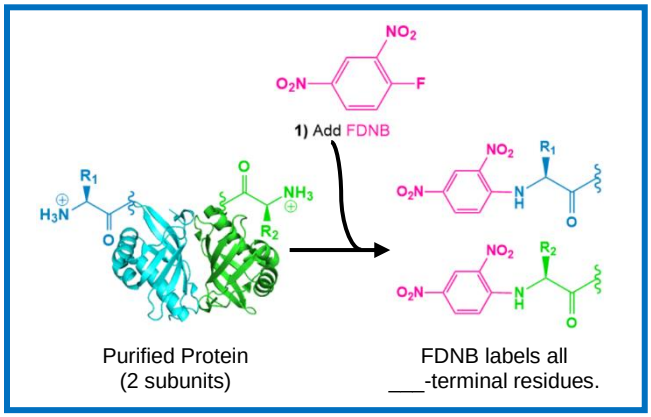
CONCEPT: FDNB

●*1-fluoro-2,4-dinitrobenzene* (_____) covalently labels *all* free ____-terminal aa-residues on *all* polypeptide chains.

□ Also known as _____ reagent since Frederick Sanger first used it to identify the *N-terminal residue*.

EXAMPLE: Effect of FDNB on proteins.



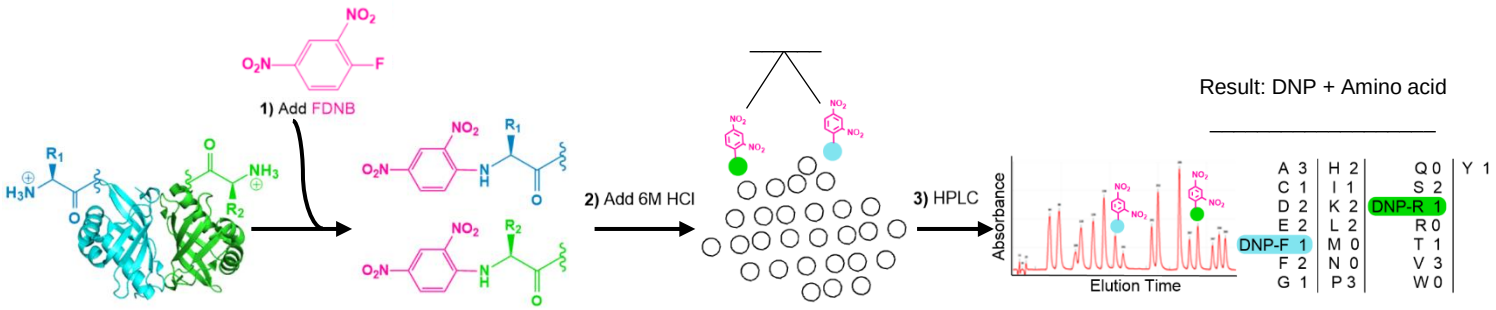
FDNB + Amino Acid Hydrolysis

●FDNB treatment followed by amino acid hydrolysis results in free amino acids & *dinitrophenyl* (_____) derivatives.

□ DNPs are easily analyzed via HPLC or mass spectrometry to _____ *all* N-terminal amino acids.

□ Amount/types of DNPs detected indicates the number of _____.

EXAMPLE: FDNB Reveals N-terminal Residues & Number of Subunits.



Conclusion:

- Two DNPs indicates ____ subunits.

- N-terminal Residues of Subunits = ____ & ____

●*Dansyl chloride* & *dabsyl chloride* are used in a similar way to FDNB.

●Amino acid composition can be used to _____ *protein fragmentation* prior to sequencing.

PRACTICE: The peptide Lys-Leu-Leu-Arg-Phe is incubated with FDNB. After exposure to 6M HCl, the solution contains:

a) A free Lys labeled by FDNB & an unlabeled tetrapeptide.

b) 5 free amino acids labeled by FDNB.

c) 5 free amino acids with Lys & Arg labeled by FDNB.

d) Options a, b & c are all incorrect.

CONCEPT: FDNB

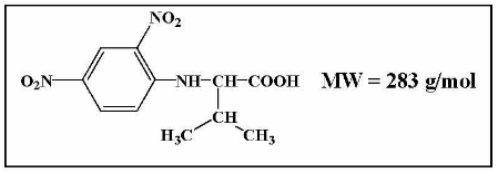
PRACTICE: After purifying a protein, you react the protein with 1-fluoro-2,4-dinitrobenzene (FDNB or Sanger's reagent) then with 6M HCl. You obtain DNP-Arg and DNP-Asp. Which of the following is an appropriate conclusion of the results?

a) The protein's N-terminal residue is Arg & its C-terminal residue is Asp.
b) The protein's N-terminal residue is Asp & its C-terminal residue is Arg.
c) The protein has two subunits; one with an Arg N-terminal residue and one with an Asp N-terminal residue.
d) Perhaps the protein has not been completely purified after all.
e) c and d.

PRACTICE: A tetrapeptide of unknown sequence, containing 2 moles of glycine (Gly) and 1 mole each of leucine (Leu) & alanine (Ala), gives a dinitrophenyl derivative of Gly (DNP-Gly) after treatment with 1-fluoro-2,4-dinitrobenzene (FDNB) and then 6M HCl for complete hydrolysis. Partial hydrolysis of the tetrapeptide gives Gly-Ala and Gly-Gly fragments. What is the amino acid sequence of the original tetrapeptide?

a) Ala-Gly-Gly-Leu.
b) Gly-Ala-Gly-Leu.
c) Leu-Gly-Gly-Ala.
d) Gly-Gly-Ala-Leu.

PRACTICE: A 660 mg sample of a protein with quaternary structure (MW=132,000 g/mol) was treated with excess 1-fluoro-2,4-dinitrobenzene (FDNB) under slightly alkaline conditions until the reaction was complete. After FDNB treatment, the peptide bonds of the protein were then completely hydrolyzed by heating it with concentrated 6M HCl. The resulting solution was found to contain 5.5 mg of DNP-Val (shown in the figure below):



2,4-Dinitrophenyl derivatives of the α-amino groups of the other amino acids were not detected. Assuming 100% yield for the reaction of FDNB with the protein, determine the number of subunits in this protein.

a) 1. b) 2. c) 3. d) 4. e) 5.