060113 Quiz 1 Morphology of Complex Materials

1) Explain how a protein displays structural hierarchy.
   a) List 4 levels of structure for a protein
   b) Describe each of these levels of structure
   c) Explain what self-assembly means in the context of a protein.
   d) How is structure related to function in a protein? (For example in a membrane protein.)
   e) How could evolution act on the structural hierarchy of a protein?

2) Amino acids are the mer units of a protein.
   a) Give the generic chemical structure for an amino acid (labeling the alpha carbon).
   b) Show how a condensation reaction could proceed between two amino acids to result in a dipeptide. Label the C and N ends of this dipeptide.
   c) Cystine is an important amino acid. Sketch the structure of cystine and explain the importance of cystine in protein structure.
   d) Proline is an important amino acid. Sketch the structure of proline and explain the importance of proline to protein structure.
   e) Give the structure of glycine and explain where glycine units might occur in the secondary structure of a protein.

3) In polymers and in proteins bond rotation angles have been found to govern, *to some extent*, chain conformation. In polymers bond rotation angle is the basis of the rotational isomeric state model (RISM) of Flory that, *through matrix math*, describes short-range interactions; and in proteins bond rotation angles are the basis of Ramachandran plots for elucidation protein conformation. (We will, *hopefully*, see that these two implementations of bond rotation angle have some similarities but also striking differences which parallel the similarities and differences between Polymer Science and Structural Biology.)
   a) Sketch a protein chain labeling the three bond rotation angles of importance to chain conformation.
   b) Explain why one of these angles is generally ignored in the Ramachandran plot.
   c) The following plot (Figure 1 below) shows a Ramachandran plot for a protein displaying a prominent feature (upper left black enclosing line and black crosses) and a weaker feature (central left black enclosing line and grey crosses). The little squares to the right are glycines. Explain what these two features are and why glycines might be located away from these two features.
   d) What typically holds an α-helix or a β-sheet together? Show how this feature is shown in Figures 3 and 4 below and explain what the structures are (Label the atoms you can).
   e) The native state of protein G is shown to the right below, Figure 2. Where would you expect hydrophobic and hydrophilic groups to occur in this structure? Sketch one hydrophobic and one hydrophilic amino acid which might be expected in these two regions of the protein.

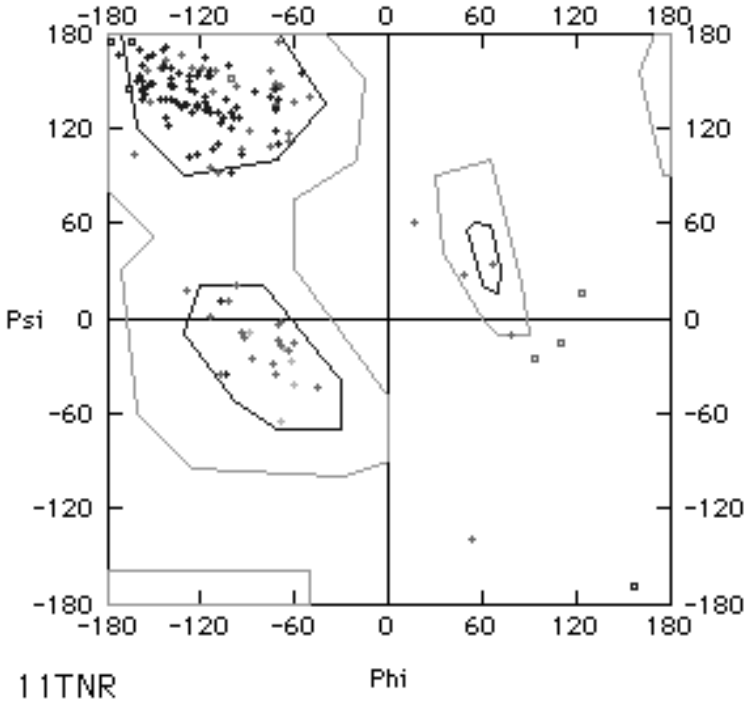


Figure 1

Figure 2

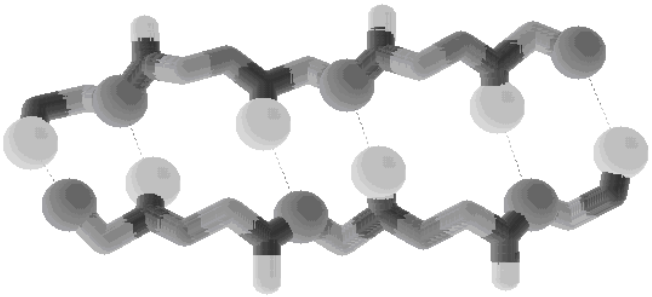


Figure 3

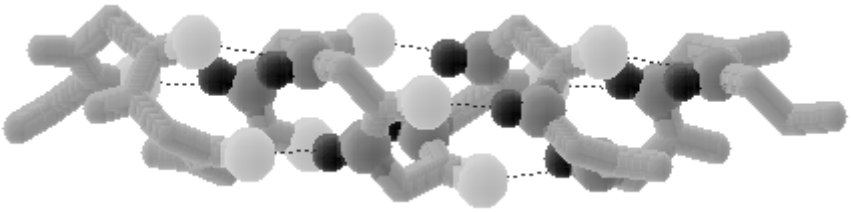


Figure 4

ANSWERS: 060113 Quiz 1 Morphology of Complex Materials

1) a) Primary Structure- Sequence arrangement of amino acids
Secondary Structure- Hydrogen bonded structures such as alpha helix, beta sheet and super secondary structures such as beta barrels.
Tertiary Structure- Larger scale structures composed of secondary structures bonded by disulfide bonds, by hydrophilicity, hydrogen bonds and by static charge. Tertiary structure defines the difference between a folded native state protein and an unfolded protein. Tertiary structure refers to the final native state of a single protein molecule.
Quaternary Structure- Generally a collection of proteins and other organic and inorganic species that are bound by hydrogen bonds, hydrophilicity and static charge to produce a single functional unit such as a ribosome.

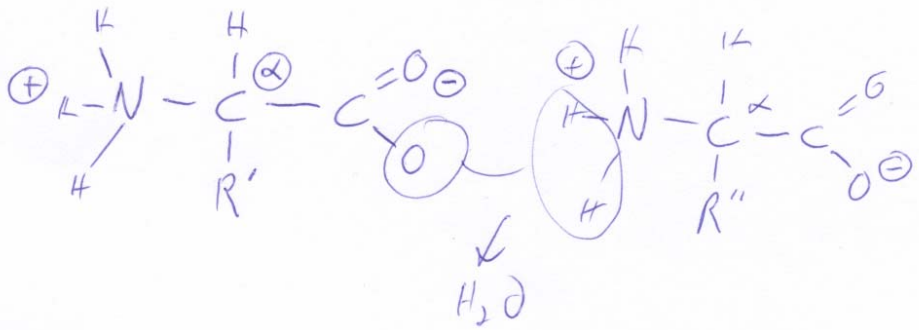
b) above

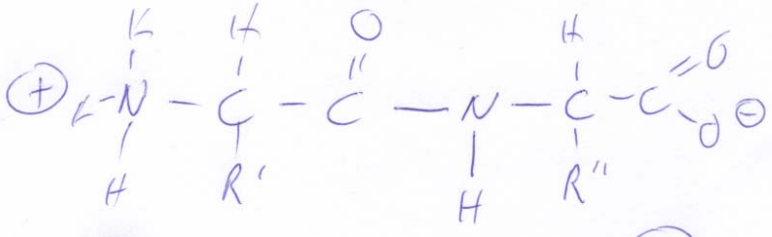
c) Self-assembly, in the context of a protein, indicates that once the amino acid primary structure is produced at the ribosome there is limited external organizational control over the formation of secondary structures by hydrogen bonds, or of tertiary structure. The tertiary and quaternary structures of the final protein are encoded to a large extent in the amino acid sequence and the higher level structures assemble themselves.

d) The spatial location of hydrophilic and hydrophobic groups in the native state folded protein decided by the primary, secondary and tertiary structure determines the functionality of a protein. For example, a membrane protein might be expected to display two hydrophilic end domains with a hydrophobic central domain that could be embedded in an amphiphilic membrane. The quaternary structure might involve a beta barrel with a hydrophilic core that could allow transport of polar molecules through the cell membrane.

e) Evolution simply observes the functionality of basically random modifications of the amino acid sequence associated with random modification of DNA in terms of survivability of the organism as a whole. Enhancement in survivability encourages a particular modification (mutation) since the carrier of this modification (organism) will be more likely to reproduce and pass on the modification to later generations. The evolutionary model has become a major pathway to understanding protein morphology (generally 20% of most current textbooks on proteins).

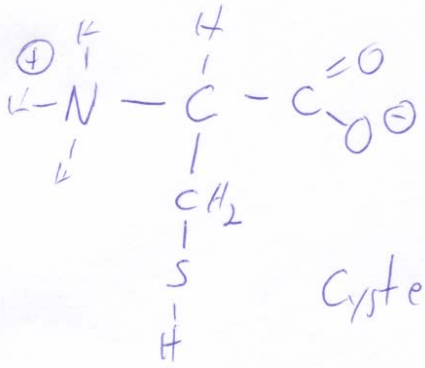
2a)





N C

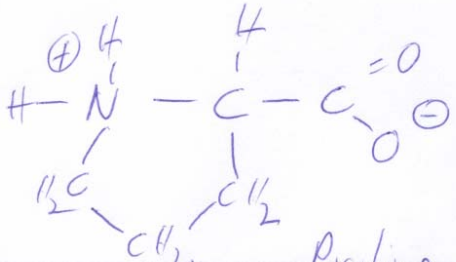
b)



