Bioanalytical NMR has developed significantly over the past decade. One important characteristic of NMR is its quantitative capabilities. The following questions are focused primarily on NMR based quantitation.

1. (16 pts) There are a number of methods of quantitation that involve the use of standards. Briefly describe the quantitation process using following:

   a) Internal standard:  
   A known amount of a compound, different from the analyte is added to the sample. The analyte concentration is found from the ratio of signals and standard concentration.

   b) External standard: In some cases, internal standards are unavailable or inconvenient (or expensive). The analyte concentration can be determined by comparing it to the same analyte measured in a separate experiment. This is especially useful in qualitative analysis for identification, but can also used for quantitation. A better approach is the calibration curve (see d) below).

   c) Standard addition: Standard addition is useful when there are multiple signals that complicate the analyte determination. Analyte is added to the sample at known concentration, and the signals are graphed. A linear extrapolation to zero signal gives the unknown concentration. A linear response to the analyte concentration is required.

   d) Calibration curve: A calibration curve is constructed by measuring the analyte at different concentrations. Then the analyte signal in the sample is compared to the curve to calculate the unknown concentration. Normally, a blank measurement is made and subtracted from the analyte signal before calculating the concentration.

2. (10 pts) What is the difference between a primary and a secondary standard? Can you give an example of each?

   Primary Standards are of known composition, usually 99.9% pure, do not decompose under normal storage conditions and typically can be weighted. There are actually few primary standards. For example, there are only 5 for acid-base reactions!

   Secondary Standards are used when they are more convenient or in some cases closer chemically to the analyte of interest. Secondary standards are typically less stable over long periods of time and must be referenced to the primary standard on a regular basis.

   FYI, standard reference materials are a related set of materials for which no good standards exist, i.e. the NIST “standardized dirt” reference material.

3. (10 pts) Give an example of a common standard used for NMR. How would you make sure that it is a quantitative standard?

   TMS (tetramethylsilane), TSP (trimethylsilyl-propionate) or DSS (dimethyl-2-silapentane-5-sulfonate) are typical chemical shift reference standard compounds. They can also be
used for quantitation. TFTMP (difluoro-1-trimethylsilyl)methyl-phosphonic acid) can also be used.

4. (10 pts) Why is it usually sufficient to use a single standard to quantitate all analytes of interest in the NMR spectrum?

The NMR signal is proportional to the number of magnetically equivalent nuclei in the sample. Therefore, it does not matter which compound those nuclei are associated. For example, two compounds at the same concentration, both with methyl groups (that appear at different chemical shifts) will have the same \(^1\)H intensity. Similarly for other nuclei in the sample.

5. (14 pts) Recently, it was shown that the solvent (water) could be used as a useful quantitative standard, instead of an added compound.

a) Why can water be used as a quantitative standard in aqueous solution?

From 4 above, if the concentration of the water is know (and normally it is or can be calculated, then its signal can be compared with that of the analyte to compute the analyte’s concentration.

b) Discuss one advantage of such an approach
There is no need to add an additional concentration standard.

c) Discuss one possible disadvantage of this approach

The water resonance is pH dependent, and so it can’t be used as a chemical shift standard. The water signal is very big, so its signal normally needs to be acquired separately, which will add noise. And sometimes it may be hard to use water in conjunction with organic solvents, such as with hydroscopic materials.

6. (10 pts) Quantitation in samples such as urine is difficult because of a variable dilution problem. Can you think of a way to reduce this problem?

One can normalize to the total sum of the signal. One can use a compound, such as creatinine, that doesn’t vary much from patient to patient. This approach is often used in medical studies. One could also dry the sample and then reconstitute on a known amount of solvent. Alternatively, one can collect 24 hr urine, which will normally reduce the dilution effect at least somewhat.

7. (10 pts) For quantitation of analytes in blood samples by NMR, a different problem exists, namely the presence of lipids and proteins that contribute large, broad lines to the spectrum and baseline. Can you think of a way to improve this situation?

One can filter the sample. One can also use a different NMR pulse sequence, like the CPMG (Carr-Purcell-Meibloom-Gill) which relaxes the protein signals and leaves the small molecules. Another approach (though less widely used) is a diffusion edited experiment (DOSY, diffusion ordered spectroscopy), which separates small molecule signals from those of large molecules based on their diffusion coefficients.
8. (10 pts) How would you perform quantitation in an LC-NMR experiment, where there are dilution steps and where not all of the analyte may be present in the NMR detection coil at one time?

Use of an internal standard would be advised. One would have to be careful, since one would have to take into account the fact that both the analyte and standard might not all be present in the NMR detection coil at the same time, and that the chromatographic peak widths could be different. Alternatively, one could use a labeled internal standard and use the isotope label to distinguish the NMR signals of analyte and standard.

9. (10 pts) Describe the differences between relative and absolute quantitation for an analyte in a complex sample. Which do you think would be better for comparing across different samples sets over time or geography?

