081017 Quiz 3 Morphology of Complex Materials

1) Explain the following terms: (for states comment on biological activity and relative size of the structure)

a) Native State
b) Unfolded State
c) Denatured State
d) Free Energy Landscape
e) Levinthal Paradox
f) Directed Process
g) Stop Flow Kinetics
h) Molten Globule

2) Give the structure of the following compounds and explain their effect on proteins.

a) Iodoacetate
b) β-mercaptoethanol (catalytic and concentrated)
c) urea
d) guanidine
e) Explain how Anfinsen used these compounds with RNase to produce 1) a denatured state then 2) an intermediate state with no biological activity; followed by 3) an intermediate state with 1% activity and finally 4) recreation of the native state.

3) Proteins in cells (in vivo) probably fold by significantly different pathways compared to in vitro proteins. For example the concentration in vitro is generally on the order of 1 mg/ml while the total protein concentration in cells is on the order of 350 mg/ml.

a) A protein in its native state is 5 to 10 nm in diameter and is extremely dense, while in the unfolded state may be 50 to 100 nm with an extremely loose structure. Such a protein might weigh 5×10^{-19} mg. At what concentration will unfolded and folded proteins have strong interactions between different chains (assume spheres and calculate the overlap concentration)?

b) Comment on the problems that might be encountered due to concentration of proteins in the cell.

c) Explain the following statement: "Eukaryotic genes (taken from higher cells which contain nuclei and internal organelles), when transferred into prokaryotes (bacteria, like *E. Coli*), can be expressed to form protein, but they often misfold and aggregate in the bacterial cells and form structures called **inclusion bodies**." (from St. Johns Web page)

d) Explain what a "chaperone" is and how it might assist in protein folding.

e) What type of protein interaction do chaperones generally enable during folding?

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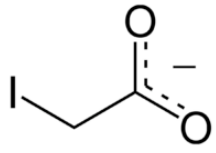
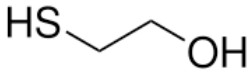
1) a) Native state is the biologically active state, generally the most compact structure observed in a folding sequence. The native state has essentially 0 conformational entropy.
b) Unfolded state is a state with no significant secondary structure, generally the least compact structure possible with the highest conformational entropy, that is, many different conformations are explored in the unfolded state due to thermal fluctuations
c) Denatured state is a state that results from chemical, thermal, physical or mechanical disruption of secondary and tertiary structure and leads to a molecule that is not biologically active. The denatured state can be related to a wide range of structures.
d) We can consider the free energy of a range of conformations that are possible across different physical, thermal and chemical conditions as constructing a free energy topology or landscape that the molecule "explores" to find the global minimum. The general shape of the free energy landscape is of a funnel with the native state at the lowest free energy.
e) Levinthal Paradox refers to a calculation of the number of possible states a protein can take and the time that would be associated with exploring all of these conformations to find the global minimum. It is a paradox that exploring all states even for a simple protein would preclude formation of native state structures in a reasonable time.
f) "Directed process" indicates that protein folding occurs loosely along a given pathway that is preprogrammed in the amino acid sequence.
g) Stop flow kinetics is an experimental method to observe protein folding involving rapidly changing the protein conditions in a flow cell to favor folding and observing the folding after flow has been frozen.
h) Molten Globule is an intermediate state with some secondary structure but little or no tertiary structure. Molten globule is close in size to the native state but lacks most biological function.

2) a)



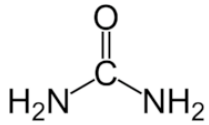
Bonds permanently with cysteine residues to prevent disulfide bonds

b)



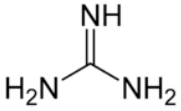
reduce disulfide bonds. At low concentrations it acts to catalyze reformation (mix and matching) of disulfide linkages, at high concentrations it disrupts disulfide bonds (reversibly).

c)



hydrogen bond donor and acceptor can disrupt secondary structure held together by hydrogen bonds such as helices and sheets.