Cysteine

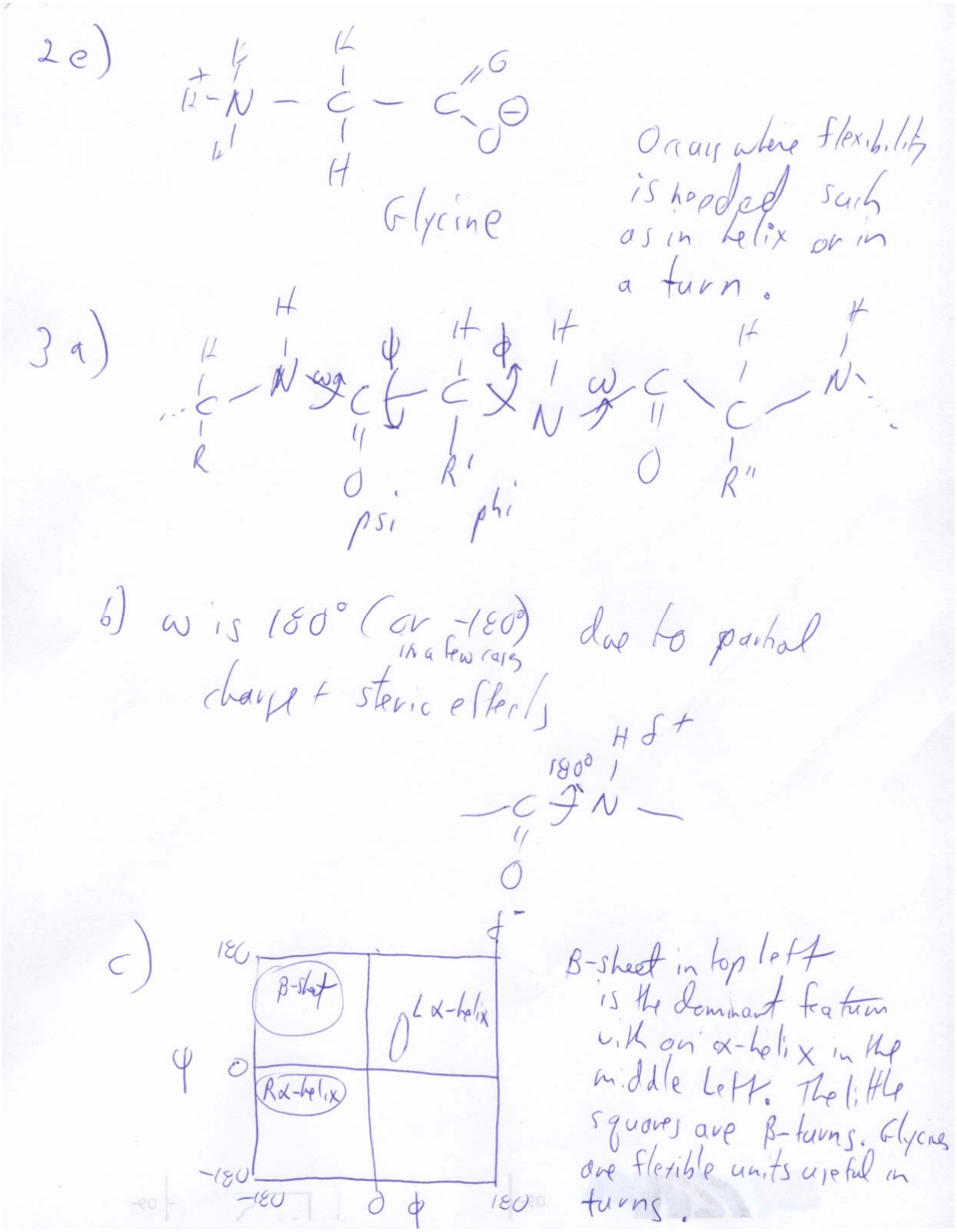
Impurtant is Disulfide bonds occur between Cysteine units along the chain

d)



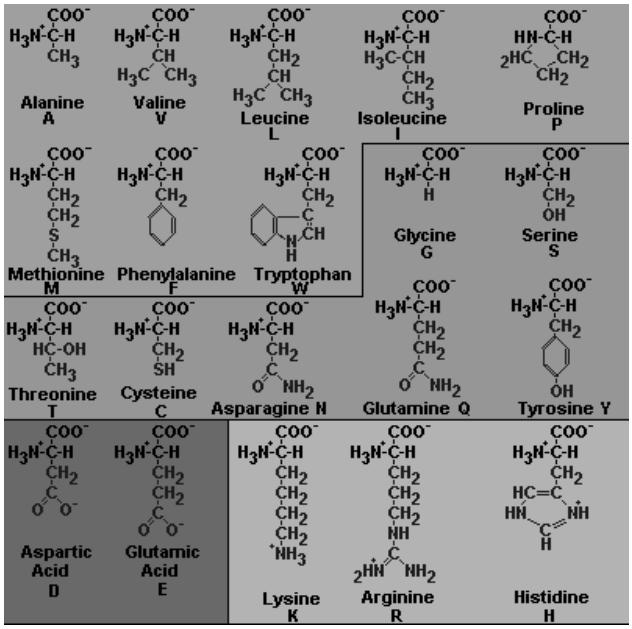
Proline

$\phi = 180^\circ$
Fixed bond angle
Rigid structure
Breaks α-helix



d) Dashed lines are hydrogen bonds between NH, dark grey ball and O, Light ball end of dashed line. Top are two Beta strands hydrogen bonded together and bottom is an apha helix.

e) Hydrophobic where the two structures touch, hydrophilic towards the outside of the structure.



Top group are hydrophobic. All others are hydrophilic.

060123 Quiz 2 Morphology of Complex Materials

1) For a protein of N = 100 amino acid residues with j ~ 8 conformations (rotational isomeric states) per residue the number of possible conformations for the protein is j^N or about 10^{89} [Pande papers on the web page]. All but one of these states corresponds to the non-native or unfolded state. It would require 10^{66} years to explore all of these conformations [R. H. Pain, *Mechanisms of Protein Folding*, Oxford Press 1994].
   a) Explain how the concept of an energy landscape can be used to resolve this problem with protein folding.
   b) We saw several simulation results that show proteins "exploring" the energy landscape. Describe what "exploring" means in this context.
   c) What distinguishes the "native state" from the "unfolded state"?
   d) Explain how Anfinsen used β-mercaptoethanol, urea and RNAase to demonstrate an intermediate state in the folding of RNAase to the native state. (Show the structure of β-mercaptoethanol and urea and explain how β-mercaptoethanol could interact with RNAase to control folding.)
   e) What are stopped-flow and continuous flow kinetics? Explain the problems associated with these two experiments.

2) On Friday we considered circular dichroism as a method to monitor protein folding.
   a) What is the difference between linearly-polarized and unpolarized light?
   b) Explain how plane-polarized light can be produced from two circularly polarized beams.
   c) For linearly polarized light explain the difference between absorption and refraction. (Absorption coefficient and the index of refraction).
   d) For plane polarized light that is passed through a protein solution explain the state of polarization of the exiting light and explain why the exiting light has this state of polarization.
   e) List 2 other methods that can be used to monitor protein folding.

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?

ANSWERS: 060123 Quiz 2 Morphology of Complex Materials

1) a) The energy landscape, as shown by Pande and Rokhsar [*Proc. Nat. Acad. Sci*. **95** 1490 (1998)], indicates that regions of conformation exist that are highly preferred energetically and that the protein molecule will gravitate towards these regions as it explores the energy landscape. It has been found from computer simulations that the energy landscape forms a kind of funnel where the protein can reach the lowest energy, native state by a number of different paths along this funnel. Local energy minima along this pathway might include various intermediate states and particularly a disorganized dense state referred to as the Molten Globule which has been referred to by Pande as a "liquid" state compared to the "crystalline" native state. In this line of thought the unfolded state is a "gas". Pande shows the energy landscape as a plot of number of "native" contacts" versus total number of contacts.

You should notice several inconsistencies in Pande's proposal that the native state/molten golubule/and unfolded state correspond to states in normal atomic or molecular systems. First, normal systems display entropy associated with free motion of subunits, that is, a gas molecule has translational entropy that is significantly larger than that associated with a mer unit in a polymer. Secondly, Pande's approach, although giving lip service to Polymer Science, largely ignores a large body of literature, a field in fact, that has extensively dealt with the physical chemistry of phase transitions in chain molecules including at least 4 noble prizes. This would seem to be a major oversight even for a biologist. The unfolded state is a liquid state and not a gas state. The molten globule and native states involve only one molecule so these are not phase transitions but isomeric states of a molecule. There are many tracks to attack what has been published by Pande and you should mention something of this from your previous training in Materials Science. The analogy with nucleation of phases in the *Current Opinion in Structural Biology* **8** 88 (1998) article is particularly troubling if you think about the details of this comparison and if you have any knowledge of homogeneous nucleation theory. There is a reason (you should recognize quickly) that Pande goes no further than to suggest this idea. (This does not mean that you do not appreciate Pande's contribution.)

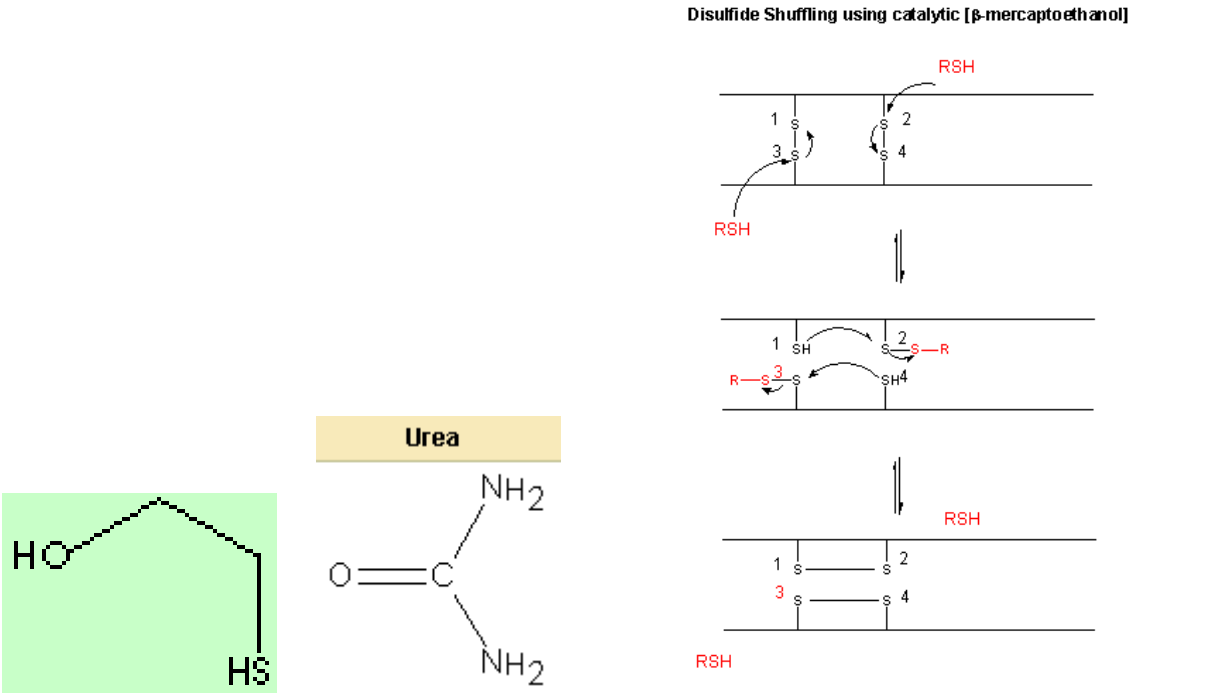
b) Exploring the energy landscape, in the context of Pande's plots of fraction of native contacts versus total number of contacts would mean swapping of partners between binary interactions of residues in the protein in a more or less random way except that the energy of the resulting state would be used to give the probability of that swap happening. This approach is common in Monte Carlo conformational simulations of synthetic polymers for instance and has been commonly used for more than 35 years. In terms of 2d graphics this probing of the energy landscape becomes a molecular dance. the polypeptide chain shakes and translates until it finds native conformations or low energy conformations that lock in parts of the chain. Finally, we usually see one part of the molecule that swings wildly, even 180 degree swings of certain residues to give the final native state. This was the antennae in the simulation we saw in class. The final native state is locked-in due to the low free energy though some slight structural vibrations can be seen.