Relative quantitation looks at the changes in concentration with respect to other samples and is relatively easy to perform. Absolute quantitation relates the analyte concentrations to absolute levels. It is more challenging, but provides a method to better compare samples across multiple studies.
Biochemistry Cumulative Exam

Title: Signal Transduction

April 30, 2011

1. (20 points). What are the binding specificities for the following protein modules:

(a) SH3 domain (PXXP)
(b) SH2 domain (XXpYXX)
(c) PH (IP3)
(d) WW (XXpXXX)

2. (15 points) How are signals transduced by GPCR? Provide a pathway.

3. (15 points). What is MAPK cascade? Explain using any one example where signal from a receptor is transmitted to the nucleus using MAPK cascade.

Show any one pathway.
4. (15 points) How do G Proteins act as on/off switch? What are the accessory proteins that are required for G Protein activity/inactivity?

5. (15 points) How do Cholera toxin and Pertussis toxin (whooping cough disease) target G Protein signaling?
• Cholera toxin catalyzes covalent modification of G<sub>sa</sub>. ADP-ribose is transferred from NAD<sup>+</sup> to an arginine residue at the GTPase active site of G<sub>sa</sub>. This ADP-ribosylation prevents G<sub>sa</sub> from hydrolyzing GTP. Thus G<sub>sa</sub> becomes permanently activated.

• Pertussis toxin (whooping cough disease) catalyzes ADP-ribosylation at a cysteine residue of G<sub>ia</sub>, making the inhibitory G<sub>a</sub> incapable of exchanging GDP for GTP. Thus the inhibitory pathway is blocked.

6. (10 points) Explain how mutations in R or C subunit of Protein Kinase A (PKA) might lead to a (a) constantly active PKA or a (b) constantly inactive PKA?

(a) The mutation that makes R unable to bind and inhibit C, so C is constantly active.
(b) The mutation prevents cAMP binding to R, leaving C inhibited by bound R.

7. (10 points) What are the differences between Ras and G<sub>s</sub>? What is the functional difference between G<sub>s</sub> and G<sub>i</sub>?

Ras is small monomeric protein, Gs is heterotrimeric.
Gs activates adenylate cyclase, Gi inhibits it.
Inorganic Chemistry Cumulative Exam

Purdue University

April 30, 2011

There are 100 possible points in this exam.

1. (10 points) Write Miller indices for each plane shown below.

(a) (111)  (b) (011)  (c) (0-11)  (d) (110)  (e) (22-1)  (f) (1-10)

2. (10 points) Metal X has a face-centered cubic structure with a cell parameter, a = 4.00 Å. Calculate the density of metal X (Atomic weight of X = 60.0 g/mol).

In a face-centered cubic structure four atoms are present per unit cell.

\[
\text{Mass} = 4 \times 60.0 \text{ g} / 6.022 \times 10^{23} \\
\text{Volume} = (4.00 \text{ Å})^3 = 64 \times 10^{-24} \text{ cm}^3 \\
\text{Density} = 6.23 \text{ g/cm}^3
\]

3. (20 points) Draw the atomic arrangement of X on the (100), (110), and (111) planes. Indicate interatomic distances in each plane. Which plane has the most densely packed X atoms? Show work.

The shaded area shows the area per X atom on each plane. The (111) plane has the most densely packed X atoms.
4. (15 points) What is the distance between the nearest (100) planes of metal X? Repeat the question for (110) planes and (111) planes.

For a cubic structure, \[ d_{hkl} = \frac{a}{(h^2+k^2+l^2)^{1/2}} \]
\[ d_{100} = 4.00 \text{ Å} \]
\[ d_{110} = 4.00 \text{ Å} / \sqrt{2} = 2.83 \text{ Å} \]
\[ d_{111} = 4.00 \text{ Å} / \sqrt{3} = 2.31 \text{ Å} \]

5. (15 points) (a) What is the shortest metal-metal distance in the crystal structure of metal X? How many neighboring metal atoms can be found with this distance from one metal atom?

(b) What is the second shortest metal-metal distance? How many neighboring metal atoms can be found with this distance from one metal atom?

(c) What is the third shortest metal-metal distance? How many neighboring metal atoms can be found with this distance from one metal atom?

6. (10 points) (a) Explain why metals usually have a good electrical conductivity.

In a metal there is no energy gap between the valence band (or occupied orbital) and the conduction band (or empty band), therefore, electrons can be easily excited to the empty band to conduct electricity.

(b) When the temperature increases, how does it affect the electrical conductivity of metal?
In general, electrical conductivity of metals decreases with temperature because the thermal vibration of metal atoms interferes with electron transport when temperature increases.

