ANALYTICAL CUME

Two dimensional gel electrophoresis (2-DE) is a widely used, high-resolution technique for the separation of polypeptides.

1. The two dimensions separate analytes by different mechanisms.
   a. What is the separation mechanism in each dimension?

   The separation mechanism is one dimension is isoelectric focusing. The separation mechanism in the second dimension is differential mobility of protein analytes saturated with SDS to make them all other the same charge to mass ratio through a porous gel matrix based on their hydrodynamic volume. (10 points).

   b. Which molecular properties of the analytes are being targeted in each dimension?

   The molecular property being targeted in the IEF dimension is the isoelectric point of the protein. The ratio of positive to negative charge is another way to think of this. The molecular property being targeted in the gel electrophoresis dimension is the hydrodynamic volume of the analyte after denaturation with SDS.

   c. In what order are the two different separations applied?

   The IEF dimension is applied first followed by the SDS-gel electrophoresis dimension.

   d. Why is it necessary to apply the separation dimensions in a specific order?

   After a protein is denatured with SDS it is relatively difficult to remove it. Moreover, SDS denaturation causes the dissociation
and potentially the resolution heterogeneous multiple subunit proteins. Finally, many protein do not renature after removal of SDS and they would not recover to their original pI. This means it is very difficult to carry out an IEF separation after an SDS-PAGE separation.

2. A gradient is involved in one of the dimensions.
   a. How is this gradient established and maintained?

   The gradient established is a pH gradient. This is accomplished by using an ampholine. Ampholines are mixtures of a thousand or more species of oligomer, each with its own unique pI. The pI range of the species in an ampholine may be as small as one pH unit or span the range from 3-10. When an ampholine is placed in a tube or gel matrix and a potential applied, each specie in the ampholine is focused to it's isoelectric point. When the ampholine has been focused it maintains a constant, stable pH gradient across the tube as long as potential continues to be applied.

   b. Properties of both the analytical system and analytes impact resolution in this dimension.
      i. What are the analytical system properties that contribute to resolution and band spreading?

      The slope of the pH gradient in the capillary. The more gradual the slope, i.e. d(pH)/d(L) where L is length or distance, the higher the resolution. Voltage is another important variable. High voltage is required to keep analytes focused. In capillary electrophoresis, EOF is another important component. Excess EOF diminishes resolution.

      ii. Which analyte characteristics impact resolution and band spreading?
One variable is diffusion coefficient. Small molecules that diffuse rapidly, such as peptides, are difficult to focus. Another is total positive and negative charge. Molecules that have a lot of charge will accumulate charge very rapidly as the diffuse away from the pI point in the capillary and be refocused quickly. Those with a small amount of charge can diffuse a long distance before they accumulate enough charge to be refocused.

c. How are analytes transferred between the dimensions without loss of resolution?

First, it is important to note that the IEF dimension is physically separated from the SDS-PAGE dimension as described in the original method. The IEF gel is then placed along the edge of the SDS-PAGE gel and electrophoreses at a right angle to the direction it was originally developed into the SDS-PAGE gel. This process allows transfer between the gels with almost no loss in resolution.

3. Sodium dodecylsulfate is used in 2-DE.
   a. What is the function of this reagent in 2-DE?

Proteins vary widely in charge and shape. If one electrophoreses a protein mixture in a sieving gel matrix these properties of proteins will have a major impact on the separation. Size and shape differences in proteins are eliminated by adding 0.1% SDS to the sample and buffer. SDS adsorbs to proteins at about 1.5g/g of protein, causing them to be of the same charge to mass ratio and globular in shape. When they are separated in a gel matrix, the separation is according to their hydrodynamic volume, or size. Glycoproteins are an exception.
b. Why is it not used in both dimensions?