c) The native state is defined by biological functionality. If the protein is active it is in its native state. It is also implied that the native state is the lowest energy state, generally

the smallest and densest packed state. The unfolded state is similar to a synthetic polymer in solution except that certain secondary structures exist in the unfolded state making the structure simpler and easier to recognize than a true random state such as would be seen in a synthetic polymer. Put differently, the unfolded state displays large and more irregular chain persistence compared to a normal polymer.

d) Anfinsen chose a simple protein that had 4 disulfide bonds that held the tertiary structure together in the native state, RNAase. He understood that he could denature this protein by breaking these disulfide bonds using urea. Mercaptoethanol is capable of bonding to these cystine groups to lock-out disulfide bonds. The mercaptoethanol can also destabilize a bonded intermediate state if a small amount of mercaptoethanol is added. Using this tool Afinsen was the first to reversibly denature and refold a protein to a native state structure making the mystery of tertiary structure controllable.

Figure: CATALYTIC SHUFFLING OF DISULFIDES WITH BETA-MERCAPTOETHANOL



e) Stopped-flow experiments are intended to study protein folding generally using spectroscopic techniques, circular dichroism or flourescense. In stopped flow the unfolded protein (perhaps too acidic to fold) is mixed rapidly (1 ms) with a diluent or other solution that encourages folding, the mixed solution is then flowed into an observation chamber. This apparatus is used due to the high cost of pure protein. It is the most expedient method with a small amount of protein. A continuous flow experiment allows for a more rapid observation of the folding but at the cost of using much more protein. There is a problem with using smaller tubes in a continuous flow experiment in that mixing is not effective in tiny tubes and it is difficult to have high linear velocities in small tubes. For the continuous-flow experiment the observation time is only limited by the velocity so folding on micro-second time scales are apparently possible.

2) a) The electric field vector for linearly polarized light is normal to the direction of propagation and restricted to a plane that includes the direction of propagation. For unpolarized light the electric field vector is normal to the direction of propagation but is not restricted otherwise.

b) If two circularly polarized light contain one component (y for instance) that is out of phase by +90° while the other circularly polarized light contains the same (y) component that is out of phase by -90° then summing the two circularly polarized beams will lead to a plane polarized beam in the x-direction.

c) Absorption leads to a reduction in the amplitude (and intensity) of the light. Refraction changes the speed and leads to a phase shift when compared with a non-refracted beam.

d) The protein solution displays circular dichroism so plane polarized light becomes elliptically polarized after passing though the solution. This is understood by considering that the plane polarized light can be decomposed into two circularly polarized beams of opposite phase (180 degrees for the y component for instance). The circularly dichroic solution preferentially absorbs one of the two circular polarized components leading to a phase shifted elliptically polarized beam. The major axis of polarization is offset from that of the plane polarized incident beam due to circular birefringence.

e) UV absorption and fluorescence are also used.

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.

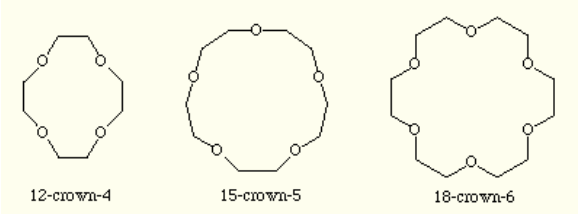
c) While the prokaryotic cells can apparently produce the peptide sequence, the polypeptides can not fold properly in the absence of other features, perhaps including molecular chaperones that provide an *aggregation-free-zone* where proteins can fold into their native states.

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.

060201 Quiz 3 Morphology of Complex Materials (*10 points each part*)

1) In class we discussed three types or classes of quaternary structure in proteins depending on the composition of the quaternary structure.
a) ;b) ;c) Describe these three types of protein quaternary structure and give an example of each. (Give as much detail as you feel comfortable with but spend no more than 5 minutes on each, a paragraph).
d) Crown ethers are organic chemicals used to chelate a metal ion (coordinate the metal ion to make it soluble in hydrophobic environments for instance. The metal ion goes in the center of the crown ether ring. Crown ethers can be integral components of hybrid catalysts for instance.



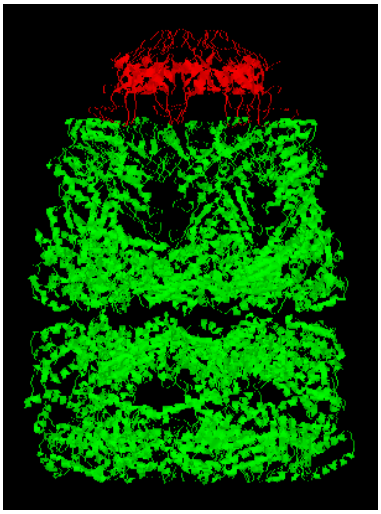
Hemoglobin contains organic molecules that have similarities to crown ethers. Give the name of the class of molecules (rings) that these molecules belong to and explain the importance of these molecules to the function of hemoglobin.

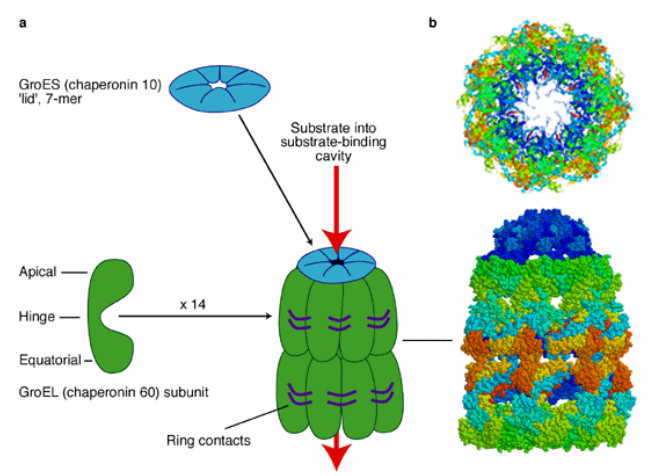
2) In class we compared protein and polymer hierarchies.
a) Polymers display short range and long range interactions. Explain what distinguishes short and long range interactions.
b) Give analogies in proteins for short and long range interactions in synthetic polymers.
c) If quaternary structure is view as a structure resulting from the interaction of multiple protein molecules, what topic in polymers relates to quarternary structure in proteins?
d) Explain why the average vector, $\langle \mathbf{r}_{i+1} \rangle_{Gaussian} = 0$, reflecting the average direction and magnitude of a step from a fixed chain step, "i", for a Gaussian (random walk) chain; while $\langle \mathbf{r}_{i+1} \rangle_{SRI}$ has a finite value, reflecting the average direction and magnirute of a step from a fixed chain step, "i", for a chain with short range interactions. How do short range interactions affect persistence length?
e) Explain how restrictions on bond rotation (rotational isomeric state theory) could lead to a larger persistence length in synthetic polymers (using your answer to part d).
f) Describe the similarities and differences between a beta sheet and the planar zig-zag conformation of polyethylene.

***ANSWERS:* 060201 Quiz 3 Morphology of Complex Materials**

1) a) Three types of quaternary structure:

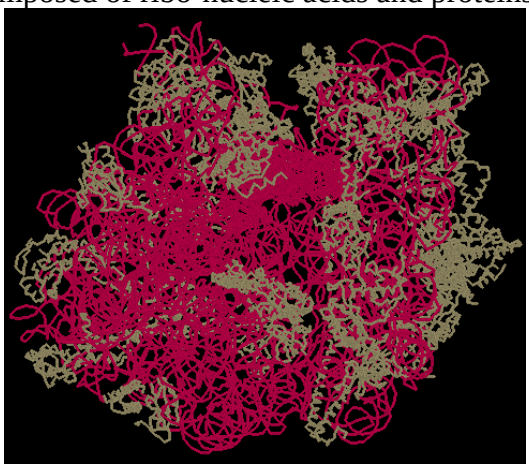
Quaternary structures composed of multiple polypeptide chains such as Chaperonin. This is composed of two units a small unit (top) and a large unit (bottom), GroES (7 protein cap) and GroEL (14 protein tube in 2 rings of 7 large proteins)





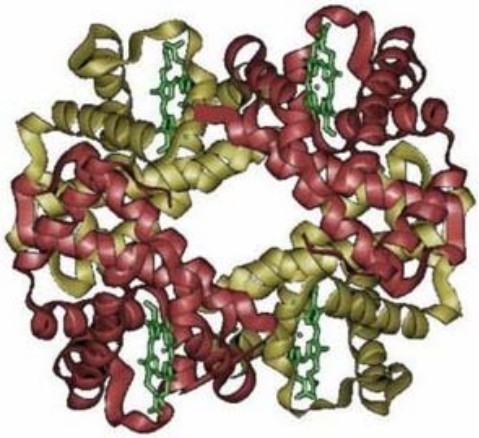
Structure of chaperonin 10 and chaperonin 60
Expert Reviews in Molecular Medicine ©2000 Cambridge University Press

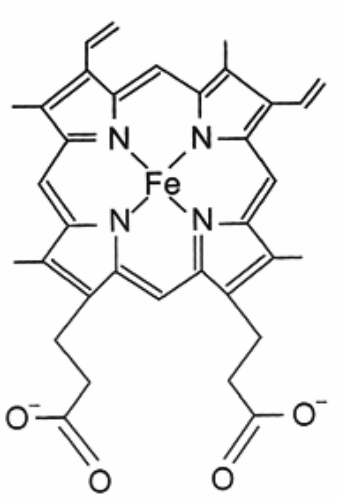
b) Quaternary structures composed of ribo-nucleic acids and proteins such as the ribosome.



