CONCEPT: ANFINSEN EXPERIMENT

●In the 1950's, Christian Anfinsen performed experiments that demonstrated _____ major principles:

1) _____ structure determines tertiary structure.

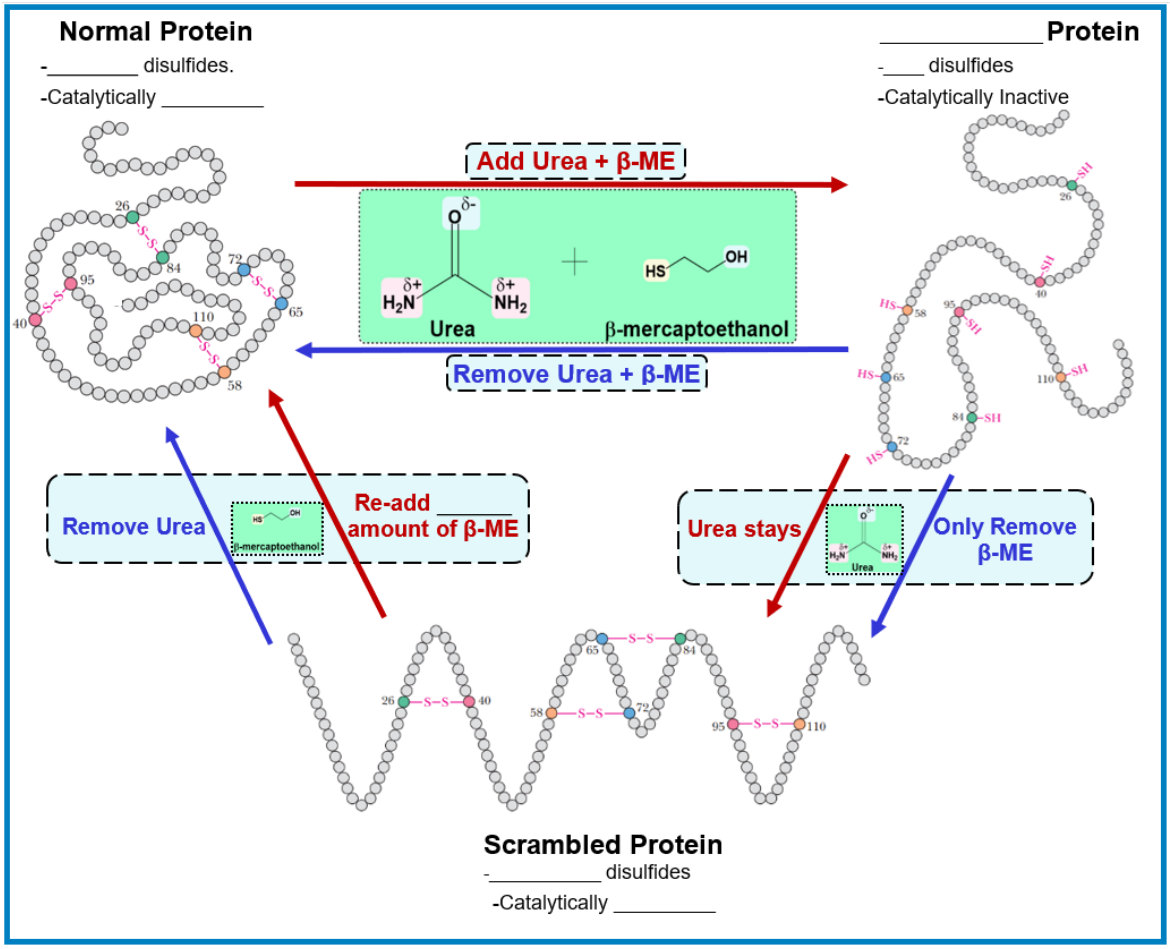
2) A protein _____ folds into its native conformation, which is its most _____ state.

●Used urea & β-ME to affect the protein structure of _____ A (RNase A).

□ _____/removal of both urea & β-ME respectively _____/renatures RNase A.

□ Subsequent removal β-ME (urea still present) results in a scrambled protein with _____ disulfide bonds.

EXAMPLE: The Anfinsen Ribonuclease A Experiment.



PRACTICE: Which of the following conclusions could Anfinsen draw from his RNase A experiment?

a) Disulfide bridges are unnecessary for the function of RNase A.

b) Kinetics is the main barrier to a protein adopting its native fold.

c) Proteins spontaneously adopt their native fold, which specifies location of disulfide bridges.

d) RNase activity cannot be destroyed by urea alone at any concentration.

CONCEPT: ANFINSEN EXPERIMENT

PRACTICE: What is likely to happen to Ribonuclease A if it is treated with both urea & β-mercaptoethanol?

a) RNase A will denature and oxidize its disulfides to generate sulfhydryl groups.
b) RNase A renatures but disulfide bonds are formed randomly between Cys residues.
c) RNase A will denature and reduce its disulfides to generate sulfhydryl groups.
d) RNase A will denature and oxidize its sulfhydryl groups to generate disulfides.

PRACTICE: Which of the following occurred when RNase A properly refolded from a denatured state?

a) The primary structure of the protein was rearranged.
b) Most of the charged, hydrophilic residues were found buried in the core of the protein.
c) The entropy of the protein structure itself was significantly increased.
d) None of the above.

PRACTICE: Which statement best supports the theory that primary protein structure dictates folding into its native state?

a) RNase A loses all enzymatic activity upon denaturing in 8M urea.
b) RNase A regains enzymatic activity upon removing urea & β-ME.
c) Purified RNase A has 100% enzymatic activity in vitro.
d) A reducing agent such as β-ME destroys disulfide bonds & eliminates RNase A enzymatic activity.