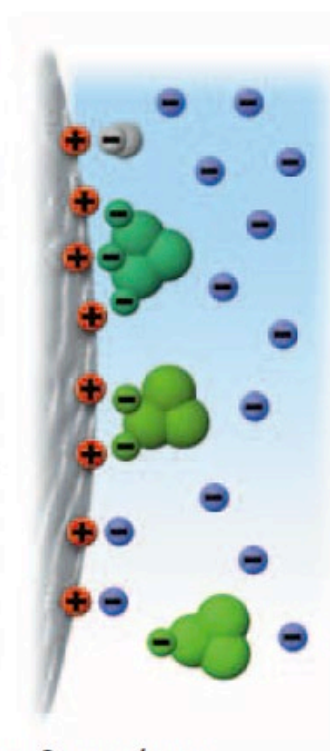
affinity chromatography



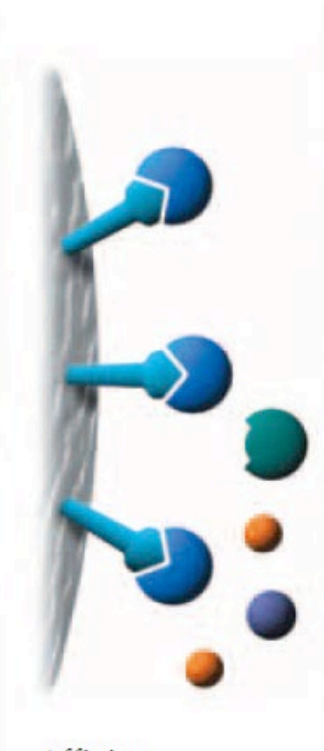
Gel filtration



Hydrophobic interaction



Ion exchange



Affinity

Change
condition
to elute

No

Yes

Yes

Yes

Sample
volume
limited

Yes

No

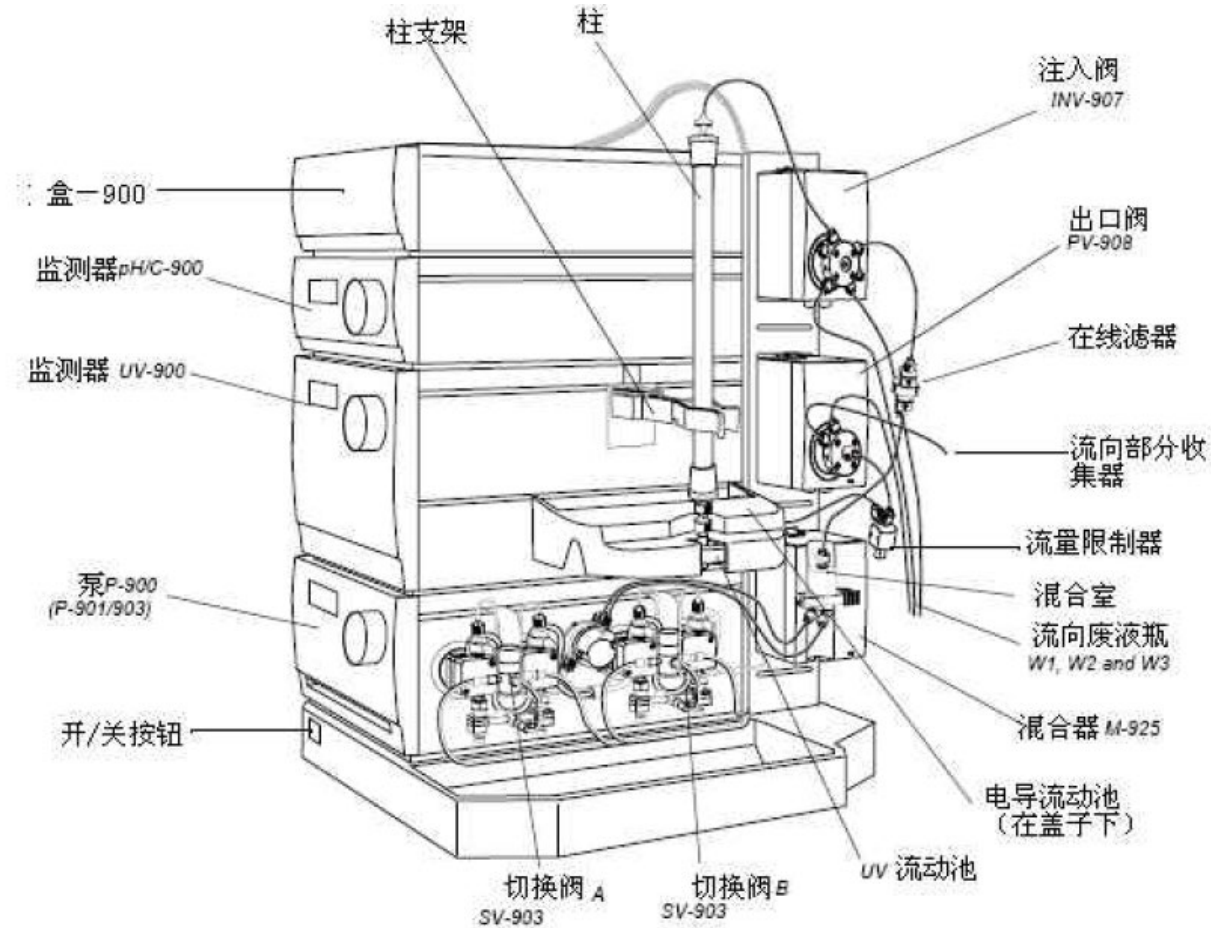
No

No

AKTA purifier 10 from GE Healthcare

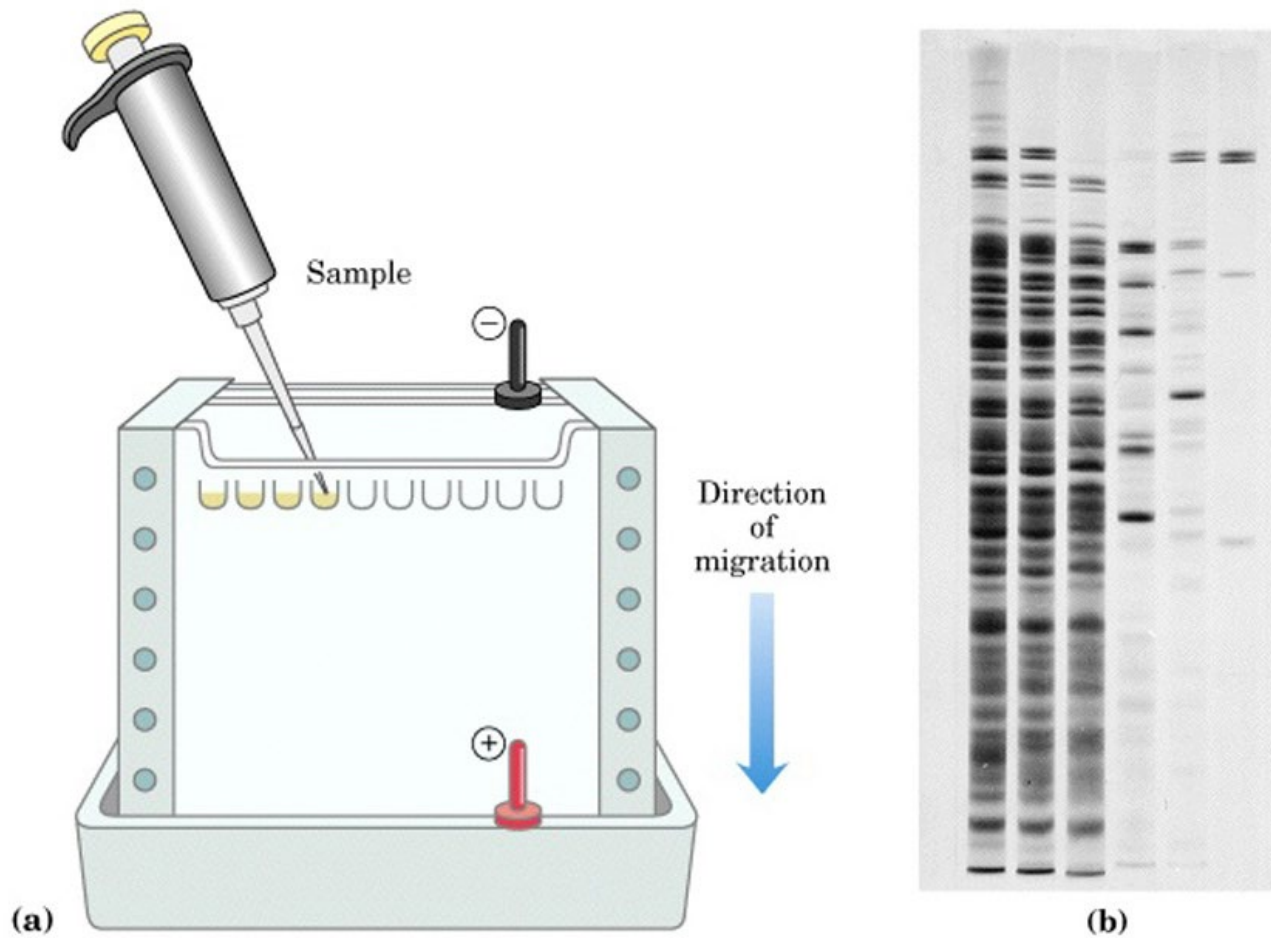


AKTA purifier 10 from GE Healthcare



The only objective:

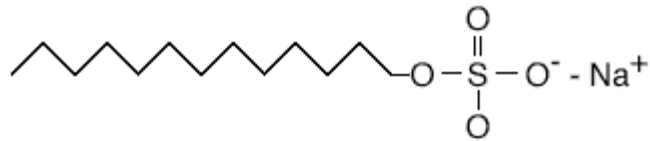
Gel electrophoresis 凝胶电泳



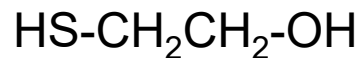
SDS-PAGE

(Sodium Dodecyl Sulfate 十二烷基磺酸钠)