The ribosome model above shows RNA molecules in red or dark and proteins in grey.

c) Quaternary structures composed of proteins and metal ions such as hemoglobin that contains the heme chelating-agent and iron. Hemoglobin is composed of 2 pairs of identical proteins, α and β, as shown below and 4 heme-molecules with 4 iron ions.





d) The heme molecule contains a porphyrin ring that chelates iron (above). The porphyrin ring locks iron in hemoglobin. Iron bonds reversibly with oxygen and carbon dioxide depending on the local and relative partial pressures of these two gasses. In high oxygen partial pressure at the lungs carbon dioxide is released an oxygen bonded. At the cell with high carbon dioxide and low oxygen, oxygen is released and carbon dioxide is bonded.

2) a) If a linear polymer chain is indexed from 1 to N then interactions between two units of similar or neighboring index are called short range interactions. Interactions between two subunits (mers) of widely different index are called long range interactions. Short range interactions occur between units that have a memory of each others direction and because of this lead to larger persistence length. Long range interactions lead to global changes in chain scaling.

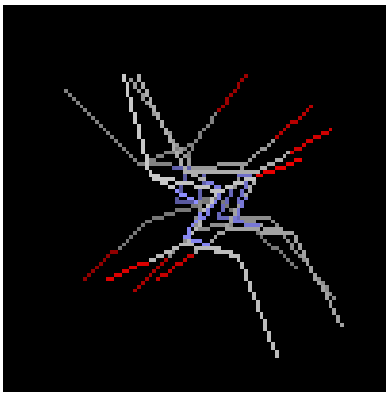
b) An alpha helix involves hydrogen bonding between peptide units of similar index and the result is a large rod like unit, the helix. The alpha helix is analogous to short range interactions. Disulfide bonds between cystine units of widely different index are analogous to long range interactions leading to changes in the global structure.

c) Quaternary structure in polymers could relate to coil overlap in semi-dilute and concentrated solutions. Coil scaling transitions at close to the overlap size might be considered a type of disordered quaternary structure. You might also consider crystalline lamellae as a kind of quaternary structure.

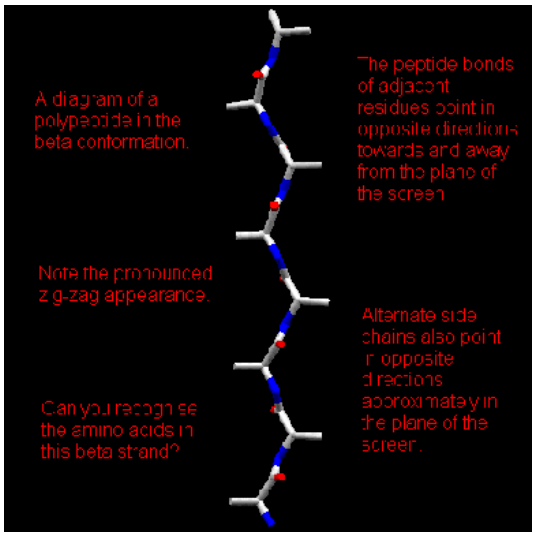
d) $\langle \mathbf{r}_{i+1} \rangle_{Gaussian} = 0$ since there is no memory of the direction $\mathbf{r}_i$ in the choice of direction $\mathbf{r}_{i+1}$ for a totally random walk. The average value of a random vector is 0. If the backward step is not allowed then $(z-1) \langle \mathbf{r}_{i+1} \rangle_{SRI} = \langle \mathbf{r}_{i+1} \rangle_{Gaussian} + \mathbf{r}_i$. So, $\langle \mathbf{r}_{i+1} \rangle_{SRI} = \mathbf{r}_i/(z-1)$, where z is the coordination number. The correlation between site "i" and site "i+1" leads to an increase in the persistence length, $b^2_{SRI} = zb^2_{Gaussian}/(z-2)$.

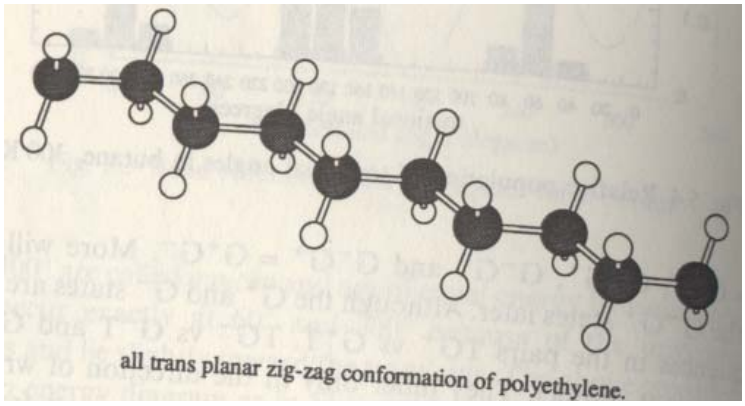
e) For a freely rotating chain z is larger than for a chain with restrictions on bond rotation. Similarly, for a chain with fixed bond angles z is smaller than a chain with free angle bonds. The reduction in z towards 2 leads to a larger persistence length, b.

f)



Beta Strand





Both the beta-strand and the planar zig-zag are similar conformations. The beta strand is not planar but is as close to planar as is possible in the polypeptide chain. Beta strands can hydrogen bond with other beta strands to form a beta sheet and the planar zig-zig zag can crystallize in polyethylene forming an orthorhombic unit cell that is similar to the anti-parallel bonding of beta-strands.

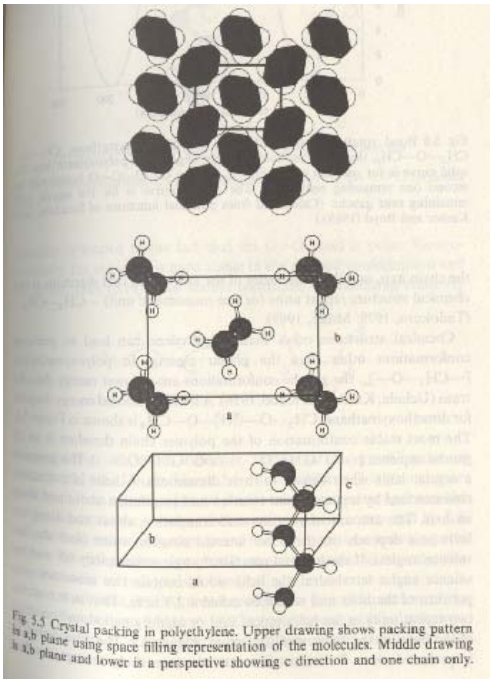
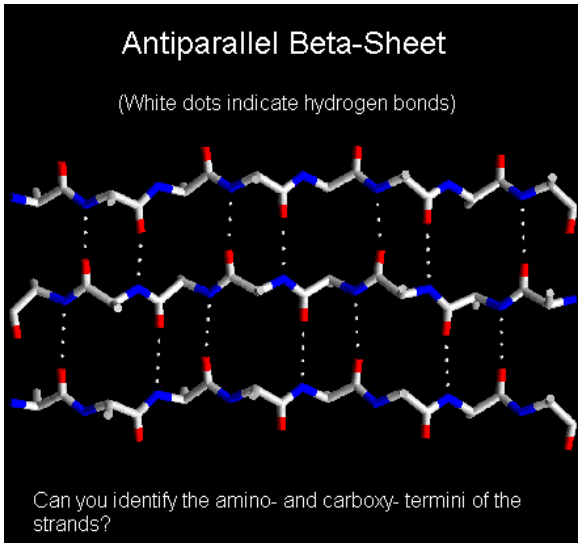


Fig. 3.5 Crystal packing in polyethylene. Upper drawing shows packing pattern in a,b plane using space filling representation of the molecules. Middle drawing is a,b plane and lower is a perspective showing c direction and one chain only.



060208 Quiz 4 Morphology of Complex Materials

A linear polymer in a semi-dilute solution displays three levels of structural hierarchy: persistence level, self-avoidance scaling level (SAW) and the screened interaction level.

1) a) Explain in general terms how tacticity effects chain persistence.
   b) What is the base unit of tacticity. Define the possible states for this unit of tacticity.
   c) Why is this base unit not used to describe tacticity?
   d) Give the triad tacticity for an atactic polymer.
   e) Define heterotactic.

2) Chain scaling in dilute solutions can display only two possible states.
   a) Briefly define these two states.
   b) How can you obtain the *Gaussian*-scaling law using a derivative of a probability function?
   c) Why is a chain obeying this scaling law called a Gaussian chain? Why is it called a Brownian chain? Why is it called a random walk coil?
   d) Show how the approach of part b can be modified for a self-avoiding walk.
   e) Kohn et al.[1] have shown that many proteins in the unfolded state display SAW scaling. Explain the following plot that Kohn uses for evidence.