SDS denatures proteins while at the same time swamping their native charge. Thus no longer have a pI after treatment with SDS and IEF separations are precluded.
1. Sketch a qualitative molecular orbital diagram for the diatomic molecule CIF assuming that the d orbitals of the chlorine atom do not overlap orbitals of the fluorine atom. Do not crowd this diagram, fill the page of your blue book with it. Show the relative energies of all of the \( n = 1 \) and 2 atomic orbitals of the fluorine atom and all of the \( n = 1, 2, \) and 3 atomic orbitals of the chlorine atom in your MO scheme. Label the molecular orbitals as bonding, non-bonding, or antibonding and indicate how they are populated. Calculate the bond order of the bond in CIF.

See Shriver & Atkins Inorganic Chemistry, pps 91-92 for a discussion of MOs in heteronuclear diatomic molecules. Common errors were:
- Showing energy of Cl 1s orbital higher or equal to energy of F 1s orbital.
- Failure to recognize the 2s & 2p Cl orbitals are core orbitals and setting their energy equal to those of the F 2s and 2p valence orbitals.
- Lack of understanding of the overlap pattern of p orbitals

2. CIF is polar with the fluorine atom at the negative end of the molecule. The related molecule, BrCl, is polar with the chlorine atom at the negative end of the molecule. Using molecular orbital ideas, explain why the chlorine atom is partially positively charged in CIF and partially negatively charged in BrCl. Do NOT use the words electronegative or electronegativity in your explanation.

See Shriver and Atkins, p 88.

3. Sketch the shapes of the following sigma molecular orbitals in Cu(NH$_3$)$_4^{2+}$:
   a. the molecular orbital containing the 4s orbital of the copper ion.
   b. the two molecular orbitals containing the 3d$_x$ or 3d$_y$ orbitals of the copper ion.
   Question b was not graded. should have read “3p$_x$ and 3p$_y$”
   c. the molecular orbital containing the d$_{x^2-y^2}$ orbital of the copper ion.

See Shriver and Atkins pps 236-237 for a general description, Common errors:
- Assuming Cu(NH$_3$)$_4^{2+}$ is tetrahedral, it is square planar
- Sketching a sigma bond rather than a molecular orbital

4. A MO scheme for the sigma molecular orbitals in an octahedral complex of Cr$^{3+}$ is shown on the next page. Describe how \( \pi \)-bonding could either increase or decrease \( \Delta_o \).

See Shriver and Atkins, pps 239-240.
November 18, 2002
Cumulative Exam – Organic Chemistry – Answer Keys

This cumulative exam is based on two recent papers. The first, by Otto et al. (Science, 2002),
describes a method to generate artificial receptors from dynamic combinatorial libraries. The
second, by Reinholdt et al. (JACS, 2002), introduces the concept of “molecular chaperones”.
The answer to the questions below is either in the papers (attached) or can be inferred from them.
Please read the questions carefully and the manuscripts, then provide your answers succinctly
but clearly.

Questions

Part 1. Science paper (50 points)

1. Define dynamic combinatorial chemistry in the context of host-guest chemistry (10
   points, 100 words max.)

A chemical library is traditionally generated from the combinatorial “covalent” association of
building blocks (diversity elements). In a dynamic library of receptors, a combination of
building blocks is in thermodynamic equilibrium with the final receptor(s). The formation of a
particular subset of receptors is driven by a guest, substrate or ligand, that bias the equilibria
toward a particular subset of host-guest complexes according to basic Le Chatelier principles.

2. Describe briefly how the selection/amplification of artificial receptors works. (15 points,
   100 words max.)

Compounds 1-3 have the ability to form reversible disulfide bonds leading thereby to a large
collection of dynamic linear and cyclic potential receptors. Addition of guests 4 or 5 to this
mixture bias the equilibria toward the formation of one main product (6 or 7, respectively).

3. How does the process of scrambling and freez(2, BuCYA_{12})ng of the dynamic library
take place? (10 points, 100 words max.)

A DCL is prepared by mixing equimolar amounts of building blocks 1-3 (10 mM overall) in
water at pH 8.5 in an open vial. Oxygen from the air is sufficient to oxidize the thiols to
disulfides. Subsequent disulfide exchange is mediated by residual amounts of thiolate anion.
Exchange ceases upon protonation or removal of the thiolate, allowing isolation and handling of
individual library members.