Sodium dodecyl sulfate,
 $\text{CH}_3(\text{CH}_2)_{10}\text{CH}_2\text{-SO}_4^-, \text{Na}^+$

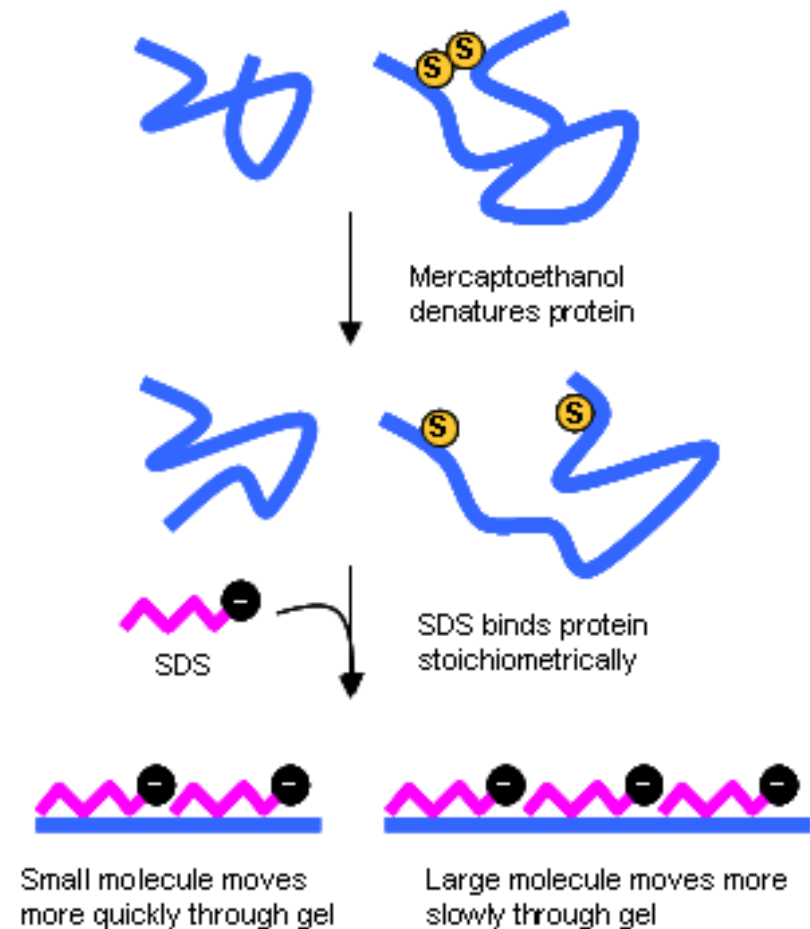
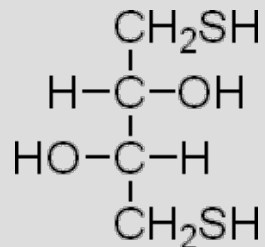


β -mercaptoethanol



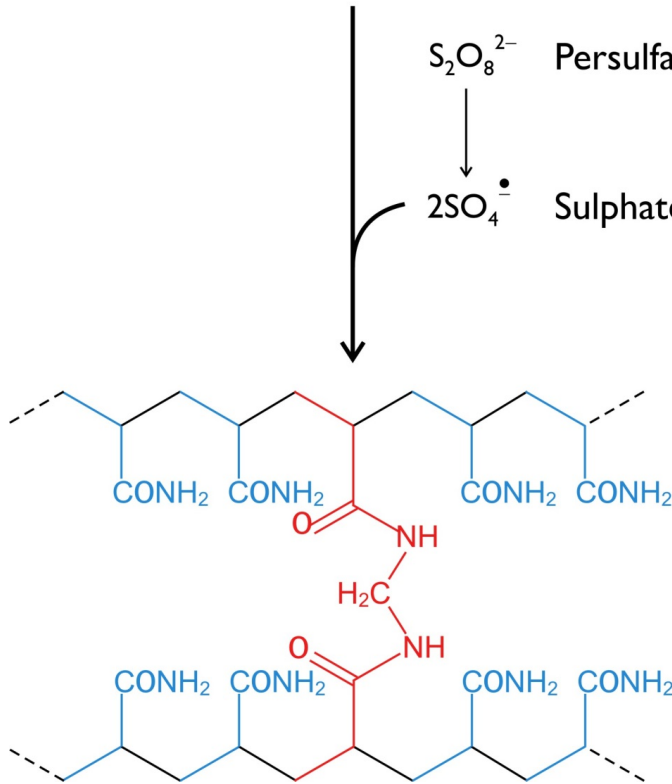
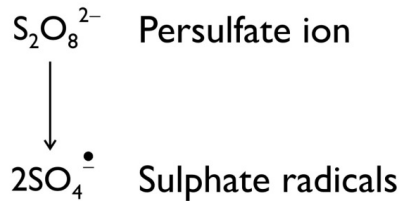
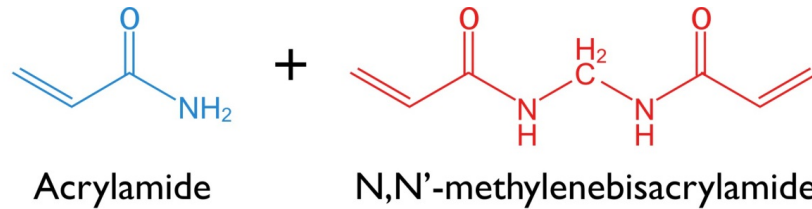
Sth to know by the way:

DTT (dithiothreitol)



SDS-PAGE

(PolyAcrylamide Gel Electrophoresis 聚丙烯酰胺凝胶电泳)



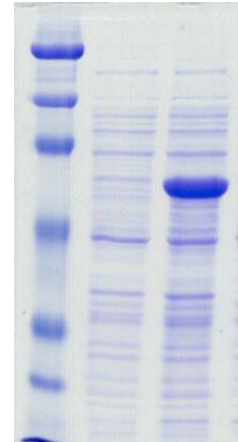
% Gel	Mass Range
7	50 kDa - 500 kDa
10	20 kDa - 300 kDa
12	10 kDa - 200 kDa
15	3 kDa - 100 kDa

Acrylamide is neurotoxic!

