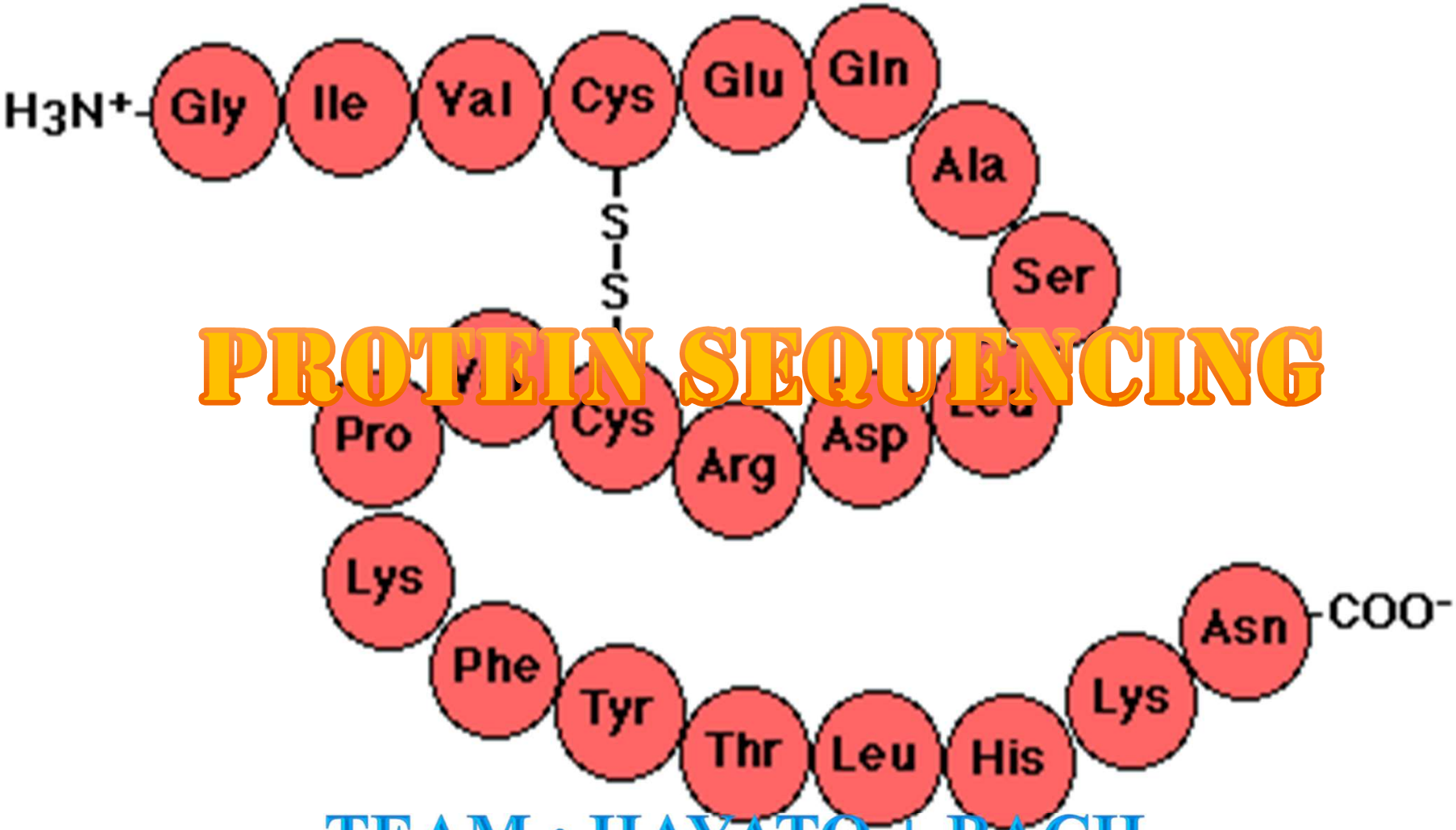
http://www.ehu.eus/biomoleculas/proteinas/prot41.htm



PROTEIN SEQUENCING

TEAM : HAYATO + BACH

PRESENTED BY : HAYATO

Introduction

- **Protein sequencing** is the method of studying the covalent structure and amino acid sequence of a mature polypeptide.
- **Protein sequencing** is a part of posttranslational modifications.
- There are three main categories of **protein sequencing** :

  - Studying N-terminus

  - Studying C-terminus (very few methods, mostly using enzyme carboxylpeptidases).

