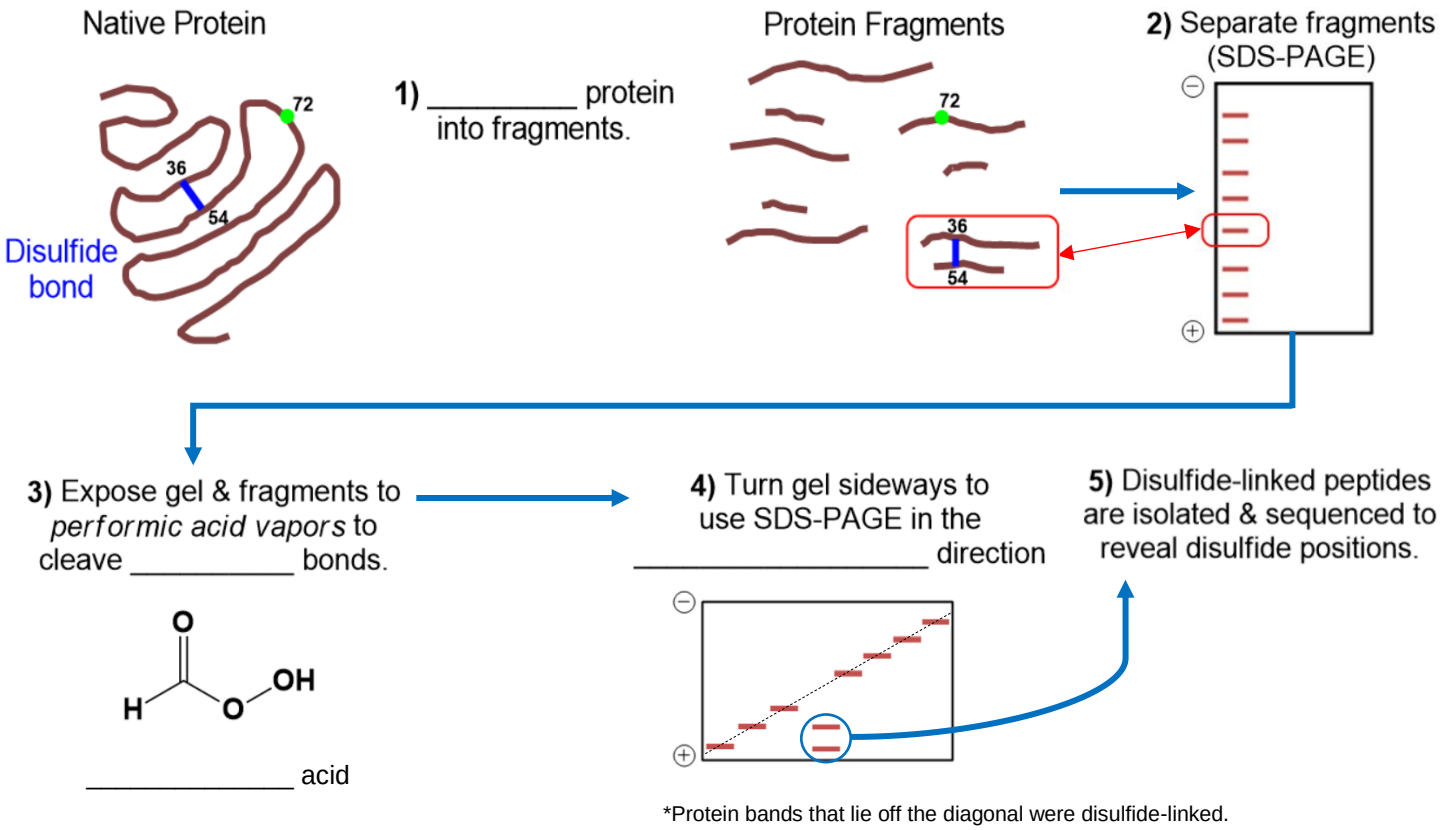
CONCEPT: DIAGONAL ELECTROPHORESIS

- _____ *Electrophoresis*: isolates disulfide-linked proteins to determine the *positions* of original _____ bonds.
  - □ Recall: Disulfide bonds covalently link R-groups of any two cysteines on the same chain or separate chains.
- Results: peptides *without* disulfide bonds will _____ diagonally due to _____ mobility.
  - □ Peptides *with* disulfide bonds will lie _____ the diagonal line due to _____ mobility.
  - □ Peptides found to be disulfide-linked can be isolated & sequenced to determine disulfide bond _____.

EXAMPLE: Diagonal Electrophoresis.



*Protein bands that lie off the diagonal were disulfide-linked.

PRACTICE: Which of the following techniques is used specifically to help determine the site of a disulfide bond?

a) Edman degradation.
b) Affinity chromatography.
c) SDS-PAGE.
d) Diagonal electrophoresis.
e) MALDI-TOF Mass spectrometry.