Visualization of protein on gel

Coomassie brilliant blue staining

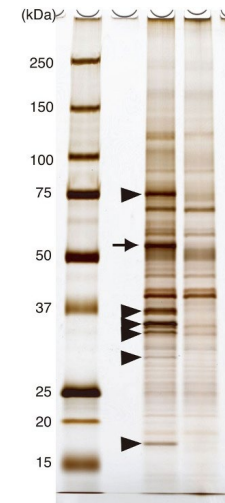
考马斯亮蓝染色



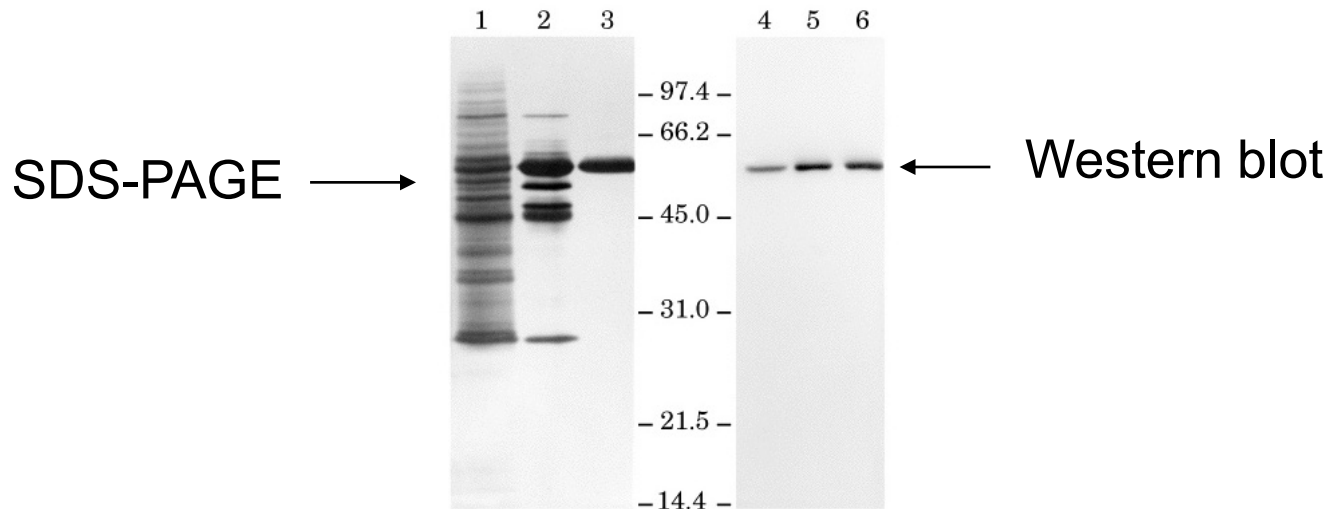
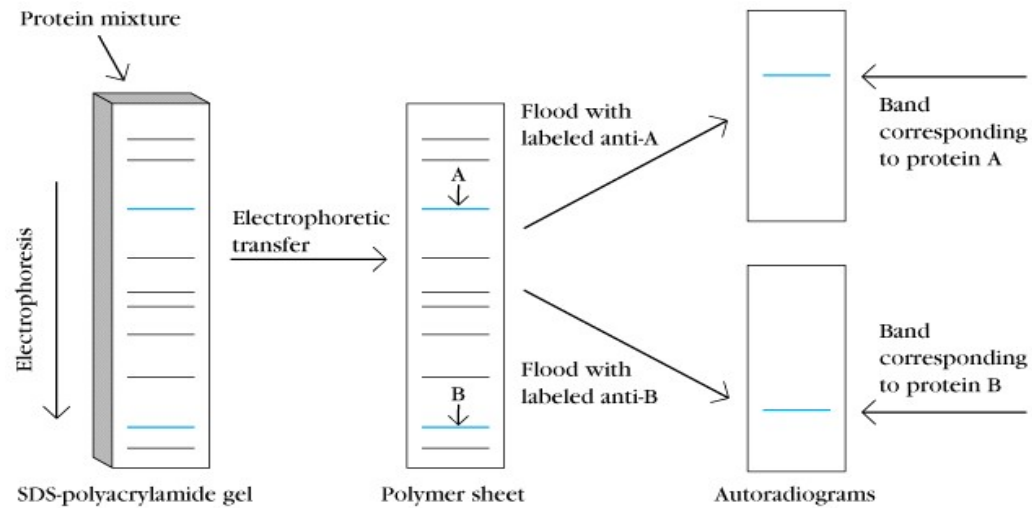
Silver staining 银染

Involving the reduction of Ag^+ to Ag ;

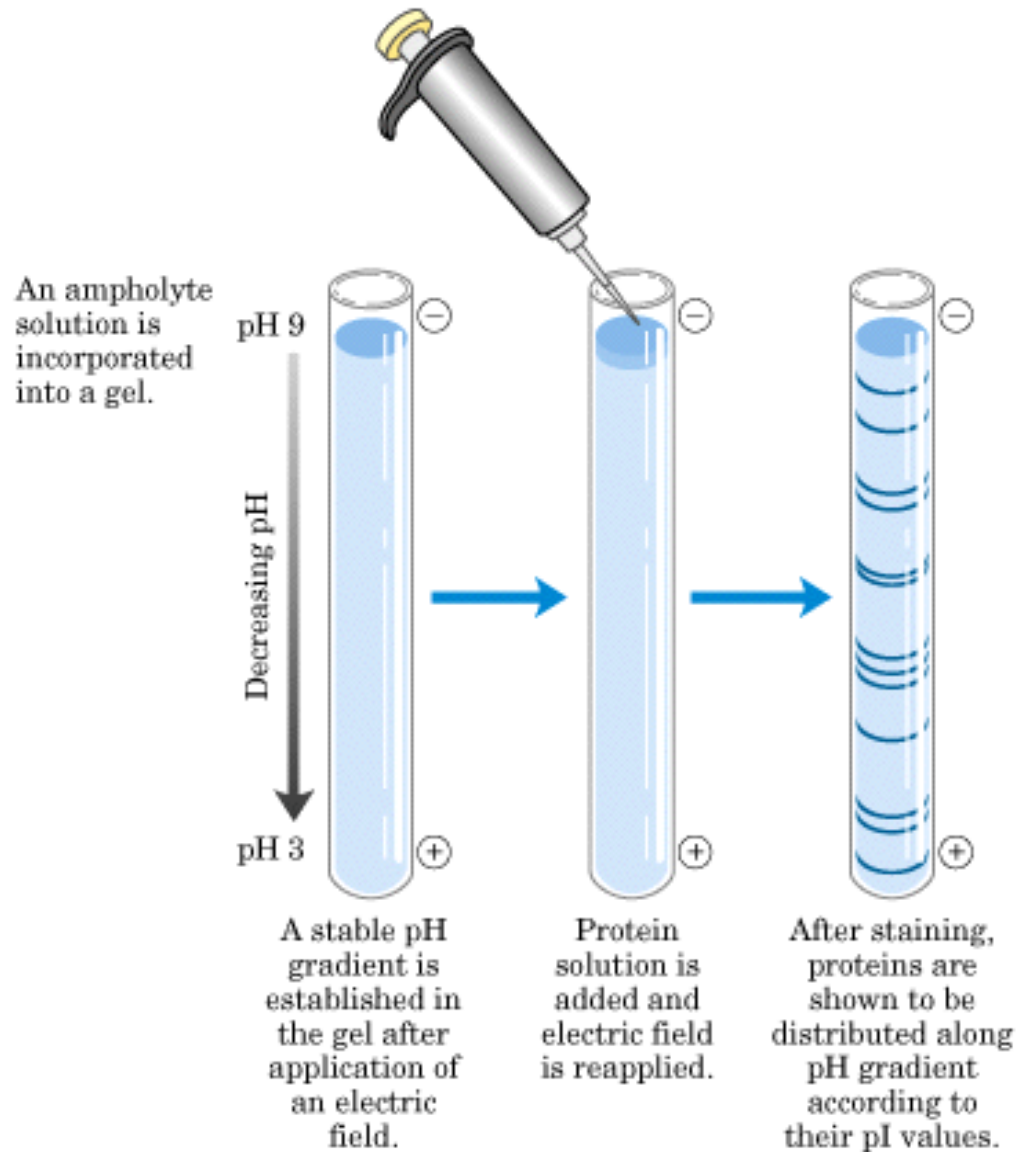
10-50 times sensitive than coomassie blue.