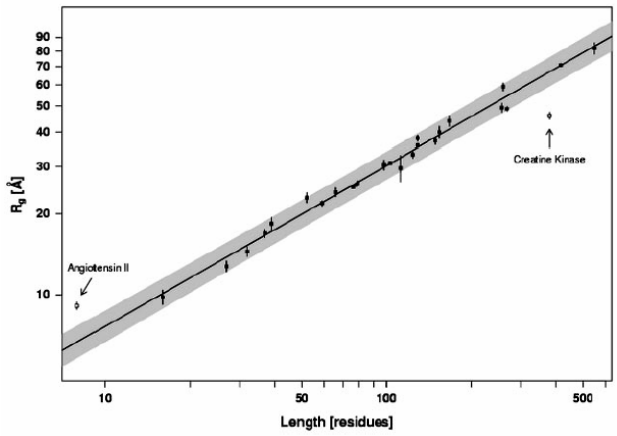


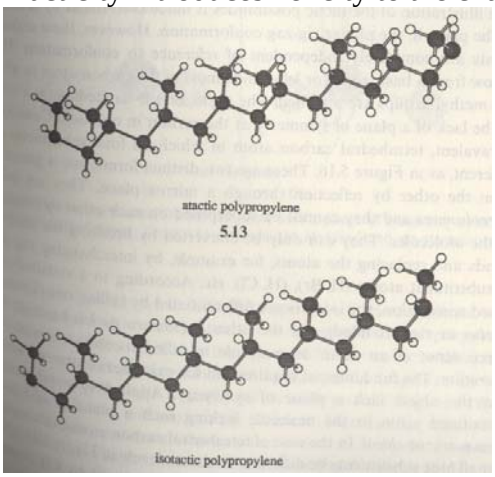
Fig. 1. The R_G of the large majority of chemically denatured proteins scale with polymer length, N, by means of the power-law relationship $R_G = R_0 N^{\nu}$. Two statistically significant outliers, creatine kinase and angiotensin II, are indicated. The solid line, which is the least-squares fit ignoring the two potential outliers, produces an exponent, $\nu = 0.598 \pm 0.028$ (95% confidence interval), that is indistinguishable from the 0.588 predicted for an excluded-volume random coil. The shaded region represents the 95% confidence intervals for future measurements, assuming that the errors about (log)R_G are normally distributed around the fitted relationship. Only the measurements for creatine kinase and angiotensin II fall outside this predictive interval, and, thus, only these measurements can be said to represent unambiguously significant deviations. Error bars indicate the reported experimental (i.e., standard) deviations of the sample. These were derived by using a variety of approaches and widely varying numbers of observations and therefore provide only an approximate indication of experimental precision.

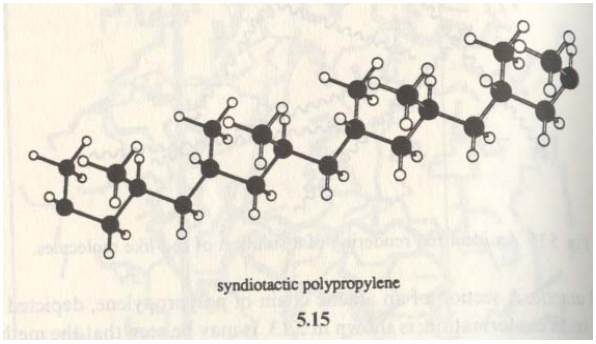
3) The concept of screening of interactions was developed by Debye for charged colloids. Debye developed the idea of a screening length that describes the distance over which interactions (such as static charge repulsion or attraction) are felt.
   a) Does the screening length increase, decrease or not change with concentration? (Explain your answer with a brief description of the nature of screening).
   b) In a polymer coil write chain scaling laws for sizes above and sizes below the screening length?
   c) Does the screening length, ξ, depend on the molecular weight of the polymer, N? Explain your answer.
   d) In the function $\xi = R_{F,SAW}\,(c/c^*)^P$ define the overlap concentration c* in terms of N, the number of persistence steps in a coil.
   e) Using the function for $R_{F,SAW}$, from (*2a*); your answer to (*3c*); and your expression for c* in (*3d*), solve for P in the expression for ξ in (*3d*).

1) Kohn JE, Millett IS, Jacob J, Zagrovic B, Dillon TM, Cingel N, Dothager RS, Siefert S, Thiyagarajan P, Sosnick TR, Hasan MZ, Pande VS, Ruczinski I, Doniach S, Plaxco KW *Random-coil behavior and the dimensions of chemically unfolded proteins. Proc. Nat. Acad. Sci.* **101** 12491-12496 (2004).

ANSWERS: 060208 Quiz 4 Morphology of Complex Materials

1) a) Tacticity introduces helicity to the chain as shown by *Paul Phillips* and Boyd below,





The interactions that lead to a regular helical structure are short range interactions and these directly control the coordination number *z*. The persistence length increases with reduction in *z* following, $b_{SRI}^2 = b^2\, z/(z\text{-}2)$.

b) The base unit of tacticity is a diad (two mer units). There are two possible states for diads, meso and racemic (m and r). Meso means that the two mer units have the same handedness, that is going along the chain the substitutions are made in the same rotational order (clockwise or counter-clockwise for instance). Racemic means that the substitutions are made in opposing rotational order.

c) NMR is the analytic technique that is sensitive to tacticity. The smallest unit of tacticity that NMR can resolve are pairs of diads or triads. A triad is composed of three mer units.

d) There are three types of triad tacticity units: *isotactic*, composed of mm triads; *syndiotactic*, composed of rr units; and *heterotactic* composed of mr and rm triads. Since there are two options to make heterotactic the distribution in a random or atactic sample has i:h:s of 25:50:25 %.

e) Heterotactic triads are composed of diads of mr and rm pairs.

2) a) Random walk state where $R \sim N^{1/2}$ b and the self-avoiding walk state (SAW) where $R \sim N^{3/5}$ b.
b) Setting the derivative with respect to the end-to-end distance R for the Gaussian probability function equal to 0 and solving for R,

$$P(R)\, dR = K\, R^2 \exp(-3R^2/(2\sigma^2))\, dR \quad \text{where } \sigma = N^{1/2}b$$

c) It is called a Gaussian chain because it is based on the Gaussian probability function. It is called a Brownian chain because it is the chain that would be obtained if the path of Brownian motion were solidified in a polymer, it is a random walk coil since it is the coil that would be obtained if a totally random walk were solidified in a linear chain.

d) The Gaussian probability function is modified by the probability of the chain not intersecting itself $p(R) = (1 - V_c/R^3)^{N\wedge 2/2} \sim \exp(-N^2V_c/(2R^3))$ for $(V_c/R^3) <<1$.

e) $\ln(R_g) = (3/5) \ln(N_{Residues})$ is the coil scaling law for a SAW chain. This might be expected for a protein in water.

3) a) Screening involves the blocking of interactions by other units of the same material (or a different material). For the case of LRI's we consider *inter*-chain interactions and *intra*-chain interactions. Increasing the concentration leads to more inter-chain interactions that block intra-chain interactions (LRI's). The blocking of interactions occurs to a greater extent at large size scales where there is a higher probability of inter-chain interference. The higher the concentration the smaller the screening length since the average separation distance between different chains becomes smaller while the average separation distance between mers in the same chain remains roughly the same.

b) Above the screening length the excluded volume is screened so the coil is Gaussian, $R \sim b_1N_1^{1/2}$. Below the screening length the chain has excluded volume so , $R \sim b_2N_2^{3/5}$. Subscripts indicate that the definition of the substructure and the number of these substructures in the chain changes with the scaling regime following renormalization of the chain.

c) The screening length, ξ, can not depend on the molecular weight since it is a size smaller than the coil size.

d) $c^* = N/R_{F,SAW}^3 = N^{-4/5}/b^3$

e) $\xi = R_{F,SAW} (c/c^*)^P = c^P N^{3/5+4P/5} b^{3P}$
so P = -3/4 since the power of N must be 0.

060215 Quiz 5 Morphology of Complex Materials

Long chain molecules display hierarchy in structure for solutions as well as in the melt. We have also seen that these molecules display hierarchy in dynamic response.

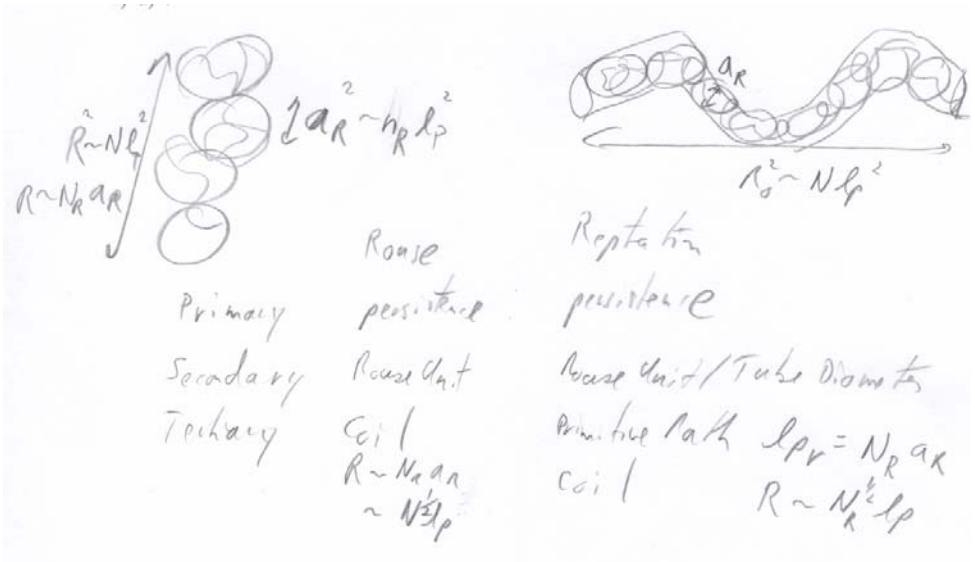
1) a) Compare the hierarchy of a Rouse chain to the hierarchy of a reptating chain by sketching a model of the chain and explaining the different dynamic levels.
b) For each of the levels of dynamics you gave in part "a", explain using mostly words how the level would respond to random thermal vibrations.
c) Modulus, E, describes the static response of a system while viscosity, η, describes the dynamic response and the ratio of dynamic to static response yields a time constant, $\tau \sim \eta/E$. Explain how a similar approach is used for a Rouse unit of size a_R using the friction factor, ξ_R, and spring constant, k_R. Give the time constant for a single Rouse unit.
d) Make a table of powers of N for τ, D, and η for the Rouse model, the reptation model, the observed behavior of a dilute solution and of a high molecular weight polymer melt.
e) Explain in words the reason for the difference between melt and solution behavior in part "d".

