071003 Quiz 2 Morphology of Complex Materials

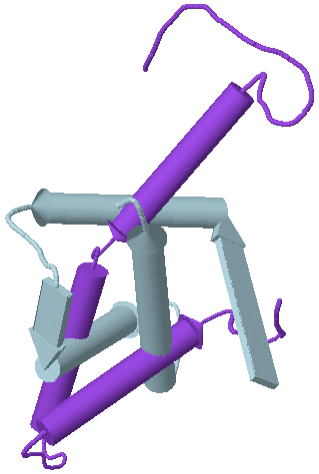
1) Explain the following terms: (for states comment on biological activity and relative size of the structure)

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.

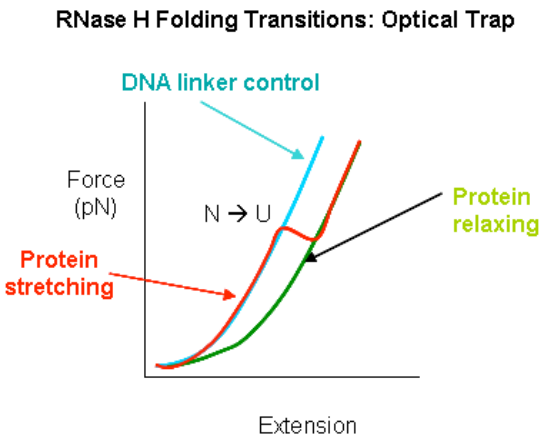
a) Iodoacetate
b) β-mercaptoethanol (catalytic and concentrated)
c) urea
d) guanidine
e) How else can protein structure be manipulated?

3) The figure below, left, shows the native (lighter) and denatured (darker) state superimposed for the protein engrailed homeodomain (http://employees.csbsju.edu/hjakubowski/classes/ch331/protstructure/olprotfold.html Religa, T. L., Markson, J. S., Mayor, U., Freund, S. M. V., Fersht, A. R. *Nature* **437**, 1053-1056 (13 October 2005).)



enHD (2 superimposed states)

RNase



Extension with DNA "Handle"

a) Is the definition of the denatured state you gave in question 1 consistent with this picture (left)?

b) The middle figure is the native state of RNase studied by Anfinsen. The oblong dark cylinders are 4 disulfide linkages while the bold dotted lines are hydrogen bonds. Explain the process that Afinsen took 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.

c) Sketch a free energy diagram along this sequence of conformational changes.

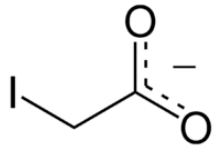
d) Explain the conformational changes that may occur between steps 3 and 4.

e) The plot to the right shows force versus extension for RNase held in an atomic force microscope cantilever by two DNA chains. The DNA extension is shown as the highest curve (light shade) while the Protein stretching crosses from this curve to the protein relaxing curve about half way on the force axis. Is this result consistent with your free energy diagram of part c? If it differs resketch the free energy diagram including features from this plot.

ANSWERS 071003 Quiz 2 Morphology of Complex Materials

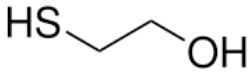
1) a) Native state is the biologically active state, generally the most compact structure observed in a folding sequence. The native state has essentially 0 conformational entropy.
b) Unfolded state is a state with no significant secondary structure, generally the least compact structure possible with 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 Gobule 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)



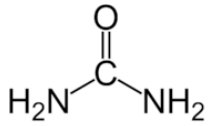
Bonds perminantly with cisteine residues to prevent disulfide bonds

b)



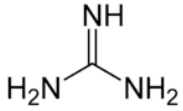
reduce disulfide bonds. At low concentrations it acts to catalyze reformation (mix and matching) of disulfide linkages, at high concentrations it disrupts disulfide bonds (reversibly).

c)



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

d)