Western blot (免疫印迹)

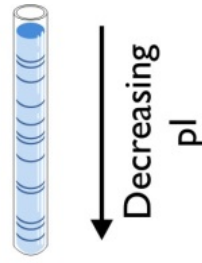


IEF (isoelectric focusing gel等电聚焦电泳)

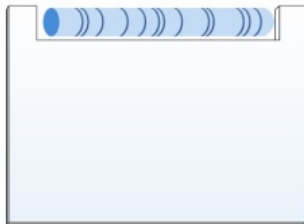


Two-dimensional gel (2D-gel)

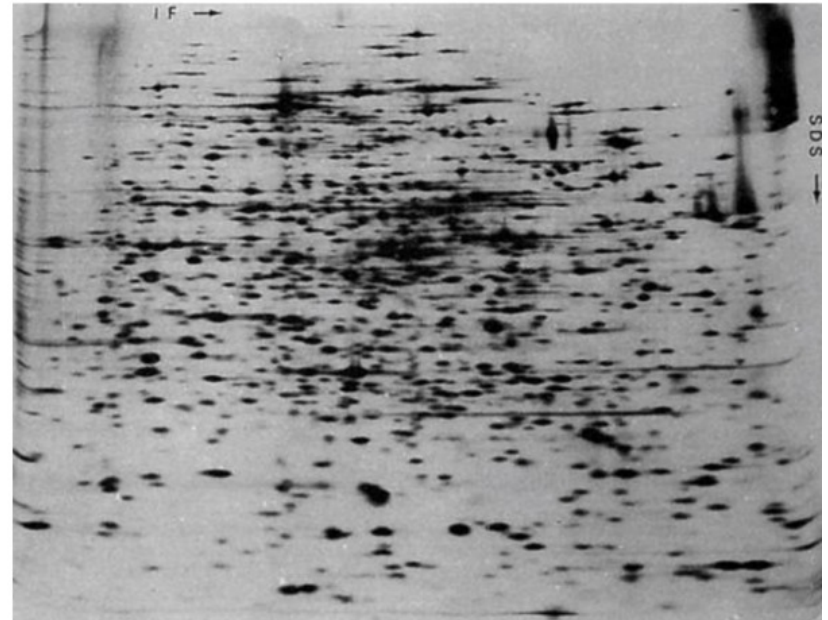
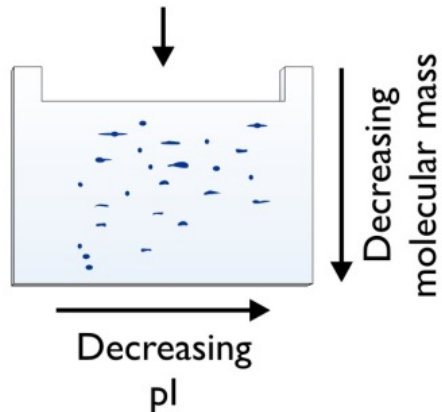
1. Separation of proteins by pI value



2. Soaking the gel in SDS solution and fitting it on an SDS PA gel



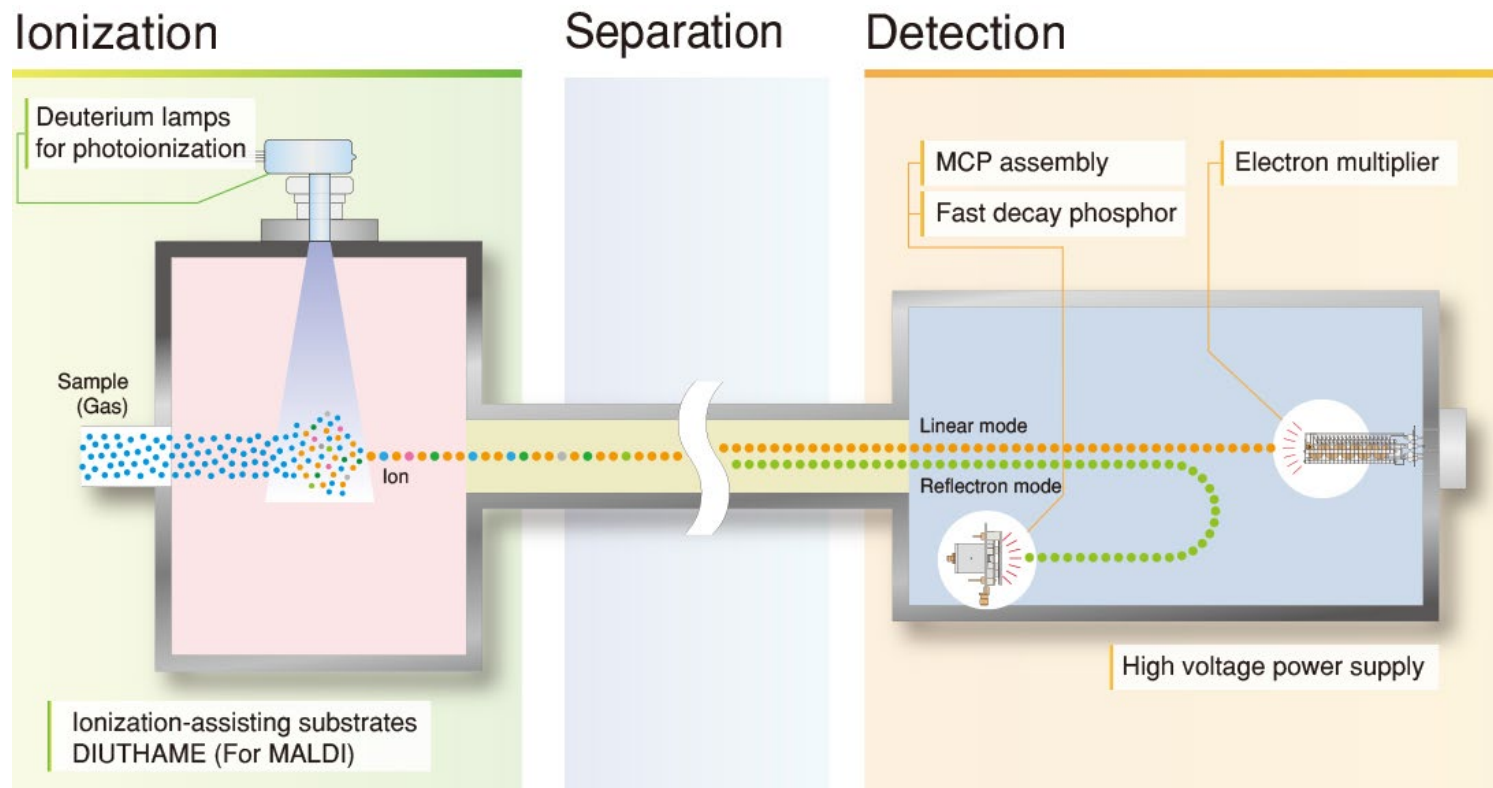
3. Separating the proteins by molecular mass with SDS PAGE