2) a) The structural hierarchy of a polymer chain in dilute solution can follow one of two models, explain briefly the difference between these two structural scaling models.
b) Are these models compatible with the Rouse model for dynamics?
c) Are these models compatible with the reptation model for dynamics?
d) How does the definition of the size of a Rouse unit, a_R, differ between the Rouse model and the Reptation model?
e) In your own words describe what you think would be the general consequence of using a self-avoiding walk in the Rouse model?

3) a) The *fluctuation dissipation theorem* (FDT) is used to describe the relationship between the molecular response to *thermal fluctuations* (thermally driven motions that are proportional in magnitude to kT) through the diffusion coefficient, D; and the bulk relaxation as described by the viscosity, η, or friction factor, ξ. The FDT postulates that $D \sim kT/\xi$. Explain the main assumption involved in the *fluctuation dissipation theorem*.
b) For a random walk (in any dimension) the RMS distance traveled is proportional to the square root of time, $\langle l^2 \rangle = D't$, where D' is for diffusion along the primitive path. Show that the reptation time, τ_d is proportional to N^3 using the random walk law, the definition of the primitive path length $l_{pr} \sim N$, and the Rouse relationship that $D_R \sim N^{-1}$.
c) The reptation tube displays Gaussian scaling, $\langle R^2 \rangle \sim N$. Show that the coil diffusion coefficient for reptation follows $1/N^2$ using the reptation time from part "b" and the distance $\langle R^2 \rangle$.
d) A protein is produced as a linear chain of amino acids residues that must undergo thermal diffusion to fold. How would you expect the time required to fold to differ for a chain in dilute solution versus a chain in a concentrated environment such as in a cell?
e) Reptation is capable of modeling the diffusion coefficient of polymers in the melt but can not fully describe the scaling of viscosity. Explain what might be missing form the dynamic hierarchy of the reptation model that could account for the discrepancy between the observed and predicted scaling of viscosity with molecular weight and explain why this wouldn't effect the diffusion prediction.

***ANSWERS:* 060215 Quiz 5 Morphology of Complex Materials**

1) a)



b) The persistence unit is rigid and does not respond to thermal vibrations.
The Rouse unit displays a decay (no momentum so no oscillation)
The Rouse coil displays a modal response like a guitar string with the majority of the response for the lowest order mode.
For reptation the Rouse units respond with the same decay but the chain relaxes with two time constants one along the primitive path (tube) that is a Rouse relaxation and a longer time lateral tube renewal.

c) The spring constant is defined using the rubber elasticity model, so $k_R = 3kT/(n_R l_p^2)$, and the friction factor is defined using Stokes Law, $\xi_R = 6\pi a_R \eta_0 = 6\pi\, n_R^{1/2} l_p \eta_0$ The Rouse unit displays an exponential decay with $\tau \sim 2\pi\, n_R^{3/2}\, l_p^3\, \eta_0/(kT)$.

d)

	τ	D	η
Rouse	N^1	N^{-1}	N^1
Reptation	N^3	N^{-2}	N^3
Dilute Soln.	N^1	N^{-1}	N^1
Melt	$N^{3.4}$	N^{-2}	$N^{3.4}$

e) Entanglements lead to the difference between melt and dilute solution dynamics. In the reptation model entanglements confine chain dynamics to a 1-d diffusion path along the primitive path created by a tube of entangling chains. It is assumed that the tube renewal time is much longer than the Rouse 1-d relaxation time for the chain to reptate through the primitive path.

2) a) Good solvent, self-avoiding walk with $R \sim N^{3/5}\, l_p$ and the Gaussian, random walk or theta-state with $R \sim N^{1/2}\, l_p$.

b) The Gaussian model is compatible but the SAW model is not since the Rouse model assumes a Gaussian Rouse unit in terms of spring constant and friction factor (in that a_R follows Gaussian scaling).

c) The reptation model has similar restrictions to the Gaussian model since it is based on the Rouse model and since it is assumed that the tube/primitive path is a random walk.

d) $a_R = n_R^{1/2} l_p$. The only difference is that a_R is defined by the average distance between chains in the melt for the reptation model so it has a value that can be calculated and that is fixed for a system of a specific composition and temperature. The reptation model calculates a_R following Rouse theory but with a restriction on the value.

e) SAW would make the Rouse unit larger making the friction factor larger if it is assumed that the SAW coil is non-draining. The spring constant for a SAW would be smaller since the coil has lower entropy (fewer states). Then the time constant, τ, from question (1c) for instance, would be larger. Overall the chain would be slower to relax and would have slower dynamics, higher viscosity, and a lower diffusion constant.

3) a) The main assumption in the FDT is that bulk relaxations such as seen in the viscosity are directly related to molecular relaxations such as seen in the diffusion coefficient. That is, the viscosity is directly related to the diffusion coefficient. The theorem implicitly assumes that there are no dynamic levels of hierarchy between the structure which is diffusing and the bulk melt.

b) $\tau_d = \frac{l_{\mathrm{Pr}}^2}{D'} \sim \frac{N^2}{N^{-1}} = N^3$

c) $D = \frac{R^2}{\tau_d} \sim \frac{N}{N^3} = N^{-2}$

d) The relaxation time in dilute solution is proportional to N while in a concentrated solution it is proportional to N^3 following the reptation model. The time required for protein folding should be proportional to the relaxation time so the time for folding should be much longer in the cell when compared to dilute solution. This effect should be greater for larger proteins.

e) The reptation model does not include long distance interactions and coupling between chain motion over a number of different chains (topologically tangled chains). These long distance tangles could give rise to a higher viscosity compared to the simple reptation model. Some authors have proposed that tube renewal could lead to the difference between the predicted N^3 dependence of viscosity and the observed $N^{3.4}$ dependence.

This doesn't effect the diffusion coefficient since diffusion is a local phenomena on the scale of a single chain while viscosity is a global phenomena that can sense larger scale dynamic levels.

060222 Quiz 6 Morphology of Complex Materials

1) Last week we considered dynamics using two dynamic hierarchical models associated with dilute and concentrated conditions, the Rouse and the reptation models respectively. Polymer crystallization also seems to display differences associated with concentration that may be linked to the differences seen in dynamics.
   a) Describe the structural hierarchy of polymer crystals grown from dilute conditions.
   b) Describe the structural hierarchy of polymer crystals grown from the melt.
   c) The micrographs below from Paul Ehrlich* in the 1990's shows polymer crystals grown in a semi-dilute solution. Comment on the hierarchy seen in these micrographs. How does it compare with "a" and "b" above?

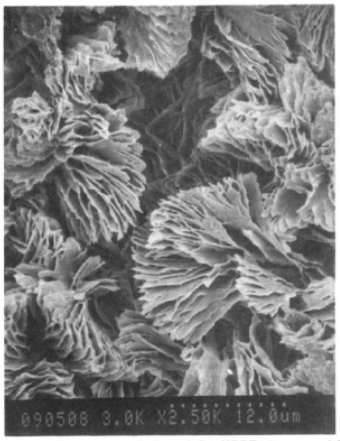


Figure 1. SEM micrograph of sample S-3, HDPE 1 (a commercial high density polyethylene with $\bar{M}_n$ = 7300 and $\bar{M}_w$ = 43 000) (rapid cooling).

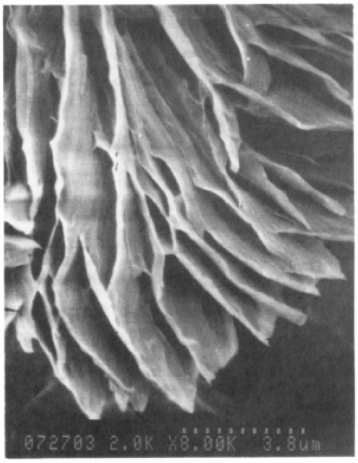


Figure 2. SEM micrograph of sample S-3, HDPE 1.

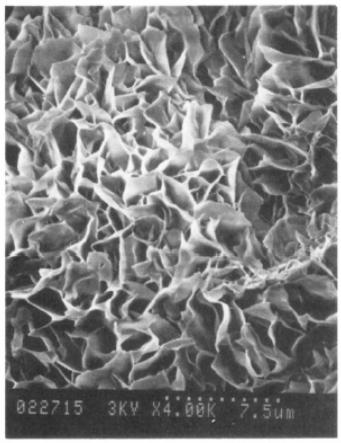


Figure 4. SEM micrograph of sample S-9, NBS 1475 (NIST standard reference linear polyethylene with $\bar{M}_n$ = 18 310 and $\bar{M}_w$ = 53 070; crystallized from a more concentrated solution).

   d) How does the dynamics hierarchy for dilute, semi-dilute and concentrated govern the structural hierarchy seen in your answers a to d?
   e) Explain how and why polymer spherulites differ from and how they are similar to dendrimers such as snow flakes.

2) The base structure seen in polymer crystals is a lamellar crystal.
   a) Obtain the Hoffman expression for lamellar thickness using the expression for the difference in the Gibbs free energy between the crystal and melt.
   b) Use the Hoffman expression to explain why polymer crystals are nanometer-sized crystals.
   c) Does the Hoffman expression work for the polymers shown in the Ehrlich micrographs above? Explain.
   d) Why are polymer crystals asymmetric (why is the lateral size larger than the thickness)?
   e) What governs the lateral size of polymer lamellae? (Define the Keith-Padden δ-parameter.)

Lamellar Structure and Organization in Polyethylene Gels Crystallized from Supercritical Solution in Propane.* Bush PJ, Pradhan D, Ehrlich P *Macromolecules* **24 1439-1440 (1991).