  - Cleavage of polypeptides into peptides.

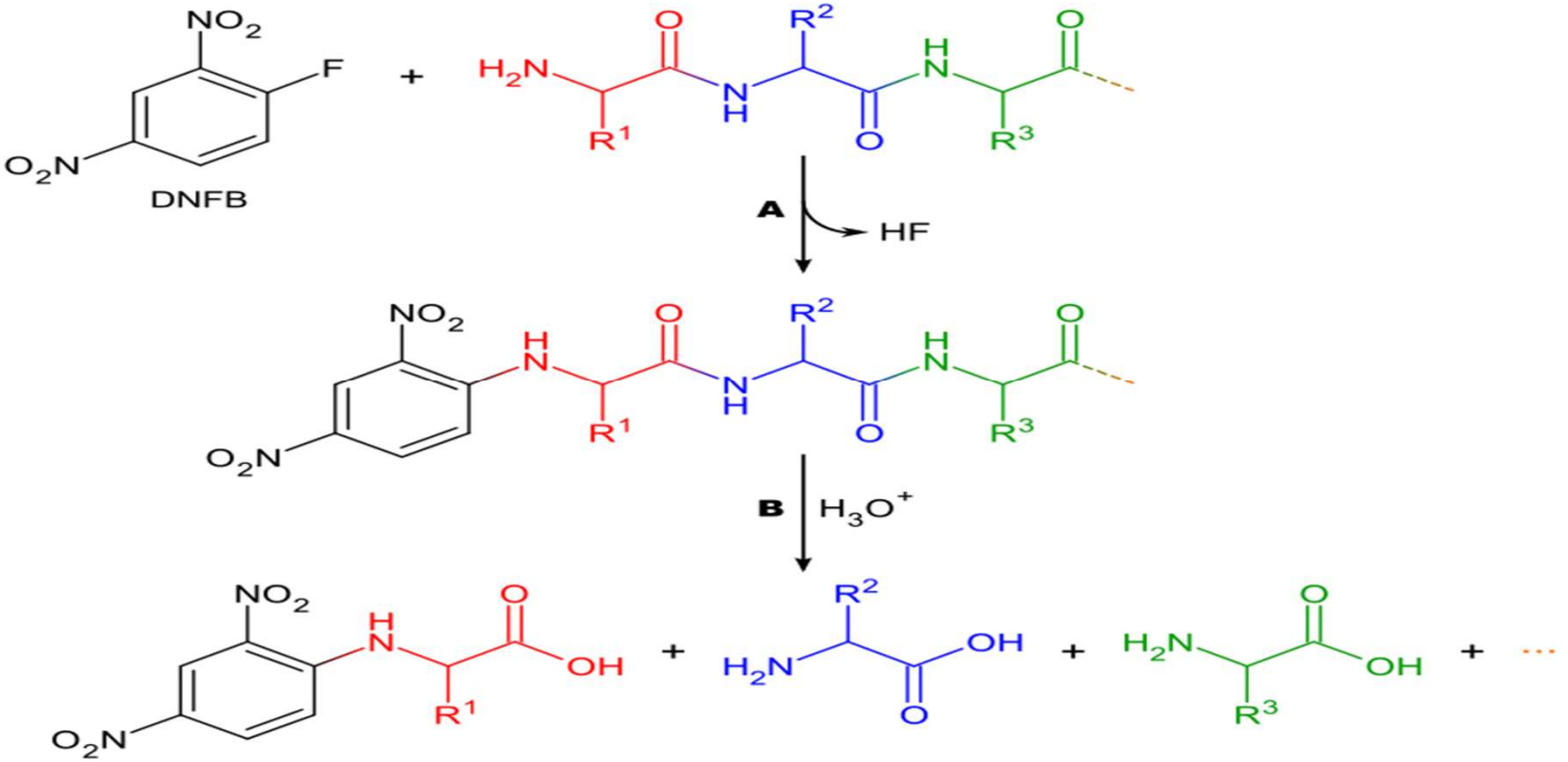
Studying N-terminus

- ***Sanger method :***

- Most common reagent is DNFB (1-fluoro-2,4-dinitrobenzene).