Approximately 1000 *E. coli* proteins on a single 2D gel

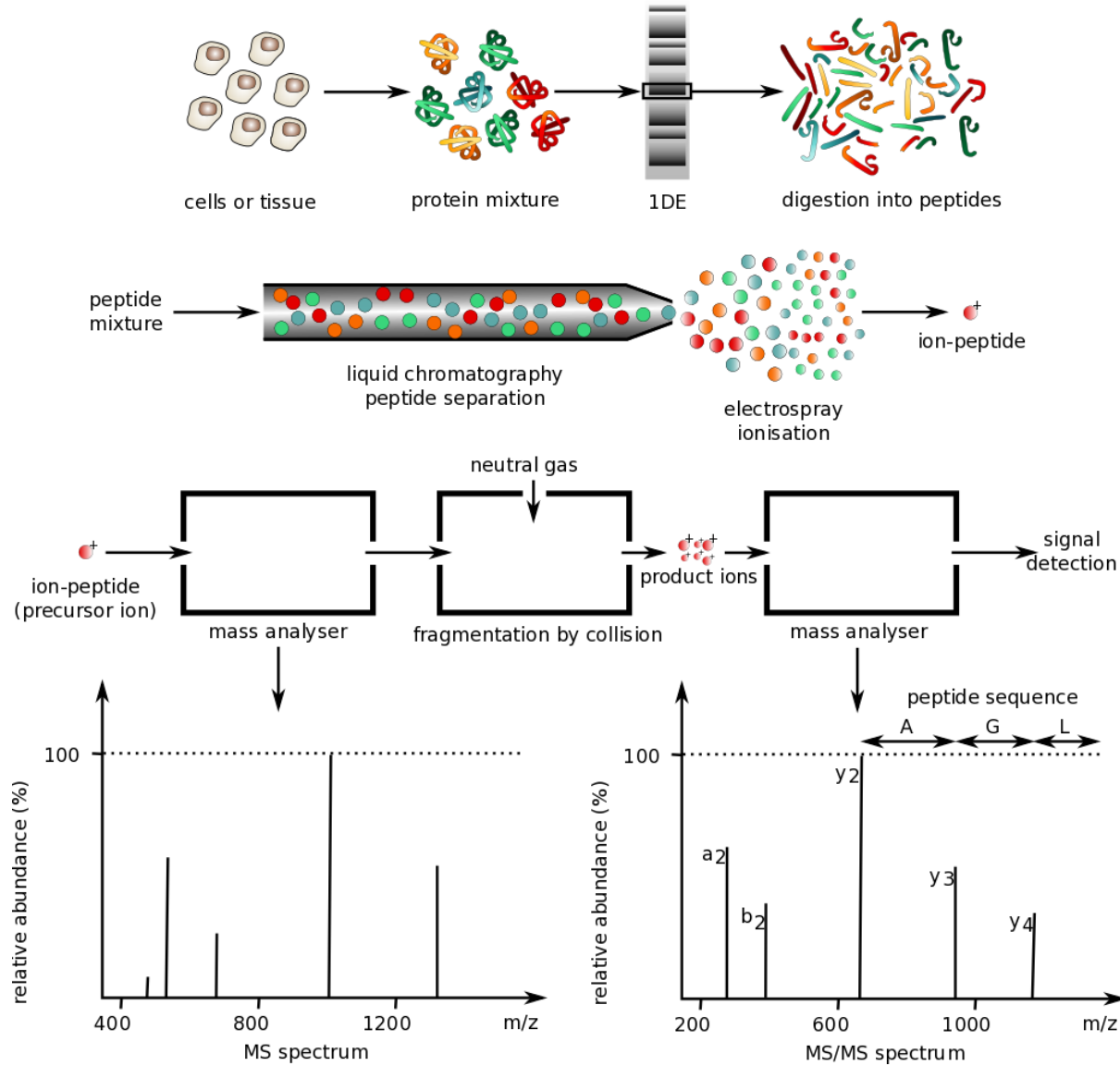
(<http://elte.prompt.hu/sites/default/files/tananyagok/IntroductionToPracticalBiochemistry/ch07s03.html>)

Scheme of mass spectrometry (MS)

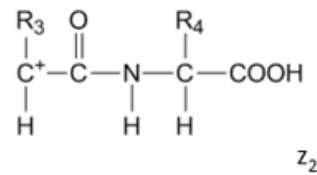
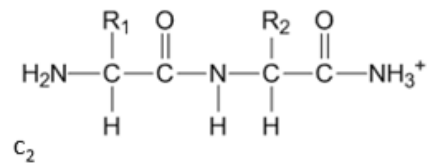
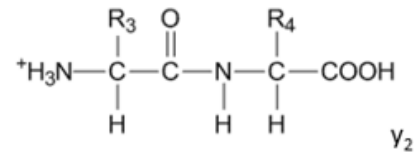
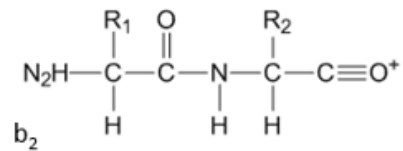
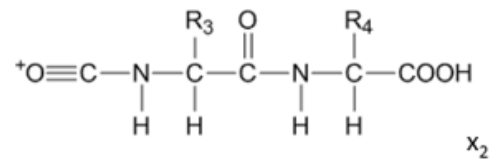
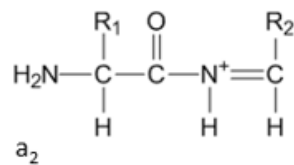
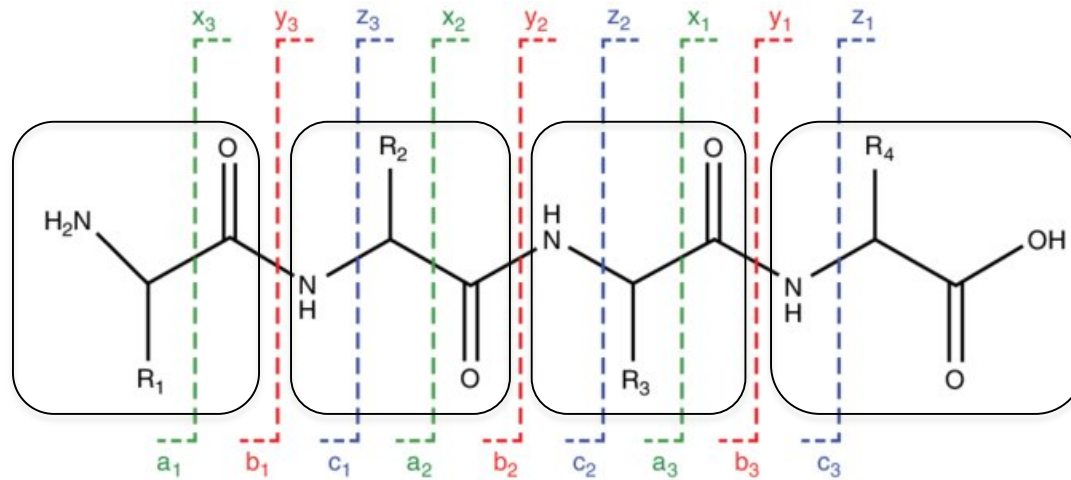


(<https://www.hamamatsu.com/jp/en/applications/Mass-spectrometry/index.html>)