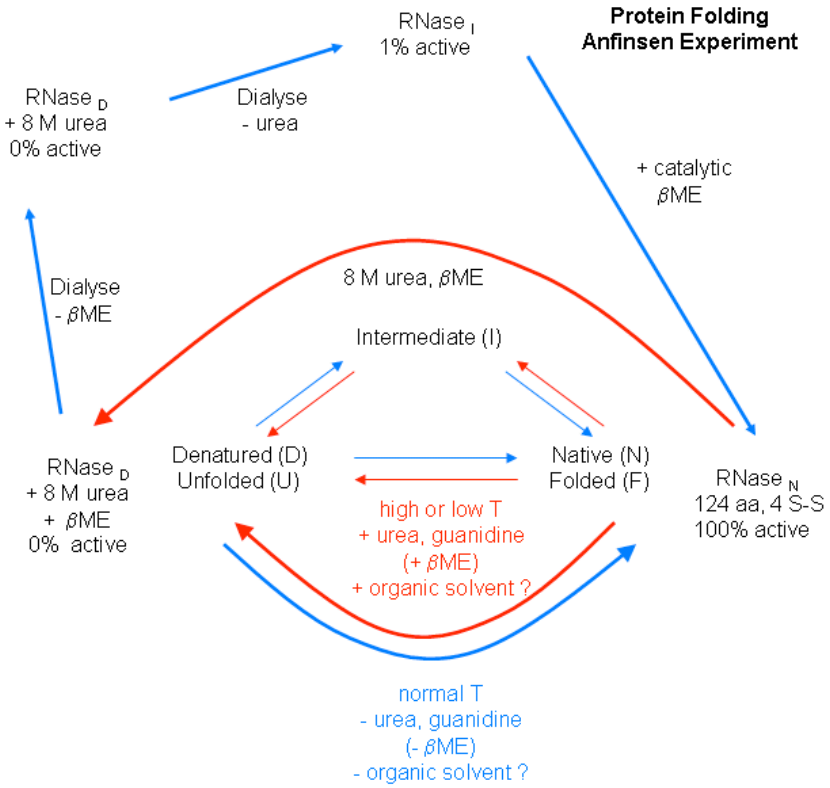
d)

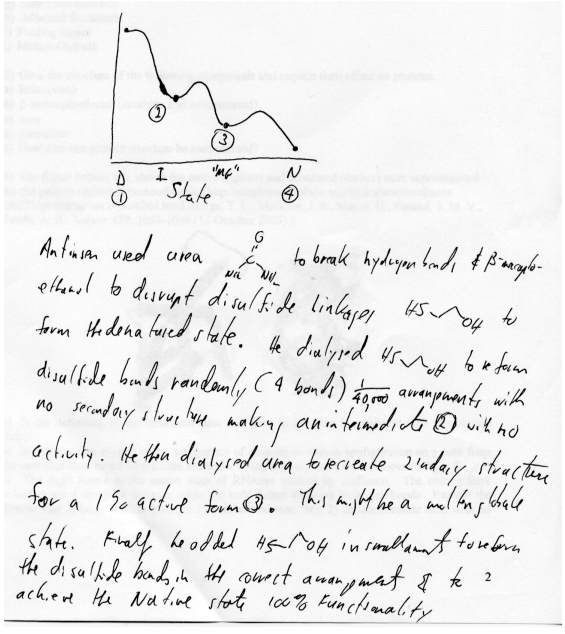


Hydrogen bonding donor. Disrupts secondary structure held together by hydrogen bonds.

e) Protein structure can be manipulated by temperature, pressure, pH, ionic conditions, and recently by mechanical manipulation applied in an AFM using optical tweezers.

Figure: Anfinsen Experiment: Folding of RNase





3) a) The overlap concentration is the concentration within a protein molecule. To calculate ϕ^* we need the mass divided by the occupied volume of the protein. Here we have been given the mass and size. Assuming that the size is the diameter the 5 nm native state has an overlap concentration of 7.6 mg/ml while the 100 nm unfolded state 0.001 mg/ml. Above the overlap concentration significant overlap between different molecules are expected (aggregation is likely).

b) The hydrophobic segments of proteins will aggregate or agglomerate non-reversibly. Inclusion bodies may form that can not be broken apart.

c) While the prokaryotic cells can apparently produce the peptide sequence, the polypeptides can not fold properly in the absence of other features, perhaps including molecular chaperones that provide an *aggregation-free-zone* where proteins can fold into their native states.

d) There are two main types of molecular chaperones. Heat shock proteins (70 kg/mole) Hsp-70, and chaperonins. Heat shock proteins act as the polypeptide chain emerges from the ribosome site binding to hydrophobic sections of the polypeptide. Heat shock proteins act as single protein chains not as quartenary structures. They bind with peptides when ATP is also bound and release proteins after cleaving ATP, using energy. Chaperonins are smaller Chaperonin-60 and chaperonin-10 for instance, but act in large quartenary structures, for instance GroEL and GroES discussed in class. Chaperonins act away from the ribosome, for example in the mitochondria or other cellular structures. Chaperonins also bind ATP and cleave ATP on releasing the native state proteins.

e) Chaperonins enable hydrophobic interactions that couldn't occur in the cytoplasm since folding that leads to hydrophobic interactions often involves revealing hydrophobic segments of the polypeptide to the cellular environment where these sites would likely bond with other hydrophobic groups on other proteins and lead to aggregation.