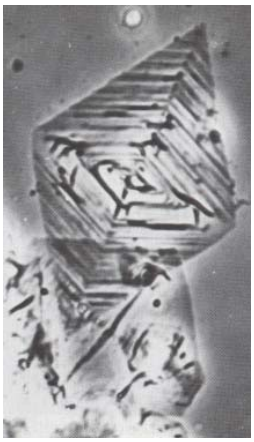
3) Chains in polymer lamellae are not normal to the lamellar surface but display a significant tilt in the lamellar crystal.

a) Sketch a cross section of a polymer lamellae showing the chains and the crystalline unit cell and indicating the c-axis.

b-e) Explain how chain tilt can be used to describe the following morphologies from Bassett and Ehrlich:

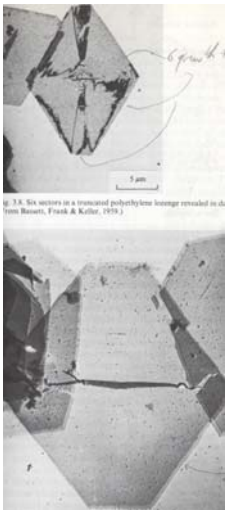
Figure 4. Axialite with dishing. HDPE 1 at 23.7% w/v, run S-3.

b)



c)

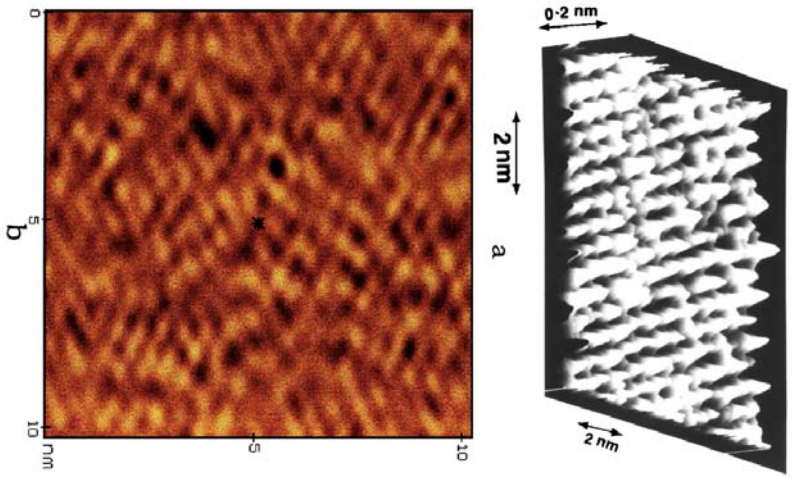
d)



e)

ANSWERS: 060222 Quiz 6 Morphology of Complex Materials

1) a) Persistence => Helix => Lamellae (i. Thickness and ii. Lateral) => Stacking/Folding
b) Persistence => Helix => Lamellae => Stacks of Lamellae in Fibers =>Low Angle Branching => Spherulitic
c) In some sense the semi-dilute solution crystals are intermediate between spherulitic crystals and single lozenge shaped crystals. Individual lamellae are seen but the do not form pyramid shapes but rather bend slowly similar to spherulitic lamellaeThe lamellae are loosely stacked and show some degree of branching but the branching is not space filling. I would appear that there is a fairly rich morphological continuum from dilute to melt crystallization. Ehrlich didn't really understand the morphological importance of his samples and he saw semi-dilute solution crystallization as a route to high surface area polymer foams for applications like gas storage.
d) The dynamic hierarchy we looked at last has implications for crystallization. The most obvious effect involves entanglements. Dilute solution single crystals do not display tie molecules and there are no entanglements. Chains fold tightly with adjacent reentry as has been shown in AFM micrographs such as that shown below (Reneker) and this is largely due to the



absence of entanglements and high mobility of polymer chains in dilute solution. In the melt chains must reptate and the reptation time is generally several orders of magnitude longer than the time required to crystallize. Entanglements are trapped and chains must span several crystals. As crystallization proceeds crystals are reeled in together since the chains are pulled into the crystals. This leads to tight stacking, switch board type reentry and dense crystalline regions. The semi-dilute samples of Ehrlich show intermediate behavior.

e) Dendrimers differ from spherulites in that dendrimers display high-angle branching along crystallographic directions (hexagonal directions for ice for instance). Spherulites display low angle branching due to twinning and epitaxial branching. Both dentrimers and spherulits grow by a fibrous motif. This is governed in both cases by a parameter like the Keith Padden δ parameter, $\delta \sim D/G$. The faster the growth rate, G, the finer the structure and the faster the transport of impurities the coarser the structure. The mechanism involving transport of impurities or thermal transfer from the crystallization front ensures low dimensional growth (non space filling).

2) a)

$\Delta G = \Delta H - T\Delta S$ so at T_∞ where $\Delta G = 0$ we have $\Delta S = \frac{\Delta H}{T_\infty}$

and for a real crystal with a finite thickness, t,

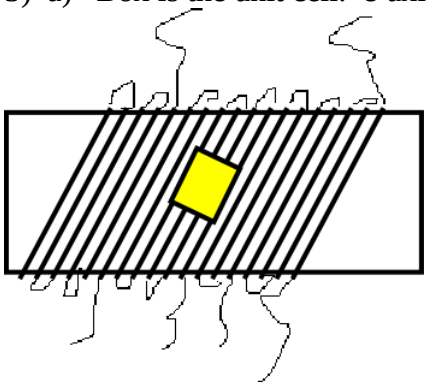
$$(\Delta G)V = abt\Delta H\left(1-\frac{T}{T_\infty}\right) - 2\sigma_e ab - 2\sigma_a bt - 2\sigma_b at = 0$$

where the last quality is for pseudo equlibrium and we ignore the a and b surface energies so,

$$t\Delta H\left(1-\frac{T}{T_\infty}\right) = 2\sigma_e \text{ or } t = \frac{[2\sigma_e/\Delta H]}{\left(1-\frac{T}{T_\infty}\right)}$$

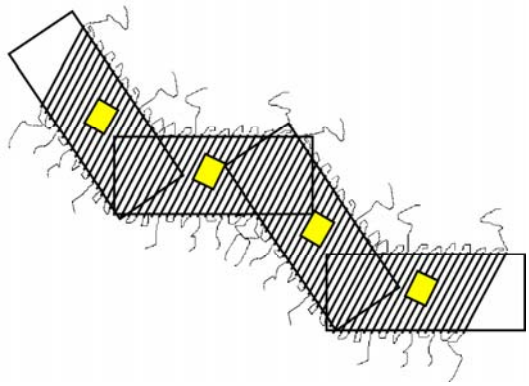
b) The numerator indicates low surface energy leads to small crystals and the denominator indicates that low temperature crystallizations lead to small crystals.
c) Hoffman works for all polymer crystals.
d) Asymmetry is due to the difference in the surface energy.
e) Keith-Padden del parameter governs lateral size due to a balance between transport of impurities and growth rate.

3) a) Box is the unit cell. c-axis is in the chain direction.



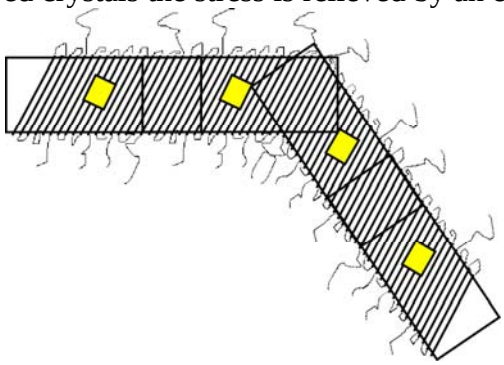
b) Chain tilt leads to a tension in the surface of the lamellae that can be relieved by bending of the lamellae if they do not have a significant lateral extent. b and d show such a bending.

c) shows a zig-zag structure which is another way the strain can be reduced.



d) Figure d shows spherulitic growth where the width is small and the length is long. Here twisting can be effective at relieving stress.

e) For pyramid lozenge shaped crystals the stress is relieved by an edge.



The edge is usually crushed out in dried samples leading to prominent crease.

060301 Quiz 7 Morphology of Complex Materials

1) In class we discussed the source of the bell shaped curve of growth rate versus temperature.
   a) Explain in words why a maximum in growth rate, G, is observed in temperature, T.
   b) Write expressions for two rate constants k_d and k_g.
   c) Derive a rate limiting function $G \sim 1/(1/k_d + 1/k_g)$ using the chemical kinetics model from class.
   d) What are the two temperature limits for polymers in the growth rate curve?
   e) Would you expect spherulites to follow such a bell shaped growth rate curve?

2) We also explored the Hoffman lamellar growth rate plot and function.
   a) Give the Hoffman expression for lamellar growth rate G.
   b) Explain what the terms are in this expression
   c) Derive the two axes used in the Hoffmann plot.
   d) Explain why this plot should be linear.
   e) What is the relationship between the growth rate function of question 1 and the Hoffmann expression.

3) Hoffmann considered three regimes of spherulitic growth.
   a) Describe the first regime.
   b) Describe the second regime.
   c) Describe the third regime.
   d) Explain how the slope in the Hoffmann plot will vary with regime.
   e) Criticize the Hoffman regime approach.

ANSWERS: 060301 Quiz 7 Morphology of Complex Materials

1a) The growth rate of a phase depends on two events, diffusion of the material which is forming the phase and conversion (surface nucleation) to the growing phase. The slower of these two rates governs the total rate. This is a classic rate limiting process. At high temperatures surface nucleation is slow and at low temperatures diffusion is slow. Between these two there is a maximum where nucleation isn't so slow and diffusion also isn't too bad.

b) $k_d \sim \exp(-U^*/kT)$ and $k_g \sim \exp(-E_g/kT)$

c) We have that the rate (1) $R = kd\,[A] = kg\,[B]$ and that (2) $Cf = Ai + Bi$ using (1) in (2) we have $Cf = Ai\,(1 + kd/kg)$ or that $Ai = kg\,Cf/(kg + kd)$ if this is used in (1) we obtain:
$Ri = kd\,Ai = kdkg\,Cf/(kd+kg) = Cf/(1/kd + 1/kg)$

d) Glass transition and equilibrium melting points

e) Yes. The bell shaped rate limiting law works for all phase separations since all phase separations depend on a balance between nucleation and diffusion. (that is except for spinodal decomposition and phase separation in supercritical fluids I think off hand).

2) a) $G = \exp(-U^*/kT)\exp(-Df/kT)$
b) U* is the activation energy for flow and Df is the free energy associated with a seed, 2st where s is the surface energy and t is the lamellar thickness.
c) If we use the Hoffmann expression for t, $t = (2se/DHf)(Tinf/(Tinf-T))$ we obtain
$G = \exp(-U^*/kT)\exp(-4se\ s\ Tinf/k(T(Ting-T)))$
taking ln of both sides
$\ln G - U^*/kT = -4se\ s\ Tinf/k(T(Ting-T))$
The Hoffmann plot is the left function versus $Tinf/(T(Ting-T))$
d) It is linear because the function is linear in these terms.
e) The Hoffmann law isn't rate limiting. It is a probability expression. The function will not show a maximum.