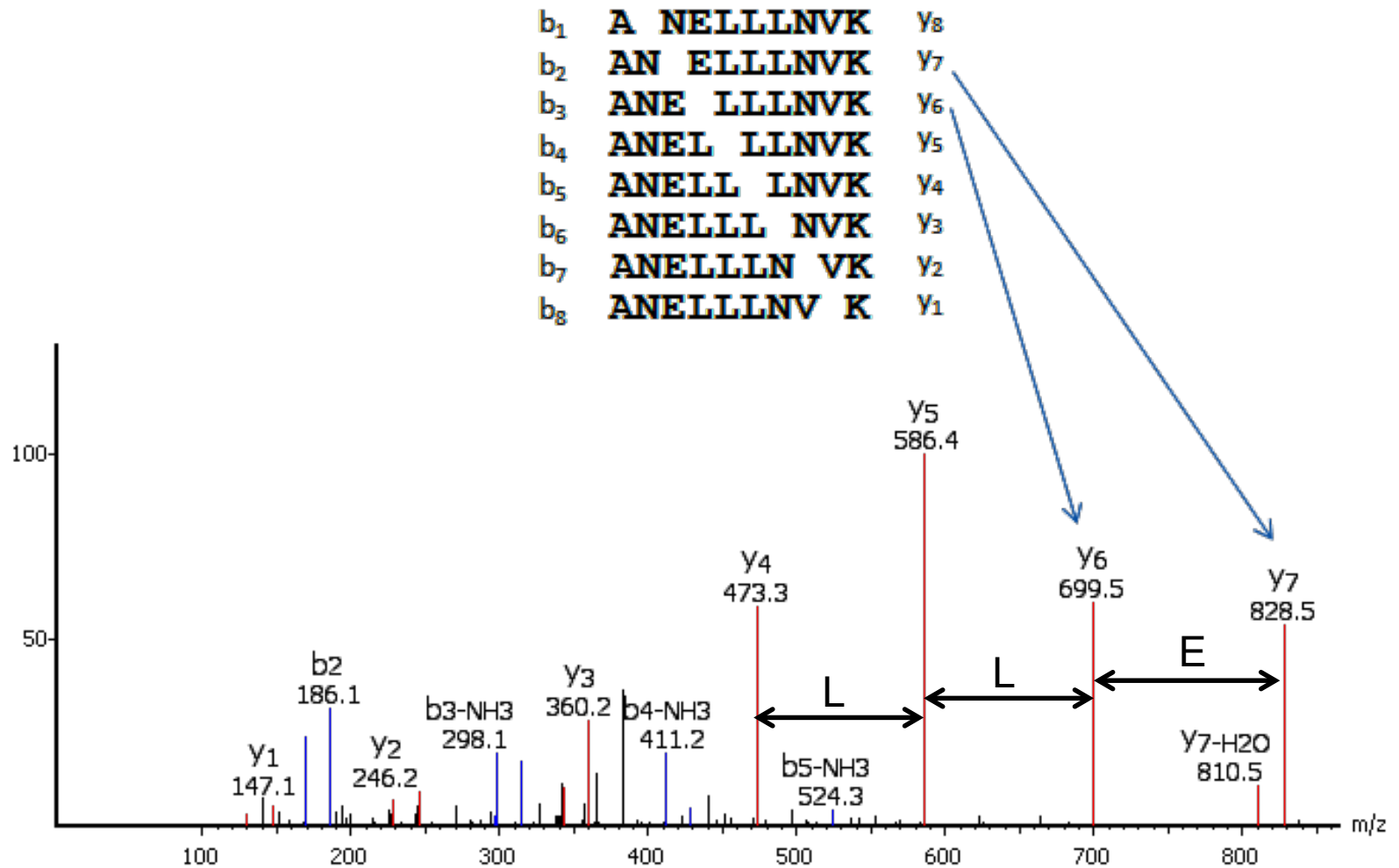
LC-MS/MS



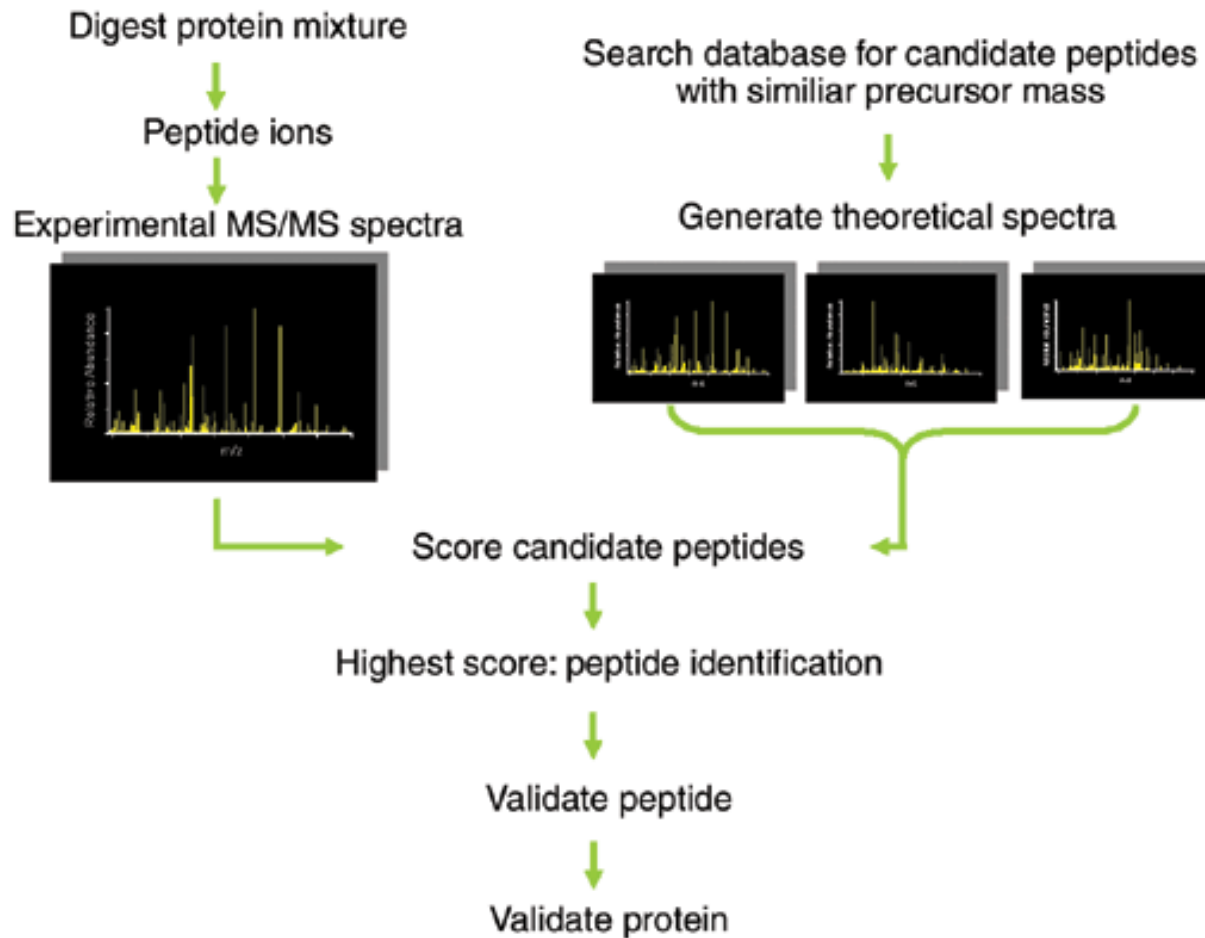
Peptide fragmentation in MS/MS



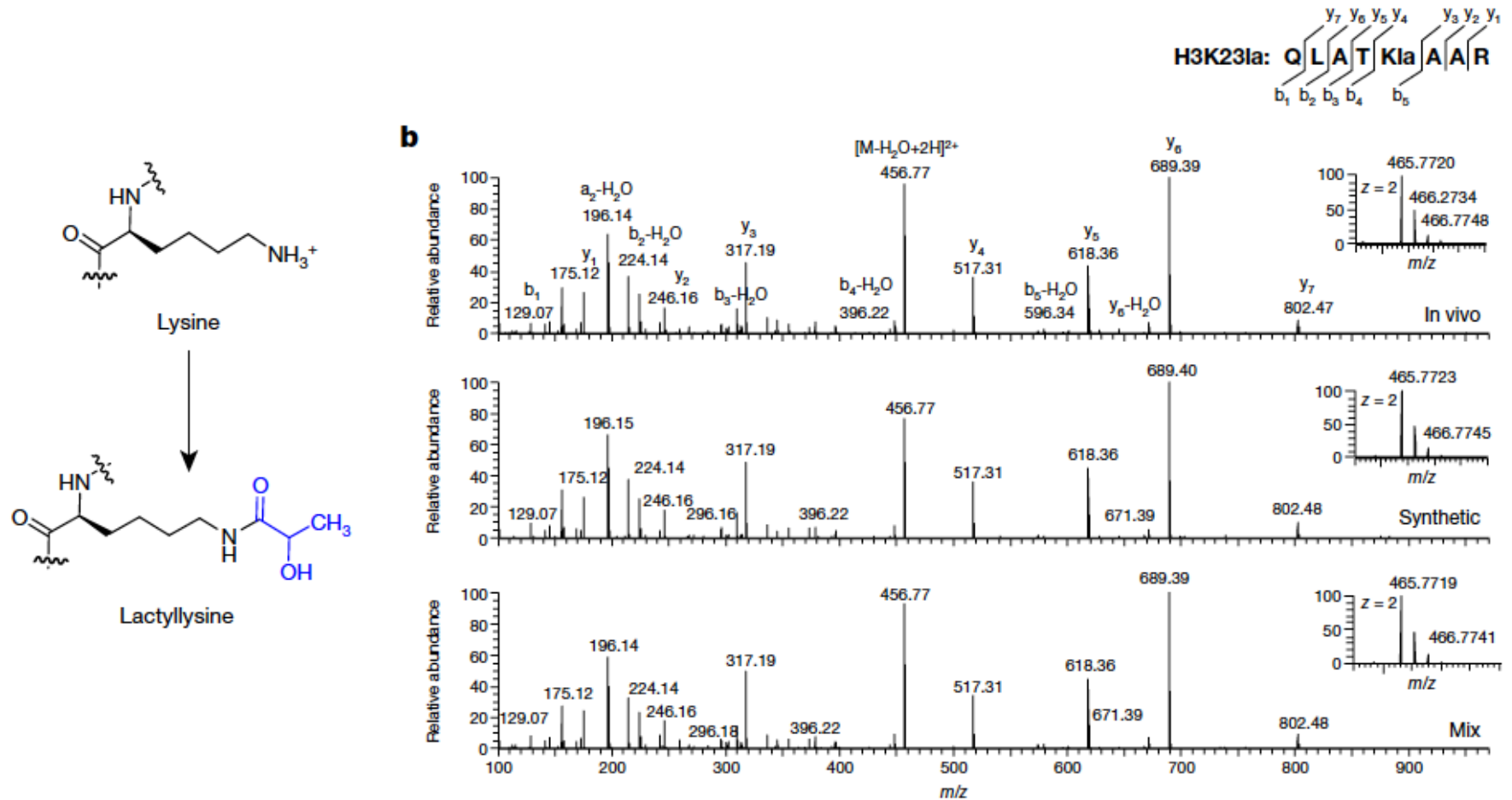
MS/MS spectrum