- The reagents produce coloured derivatives and only qualitative analysis is required.

- They all react with amine groups and will therefore also bind to amine groups in the side chains of amino acids such as lysine - for this reason it is necessary to be careful in interpreting chromatograms to ensure that the right spot is chosen.

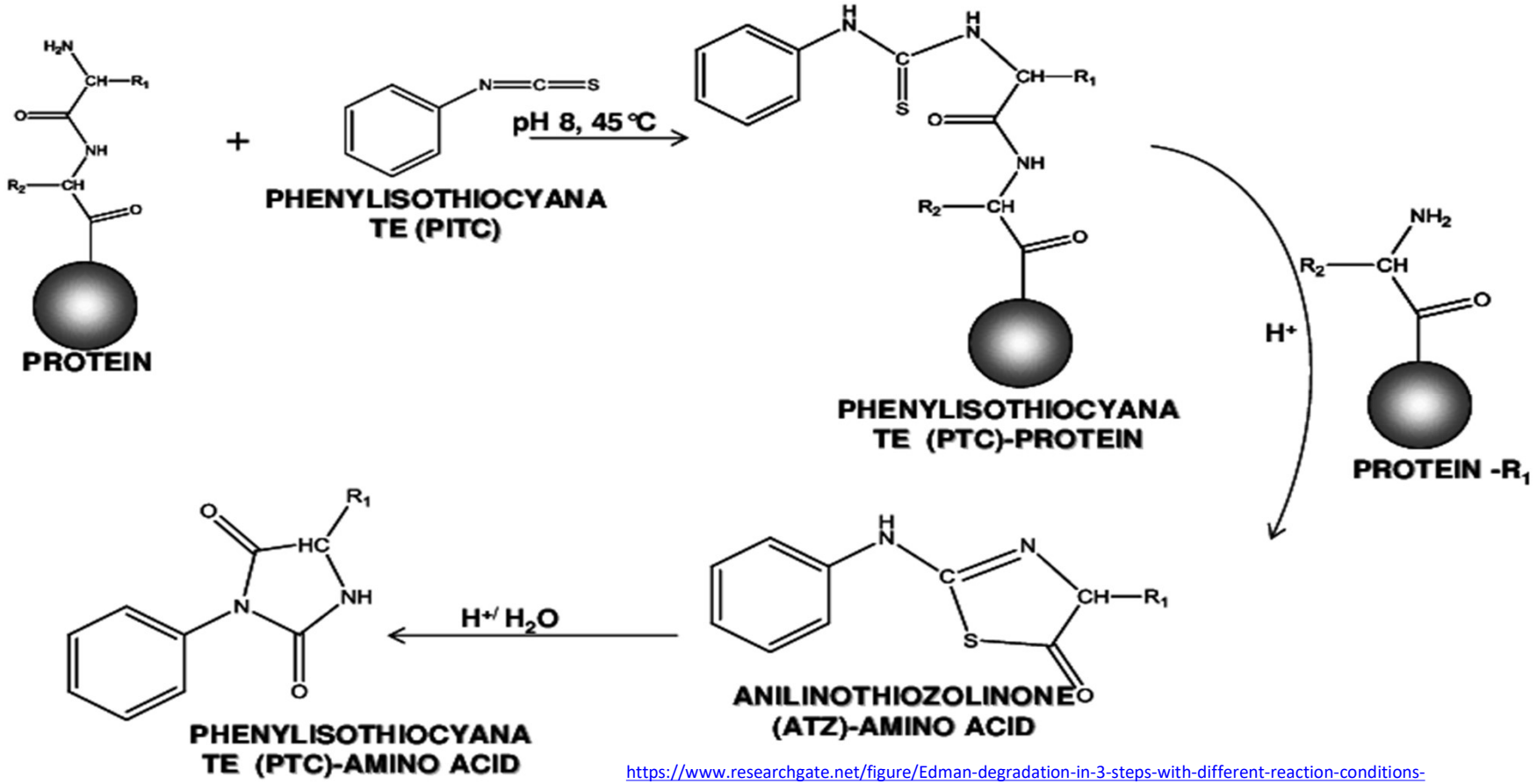


Studying N-terminus

- ***Edman degradation :***

- Can sequence peptides up to 50 amino acids long.