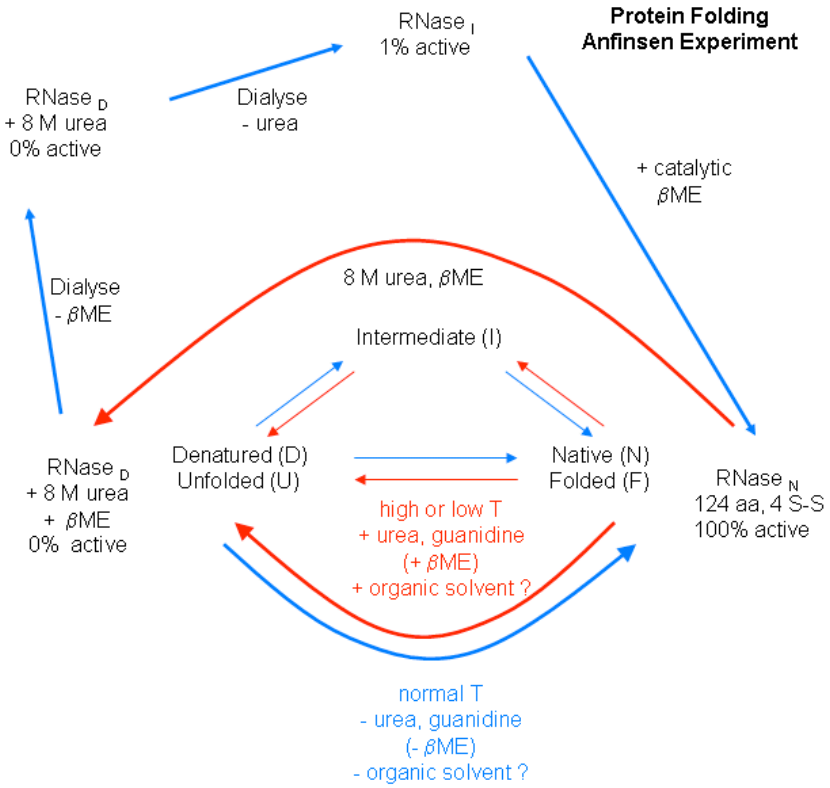


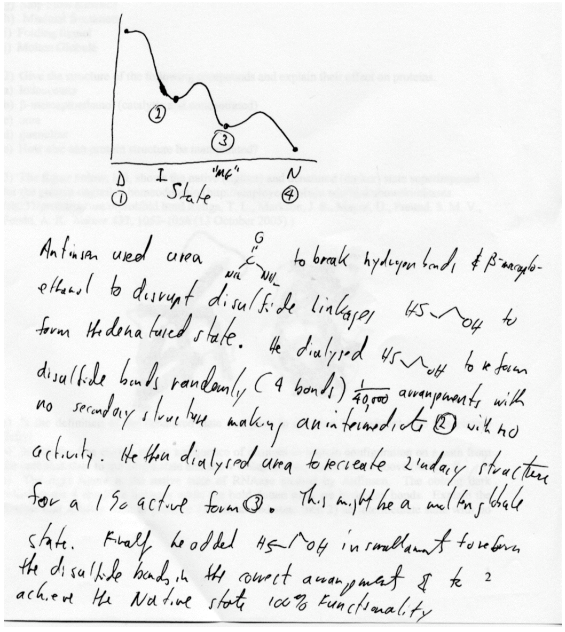
Hydrogen bonding donor. Disrupts secondary structure held together by hydrogen bonds.

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

3) a) The state shown as denatured is an intermediate state that may have some biological function since it is very close to the native state in size and secondary structure so it is probably not consistent with a fully denatured protein description above.
b) c)

Figure: Anfinsen Experiment: Folding of RNase





d) Between steps 3 and 4 the disulfide linkages are shuffled and reach the lowest energy conformation of the native state. This is achieved with catalytic amounts of β-mercaptoethanol.

e) The plot is somewhat different since this does not involve chemical denaturing but a physical process where the chain is strained from the two ends. The published free energy diagram is shown below. The large double cross peak corresponds to the major shift from the DNA mechanical propert curve. A smaller shift between I and U is not visible in the force plot without magnification of the scales.