MS/MS spectrum database searching

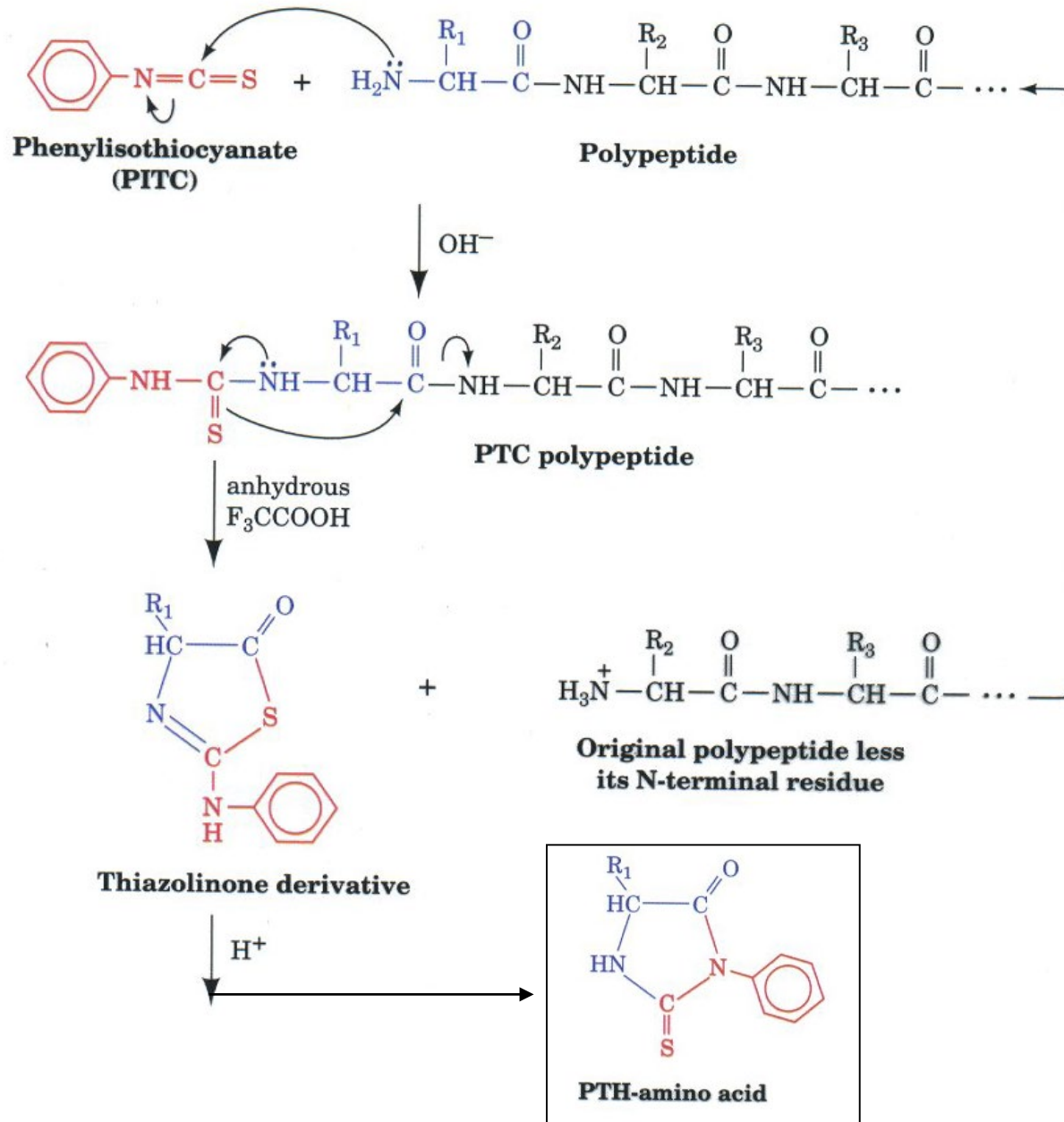


The identification of histone lactylation

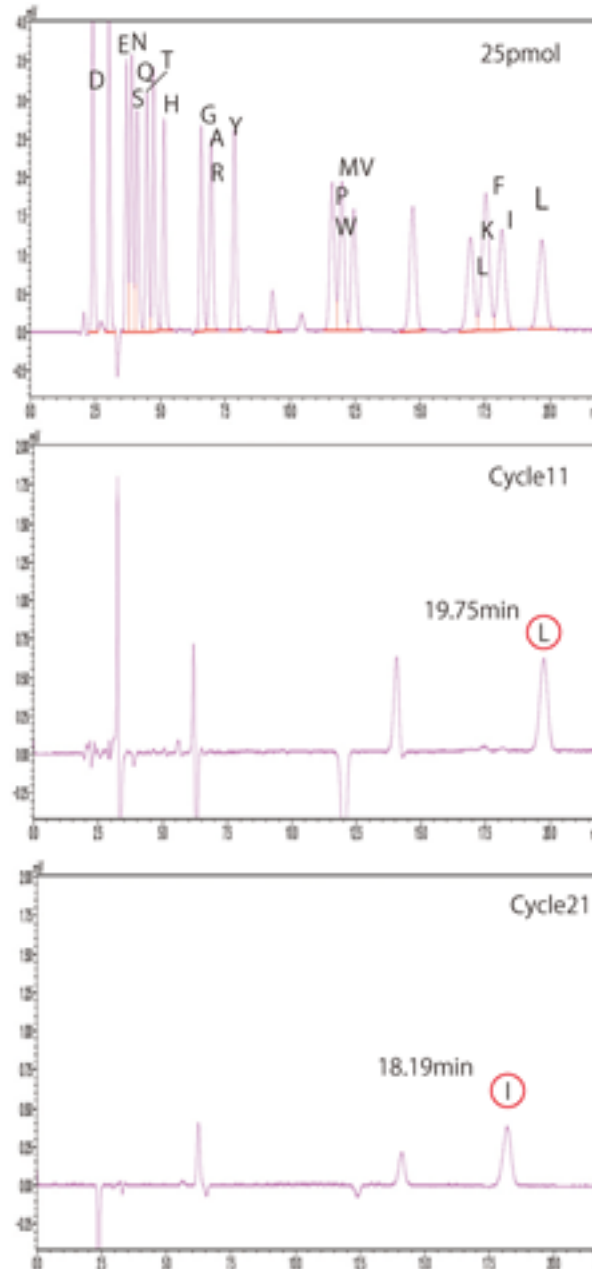


(Nature, 2019, 574, 573)