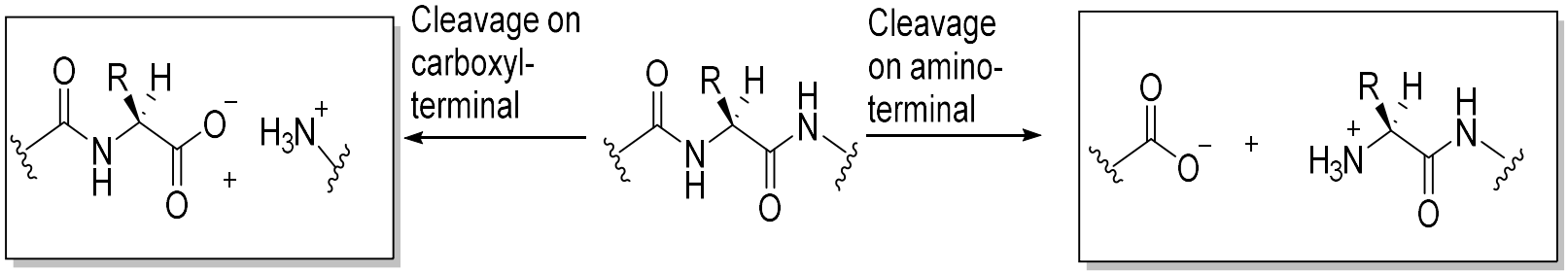
- Relies upon the removal of the N-terminus amino acid residue from the polypeptide as the (PITC)-amino acid. The (PITC)-amino acid from each step can be positively identified by high-pressure adsorption chromatography.



https://www.researchgate.net/figure/Edman-degradation-in-3-steps-with-different-reaction-conditions-1-mildly-alkaline_fig40_283380515

Cleavage of polypeptide into peptides

- ***Using specific enzyme :*** Protein needs to be unfolded before cleavage.

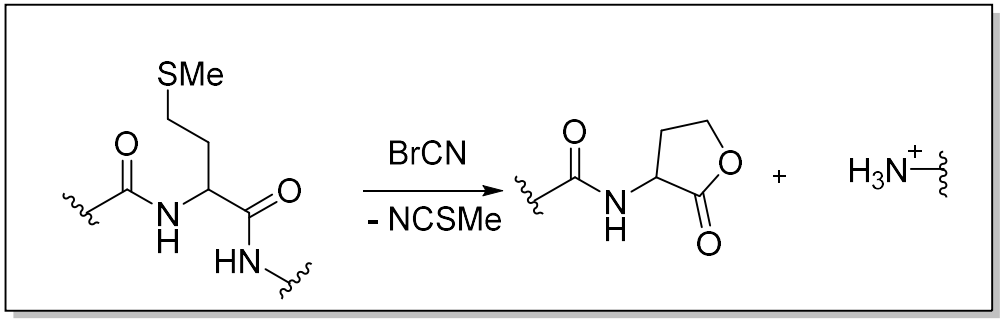


Specific enzyme	Cleavage site	R group (amino acid)
Citraconylation + trypsin	C-terminal	Arg
Trypsin	C-terminal	Lys, Arg
S.aureus protease	C-terminal	Glu, Asp
Chymotrypsin	C-terminal	Phe, Tyr, Trp (aromatic)
Thermolysin	N-terminal	Leu, Ileu, Val, Phe