4. Provide a reasonable mechanism for step (iii) in Fig. 2? (5 points)

A possible mechanism is shown below:
5. How did the authors carry out the analysis and characterization of the selected receptors? (5 points)

ESI-MS and LC-MS

6. What is the difference in product outcome between adding templates 4 or 5 to pre-equilibrated and non-equilibrated dynamic libraries of 1–3. (5 points)

Nothing. The composition of the dynamic mixture obtained after adding guests is the same, irrespective of whether the guest is present from the start of the experiment or added to the preformed library.

Part 2. JACS paper (50 points)

7. What is the difference between kinetically- and thermodynamically-driven self-assembly? (15 points, 100 words max.)

Thermodynamically-driven self-assembly leads in a defined sequence of steps to the formation of the most stable species. The resulting aggregate is located in a global energy minimum and is in a dynamic equilibrium (exchange) with minor (and higher energy) intermediate species present in the same solution. Kinetically-driven self-assembly leads to the generally irreversible formation of a kinetically stable high-energy intermediate. The resulting aggregate is said to be trapped in a local energy minimum.

8. What is a molecular chaperone? (15 points, 100 words max.)

A molecular chaperone is a molecule that reduces the energy barrier leading to the formation of the thermodynamic product. In this case, the molecular chaperone stabilizes one or more intermediate states en route to the final product. 

FYI, a molecular chaperone is not a “protein-like” structure, nor does it assist in the folding of a “protein” on its way its bioactive conformation. Students who gave this answer earned partial credit (10 points).

9. Propose a reasonable explanation as to why DEB acts as a molecular chaperone in this study (20 points, 100 words max.)

One possible answer: From the paper we know that DEB and compound 2 lead to the thermodynamic product (2,DEB,12), whereas BuCYA and 2 lead to the kinetic product (2,BuCYA,12). Addition of DEB as a molecular chaperone to BuCYA and 2 acts by generating catalytic amounts of the thermodynamic product (2,DEB,12) from which DEB is then replaced with BuCYA. The equilibrium is shifted toward the latter aggregate because BuCYA leads to a kinetically more stable aggregate with compound 2 (2,BuCYA,12). Thus, DEB acts a template for the formation of the thermodynamically and kinetically most stable species.
Physical Chemistry Cumulative Exam
You must show all reasoning and give full explanations to obtain any credit.

1. Consider the nonlinear dipropylamine molecule. It has the following chemical structure:

\[
\begin{array}{c}
\text{H} \\
\text{CH}_3-\text{CH}_2-\text{CH}_2-N-\text{CH}_2-\text{CH}_2-\text{CH}_3
\end{array}
\]  

(1.1)

(a) Dipropylamine has:

5 (i) \( T \) translational degrees of freedom where \( T = 3 \)

5 (ii) \( R \) rotational degrees of freedom where \( R = 3 \)

5 (iii) \( V \) vibrational degrees of freedom where \( V = 60 \)

The rest of Problem One will deal with two ideal gases. Each gas is composed of \( N \) molecules in a volume \( V \) at temperature \( T \). For gas 1, all of the molecules are dipropylamine. For gas 2, one-half of the molecules are dipropylamine and one-half are a different substance \( X \).

The IR spectrum of gas 1 is depicted in Figure 1.

\[\text{Figure 1}\]
(b) From statistical thermodynamics, one may show for either gas that at high $T$ the constant volume heat capacity $C_v(T)$ may be written as

$$\lim_{T \to \infty} C_v(T) = ynR$$

where $n = N/N_A$ is the total number of moles, where $R = N_A k$ is the gas constant, and where $y$ is numerical parameter.