3) abc) The regimes are in the notes. In I $i << g$ and i rate limits growth $G \sim i$, in II $i \sim g$ and $G \sim i^{1/2}$. In III $i >> g$ and growth is all seeds, $G \sim i$ again.
d) The slope in the Hoffmann plot is basically ln G so the slope in II is half the slope in I and in III since $\ln i^{1/2} = 1/2 \ln i$.
e) The problems with Hoffman are in the notes. Basically there isn't enough data to support the theory, it is difficult to access shallow quenches and deep quenches so data on I and III is mostly absent. The definition of U* is weak. Several other issues were mentioned in the notes.

060308 Quiz 8 Morphology of Complex Materials

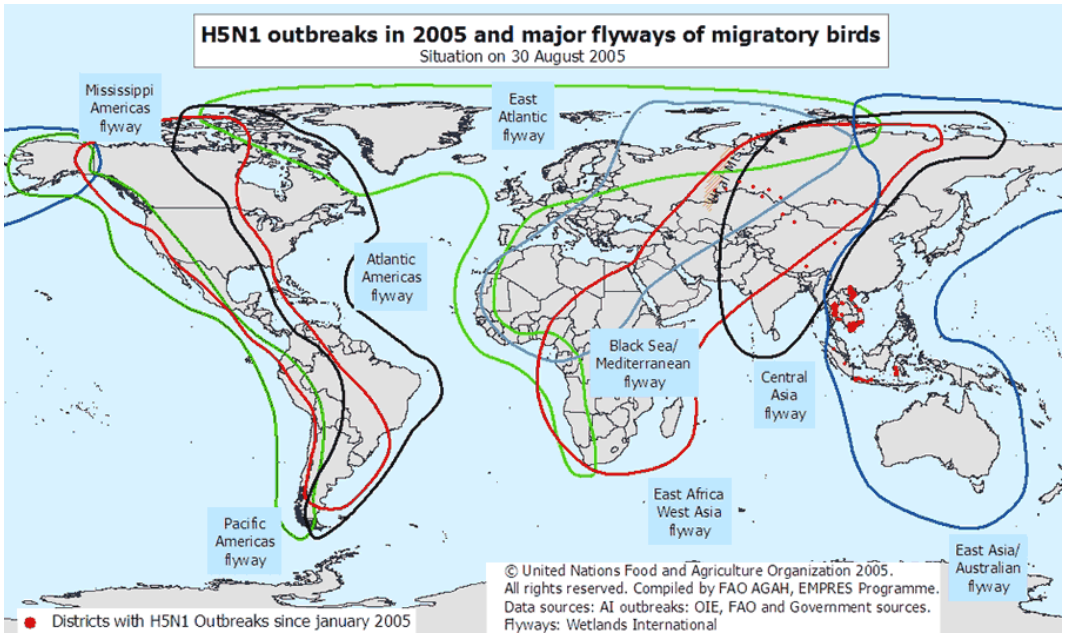


Figure 1. Migratory flight paths.

Above is a map of migration routes for birds which can carry the dreaded bird flu pandemic. Public Health scientists worry most about locations in central China since they connect with most of the world in a single year. The human population is like a supersaturated phase which can be nucleated (infected) at random locations along these flyways almost instantaneously (spontaneous nucleation). The World Health Organization has information concerning the fraction of the overall population infected as a function of time from hospital records, ϕ_{inf}. By constant monitoring it is possible to know the exact time of mutation of a human virulent strain (t_0 for "*nucleation*").

1) Assuming constant infection rate in terms of distance per time, dr/dt, from a "*nucleation*" site and complete infection of a region after an infection front passes through, derive an expression for ϕ_{inf} as a function of time that relies on the geometry of spreading by:
   a) Writing the Poisson distribution and explain the use of this distribution for this situation.
   b) Writing an expression for the average number of infection fronts that have passed through a random point at time t, <F> if infection spreads in 2d rings at a rate of dr/dt.
   c) Using the expression from "b" to write an expression for the expected behavior of ϕ_{inf} with time.
   d) If it is observed that ϕ_{inf} follows (1 - exp(-kt)) where t is time and k is a constant, what can be said about the transmission of the flu (perhaps along trade routes)? What is *k*?
   e) Could sporatic nucleation explain the behavior seen in part "d"? How or Why?

2) We discussed the hierarchy of aggregate materials such as the cosmic dust aggregate shown below, fig. 2 a.
   a) Explain the hierarchy of an aggregate structure and
   b) Compare it with the hierarchy of a linear polymer chain in solution.

c) What are the differences between these two structures?

d) If a chain aggregate (like the cosmic dust) is stretched would it behave in the same way as a polymer chain in thermal equilibrium? Explain your answer.

e) Explain the terms minimum path, p, minimum dimension d_{min}, connectivity ratio c and mass fractal dimension d_f.

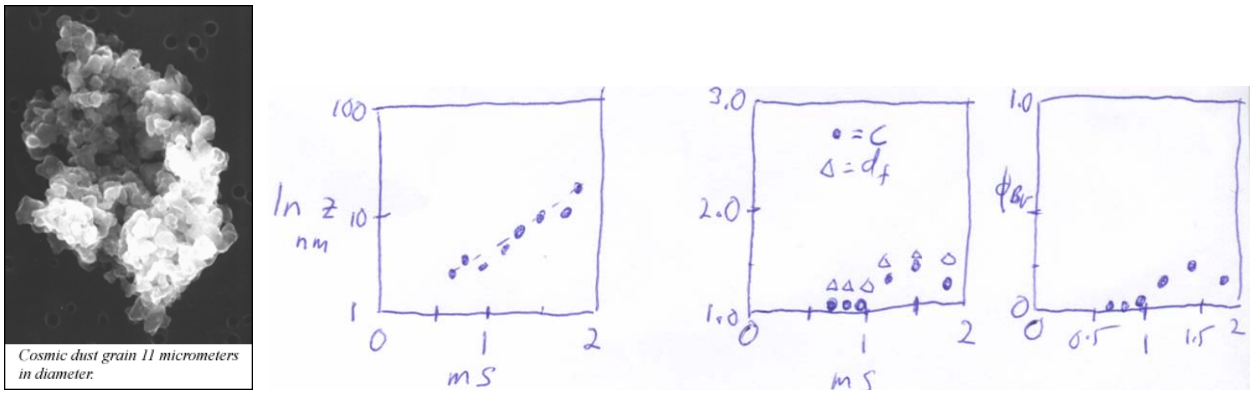


Figure 2. a) From NASA, b), c) and d) sketch of behavior of nanoparticle growth in spray flame.

3) The plot above (2b,c,d) shows the aggregation rate, aggregate size, z, versus t for alumina aggregates growing in a spray flame. The process occurs at about 1500 K and aggregation is complete in 1 ms.

a) If it is assumed that the fraction aggregate, ϕ_{Agg} is proportional to the average z (first figure, z is the mean number of primary particles in an aggregate), and that the behavior in the first plot is linear, what is the geometry of growth and type of nucleation for these aggregates based on the Avrami equation (that is if $\ln\phi_{Agg}$ is linear in t). Explain your answer.

b) Does the value of c in the third figure support your idea? Explain.

c) Does the value of d_f support your idea? Explain.

d) The branch fraction, ϕ_{Br}, in Figure 2d is defined by $\phi_{Br} = (z-p)/z$. Explain this function using a sketch of an aggregate.

e) What is ϕ_{Br} for a 6 arm star polymer?

ANSWERS: 060308 Quiz 8 Morphology of Complex Materials

1a) $p(F) = \frac{\langle F \rangle^F \exp(-\langle F \rangle)}{F!}$

The Poisson distribution gives the probability that a low likelihood event will occur F times if the average number of times that it occurs is <F>. For our case we are interested in p(F=0) given by exp-<F>. since the total infected fraction is 1 - p(F=0).

b) $\langle F \rangle = N \pi (t\, dr/dt)^2$, where N is the number of nucleation sites per area.

c) $\phi_{inf} = 1 - \exp(N\pi(dr/dt)^2 t^2)$

d) If ϕ_{inf} is linear in time then nucleation is spontaneous and growth is linear. For linear growth k is N(dr/dt) where N is the number of nuclei per length in the system. Linear growth might occur if growth of infection occurred along highways or trade routes in a straight line rather than in 2d space.

e) Sporatic nucleation could not explain the behavior in part "d" since the lowest time power is 2 for sporatic nucleation.

2) a) b)

Aggregate	Polymer
Primary particle	Persistence length
Size distribution	(Helix)
Fractal structure	Chain scaling
Branch structure	
Agglomerates	

c) Polymer hierarchy is determined by thermodynamics while the aggregate structure is determined by kinetics. The persistence length does not display a distribution in size. Aggregates tend to have a high degree of branching due to the nature of the bonding event.

d) The polymer chain obeys rubber elasticity with the chain spring constant being proportional to 3kT/p, while the chain aggregate displays Hooken behavior.

e) p is the number of primary particles in a connected path through the aggregate that has the minimum length from one side to the other. The straight distance between the two sides is R. Then, $(R/l_p)^{dmin} = p$, $(R/l_p)^{df} = z$, and $z = p^c$.

3) a) If $\ln\phi_{Agg}$ is linear in t then the Avrami power is 1 and growth is 1-d so the initial aggregates should be linear structures with c = 1 and $\phi_{Br} = 0$.

b) The value of c is close to 1 initially but later it increases to 1.2. This means that the structures become more branched later.

c) The value of d_f is 1.2 at the start of growth and approaches 1.5 at the end so it is a convoluted path structure. The growth is still linear.

d) z is the total number of primary particles and p is the number of primary particles in the minimum path. ϕ_{Br} represents the mass in branches divided by the total mass. This is typically on the order of 0.8 or higher for a branched aggregate. Here ϕ_{Br} is on the order of 0.3 so these are weakly branched structures.

e) For a 6 arm star two arms make p and the total mass is z = 3p so z-p = 2p and $\phi_{Br} = 0.67$