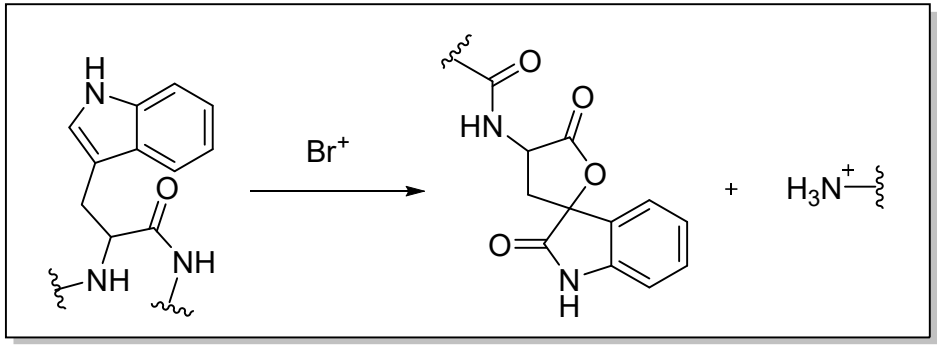
Cleavage of polypeptide into peptides

- ***Using chemical reaction :***

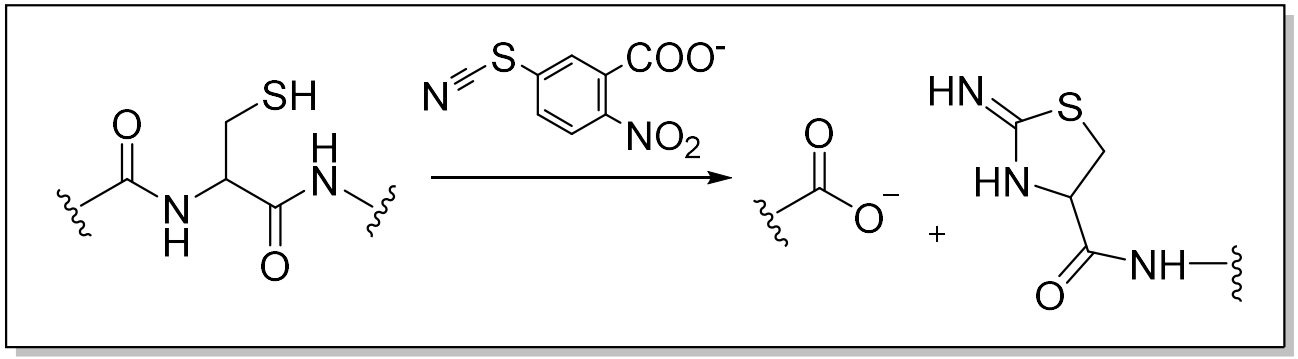
  * Cyanogen Bromide (BrCN) : *Cleavage C-terminal of Met.*



  * Mild bromination (Br^+) : *Cleavage at C-terminal of Trp.*



  * 2-nitro-5-thiocyanatobenzoate : *Cleavage N-terminal of Cys.*



A theoretical example

*A peptide segment has been purified from a digest of certain protein. Consider the following information.

(A) : Complete acid hydrolysis in 6 M HCl :

(1) : Arg, Glu, 2 Gly, Ileu, Leu, Lys, Phe, Ser, Val

(B) : React with DNFB forms (2) : DNB-Val

(C) : Using enzyme trypsin : (3): (Arg, Glu, Gly, Val) ; (4) : (Gly, Lys, Phe, Ser) (5):(Ileu, Leu).

(D) : Edman degradation :

Peptide	Cycle	
	1	2
(3)	Val	Glu
(4)	Phe	Ser

(E) : Carboxylpeptidase : Ileu

Solution :

From (B), the N-terminus is Val.

From (C) and (D) : peptide (3) is Val-Glu-Gly-Arg ; (4) is Phe-Ser-Gly-Lys

From (E), the C-terminus is Ile, so the peptide (5) is Leu-Ile.

So the peptide segment is : ***Val-Glu-Gly-Arg-Phe-Ser-Gly-Lys-Leu-Ile.***

Conclusion

-The grand strategy for determining the sequence of a polypeptide directly is to separate and sequence all of the peptides from one particular cleavage.

-However, the sequences of polypeptides are now possibly determined by sequencing DNA complementary to the messenger RNA that encodes them.

References

[1] *Structure in Protein Chemistry , 1995* – Jack Kyte – University of California, San Diego.

[2] Example is from Chemistry Olympiad of gifted high schools in the North of Vietnam 2016 (Not original).

[3] http://www.ehu.eus/biofisica/juanma/papers/EdmanDegradation.pdf

[4] https://nptel.ac.in/courses/102103017/pdf/lecture%2018.pdf

(All links are accessed on Dec.6th.2018)