(i) What is the numerical value of $y$ for gas 1? $y = 6.3$

(ii) If the substance $X$ is the deuterated isotope of dipropylamine

$$\text{D}$$

$$\text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \text{N} - \text{CH}_2 - \text{CH}_2 - \text{CH}_3$$

what is the numerical value of $y$ for gas 2? $y = 6.3$

(iii) If the substance $X$ is the fluorine molecule $F_2$, what is the numerical value of $y$ for gas 2? $y = 17/2$

(c) Will the peak positions and relative peak heights of the IR spectrum of gas 2 be the same as or different from that of the IR spectrum of gas 1, shown in Figure 1, if... Since different

Differences in molecular frequencies in... Different normal mode frequencies

2. This problem deals with NMR and electronic spectroscopy.

(a) For a spin $\frac{1}{2}$ nucleus placed in a magnetic field $B = B_0 k$, the energies of the spin-up and spin-down states split; the energy levels being given by the formulas

$$E_+ = -\frac{1}{2} g_N \beta_N B$$

and $E_- = \frac{1}{2} g_N \beta_N B$

where the nuclear magneton $\beta_N = \frac{e\hbar}{2m_p}$.

Consider two spin $\frac{1}{2}$ nuclei; namely the $^1H$ nucleus (proton) for which $g_N = 5.5857$ and the $^{15}N$ nucleus for which $g_N = -0.5661$.

(i) Given this data, explain why the following statement is true.

While NMR absorptions of the $^1H$ nucleus are spin-up $\rightarrow$ spin-down spin flips, NMR absorptions of the $^{15}N$ nucleus are spin-down $\rightarrow$ spin-up spin flips.
(ii) In a magnetic field with magnitude \( B_z = 3 \) tesla, the frequency \( \nu \) of the NMR absorption line of \(^1\text{H}\) is 127.74 MHz. What is the frequency \( \nu \) in MHz of the NMR absorption line of \(^{15}\text{N}\) in a magnetic field with magnitude \( B_z = 5.5 \) tesla?

\[
\nu_{^{15}\text{N}} = \nu_{^1\text{H}} \frac{\hbar}{\gamma_{^{15}\text{N}}} \frac{\hbar}{\gamma_{^1\text{H}}} \frac{B_z}{B_z} = \left( \frac{127.74 \text{ MHz}}{5.5 \text{ tesla}} \right) \left( \frac{0.566 \text{ MHz}}{2.1 \text{ MHz}} \right) = 23.75 \text{ MHz}
\]

(b) Consider then \( n = 2 \rightarrow n = 3 \) Balmer line of a hydrogen-like atom. At first glance the following **thirty-six transitions** would appear to all contribute to the \( n = 2 \rightarrow n = 3 \) Balmer line.

\[
\begin{align*}
2s &\rightarrow 3s, 3p_0, 3p_{\pm 1}, 3d_{0}, 3d_{\pm 1}, 3d_{\pm 2} \quad \text{(nine transitions)} \\
2p_0 &\rightarrow 3s, 3p_0, 3p_{\pm 1}, 3d_{0}, 3d_{\pm 1}, 3d_{\pm 2} \quad \text{(nine transitions)} \\
2p_{\pm 1} &\rightarrow 3s, 3p_0, 3p_{\pm 1}, 3d_{0}, 3d_{\pm 1}, 3d_{\pm 2} \quad \text{(eighteen transitions)}
\end{align*}
\]

In fact, however, because of the selection rules

\[
\Delta n = n_f - n_i \text{ is unrestricted} \\
\Delta \ell = \ell_f - \ell_i = \pm 1 \\
\Delta m = m_f - m_i = 0;
\]

only **five transitions** contribute to this line. What are these five transitions?

\[
\begin{align*}
\mathcal{R}_s &\rightarrow \mathcal{R}_0 \\
\mathcal{R}_0 &\rightarrow \mathcal{R}_s, \mathcal{R}_1, \mathcal{R}_0 \\
\mathcal{R}_{\pm 1} &\rightarrow \mathcal{R}_{\pm 1} \\
\mathcal{R}_{-1} &\rightarrow \mathcal{R}_{-1}
\end{align*}
\]