Before MS, Edman degradation sequencing

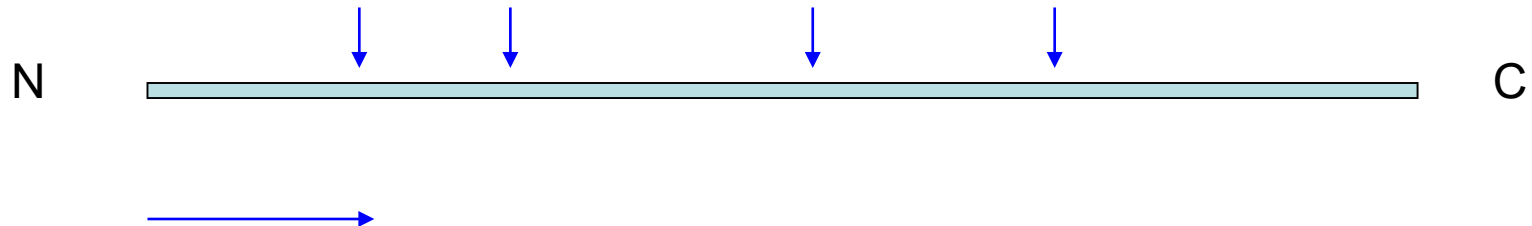


Edman degradation sequencing



<https://shimadzu.com.au/benefits-using-edman-degradation-amino-acid-sequencing>

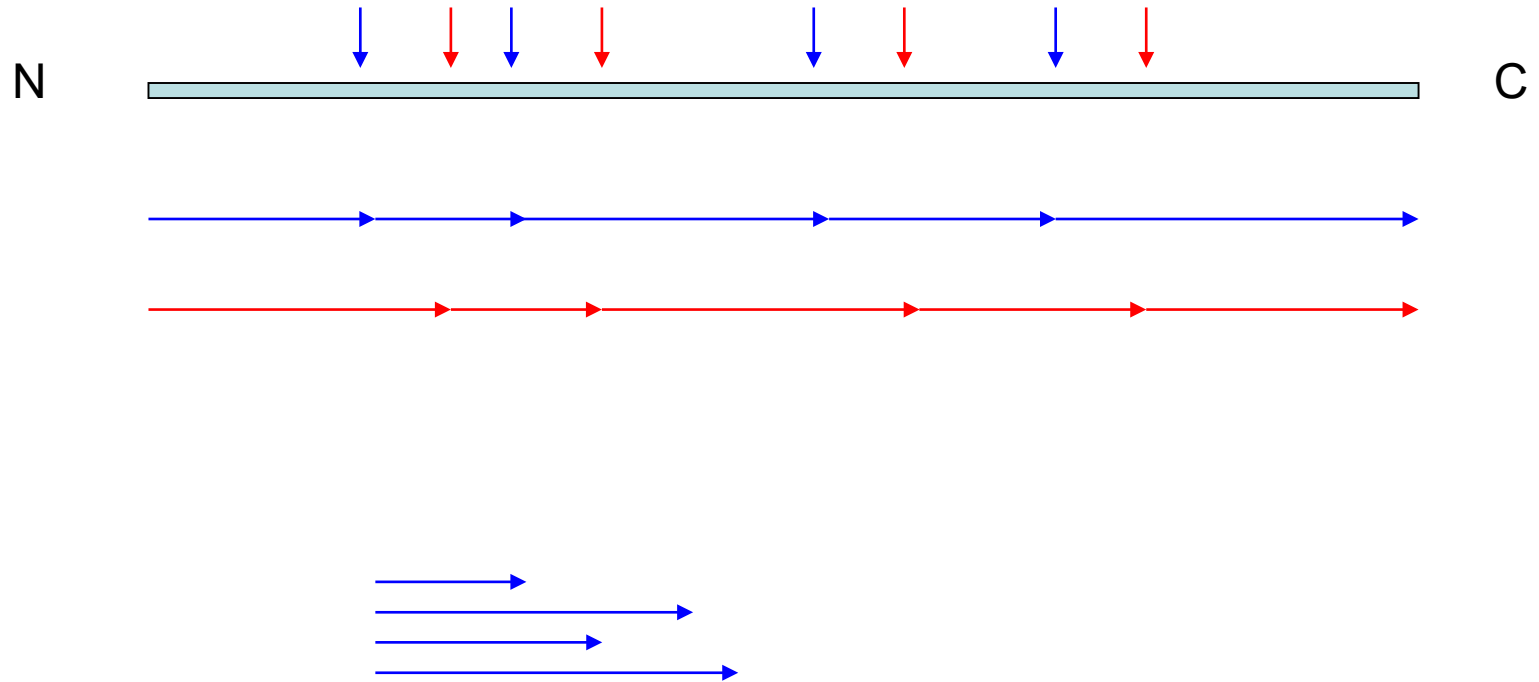
Edman sequencing = N-terminal sequencing



Need to cut protein into limited number of fragments.

Protease digestion to fragment proteins

trypsin	C-termini of Lys and Arg.
chymotrypsin	C-termini of Tyr, Phe, and Trp. Leu, Met, Ala, Asp, and Glu are cleaved at a lower rate.
pepsin	Mainly at C-termini of Phe and Leu.
Glu-C, Lys-C, Arg-C, Asp-N, etc;	
Cyanogen bromide (CNBr)	Chemical cleavage at the C-terminus of Met.



Overlapping to get complete sequence

T1 FVNQHLCGSHLVEALYLVCGER

C1 FVNQHLCGSHLVEALY

T2 GFFYTPK

C2 LVCGERGFF

T3 A

C3 YTPKA

↓ Align overlapping peptides

T1 FVNQHLCGSHLVEALYLVCGER

C1 FVNQHLCGSHLVEALY

C2 LVCGERGFF

T2 GFFYTPK

C3 YTPKA

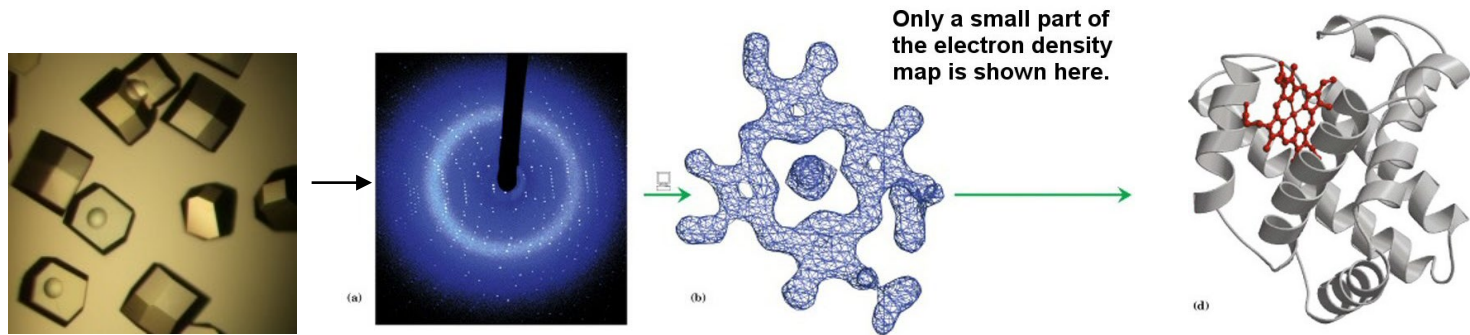
T3 A

Sequence FVNQHLCGSHLVEALYLVCGERGFFYTPKA

↑ ↑ ↑ ↑
C T C T

Three-dimensional structure determination

X-ray crystallography



NMR (Nuclear Magnetic Resonance)

Structure in solution;

For small proteins (< 20 KD);

Cryo-EM (Electronic microscopy)

For large proteins or protein assemblies.

You are ready to study proteins!