CONCEPT: DIAGONAL ELECTROPHORESIS

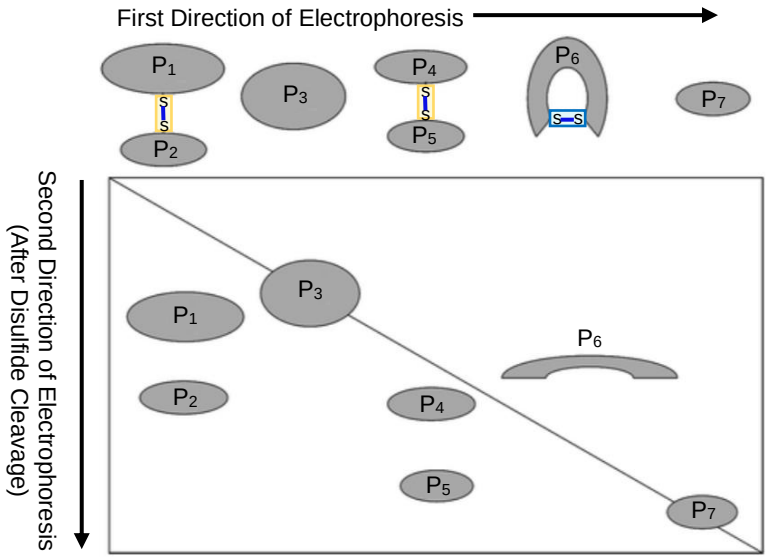
Interchain vs. Intrachain Disulfides

●Diagonal electrophoresis results differ for _____-chain (*separate* chain) & _____-chain (*same* chain) disulfide bonds.

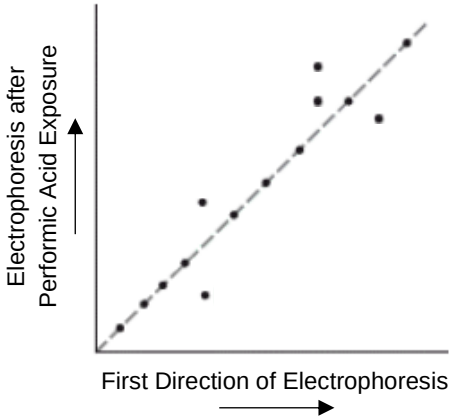
□ Upon cleavage of only interchain disulfides, peptide fragments get smaller & travel _____ in the gel.

□ Upon cleavage of only intrachain disulfides, peptide fragments change shape & travel _____ in the gel.

EXAMPLE: Interchain vs. Intrachain Disulfides.



PRACTICE: In the plot below, circle the point(s) representing peptides with intrachain disulfides.



PRACTICE: A gene encoding a protein with a single disulfide bond undergoes a mutation that changes a serine residue into a cysteine residue. You want to find out whether the disulfide pairing in this mutant is the same as in the original protein. Briefly layout a diagonal electrophoresis experiment to determine the answer.