7. (20 points) A lattice is an infinite array of identical points (i.e. each one has exactly the same environment of other points) and the points are obtained one from another by translations only. This description is applicable, equally, in one-, two-, and three-dimensional space. A unit cell of a plane (2D) lattice is a parallelogram of two unit translations, a and b, with lattice points at the corners and is perfectly representative of the lattice. There are five 2D lattice types. They are:

- **Oblique**: $a \neq b, \gamma = 90^\circ$
- **Rectangle**: $a \neq b, \gamma = 90^\circ$
- **Hexagon**: $a = b, \gamma = 120^\circ$
- **Rhombus (= centered rectangle)**: $a = b, \gamma = 90^\circ$
- **Square**: $a = b, \gamma = 90^\circ$
Draw a unit cell for the following 2D patterns (convention: choose the smallest repeat unit that is the most symmetric. (Symmetry level: square, hexagon > rhombus, rectangle > oblique) and identify a lattice type. Attach this page of the exam to your blue book. (A stapler is available at the podium).
In a recently published full paper *(J. Am. Chem. Soc. 2011, 113, 6114-6117)*, Pettus and coworkers report the total synthesis of $(\pm)$-y-rubromycin, a natural product known to be a potent inhibitor of telomerase. Several synthetic schemes from the paper are reproduced below. Answer the following questions about them.

1. (15 points) Provide a stepwise mechanistic explanation for the conversion of 7 to 9 in Scheme 2 (below). Be sure to include all likely intermediates and use curved arrow formalism to account for the movement of electrons.

**Scheme 2. Synthesis of Naphthoquinone 5 from R-Tetralone 7**

(a) LiHMDS (2.4 equiv), THF, -78° C, then NBS (2.06 equiv), -78° C to rt, 78% yield. (b) CAN (2.14 equiv), MeCN/H2O, 0° C, 60% yield. (c) NaN3 (1.46 equiv), THF/H2O, rt. (d) Cs2CO3 (1.5 equiv) PhCH3/MeOH, rt, 65% yield for 2 steps. (e) KOH (21.4 equiv), MeOH/H2O, 84% yield.
2. (20 points) The authors mention that 7 can be made in three steps from 1,2,4-trimethoxybenzene. Propose a synthetic conversion of 1,2,4-trimethoxybenzene to 7 that includes all reagents. You may use any reagent of 4 carbons or less in your synthesis. Also provide an explanation for any issues of selectivity that arise in your synthesis.

For instance:

Friedel-Crafts acylation of 1,2,4-trimethoxybenzene will effect substitution predominantly at C-5 (as shown) because it is ortho and para to two of the three methoxy groups and less sterically hindered than C-3. Clemmensen reduction will affect only the aryl ketone, leaving the carboxylic acid unaffected. The intramolecular Friedel-Crafts acylation is sterically constrained to give the desired product.
3. (15 points) Provide a stepwise mechanistic explanation for the conversion of 15 to 16 in Scheme 3 (below). Be sure to include all likely intermediates and use curved arrow formalism to account for the movement of electrons.

Scheme 3. Preparation of Methylated Isocoumarin 6

(a) Pd(OAc)$_2$, PPh$_3$, methyl acrylate (1.9 equiv), LiCl, NEt$_3$ (1.81 equiv), DMF, 80°C, 93% yield. (b) H$_2$ (1 atm), Pd/C, EtOAc, 94% yield. (c) p-TsOH (cat.), PhMe, reflux, 82% yield. (d) 14 (1.03 equiv), LiHMDS (1.0 equiv), THF, -78°C, then 13 (1.0 equiv), 60% yield, E/Z = 6/1. (e) CpTiMe$_2$ (2.19 equiv), PhMe, 70°C, 72% yield, E/Z = 8/1. (f) TBAF (1.03 equiv), THF, -78°C, 94% yield.
4. (10 points) Account for the selectivity of the reaction that converts 15 to 16 for only one of the three carbonyls present in 15.

The lactone carbonyl is the only one that isn’t conjugated to a neighboring π system, rendering it more reactive. Additionally, the lactone is further destabilized by its restriction to an s-cis conformation. These two factors conspire to make the lactone significantly more reactive toward the metallo carbene.
5. (25 points) Provide a stepwise mechanistic explanation for the reaction of 5 with 6 to form 17 and 18 as shown in Scheme 4 (below). Be sure to include all likely intermediates and use curved arrow formalism to account for the movement of electrons. Feel free to abbreviate.

**Scheme 4. Conclusion of the Total Synthesis of ((±)-γ-Rubromycin (1))**

\[
\text{CAN} \quad \begin{array}{c}
\text{NaHCO}_3 \\
\text{THF, 1 hr, r.t.}
\end{array} \quad 5 \ + \ 6 \quad \xrightarrow{(58\%) \quad (17:18 = 1:2)} \\
\begin{array}{c}
\text{BBR}_3(6 \text{ equiv}) \\
-78 \degree C \text{ to } -20 \degree C \\
1.5 \text{ hr, (61\%)}
\end{array} \quad \begin{array}{c}
\text{BBR}_3(8 \text{ equiv}) \\
-78 \degree C \text{ to } -20 \degree C \\
1.5 \text{ hr, (50\%)}
\end{array} \\
\gamma\text{-rubromycin (1)}
6. (15 points) Provide a stepwise mechanistic explanation for the reaction of 17 to form γ-rubromycin (shown below). Be sure to include all likely intermediates and use curved arrow formalism to account for the movement of electrons.
Solutions to Physical Chemistry, 10th Edition
April 30, 2011

Anthracene = C_{14}H_{10}

Number of degrees of freedom = 3(14 + 10) = 72

Number of normal modes = 72 - 6 = 66

2.

\[ P_k(T) = g_0 \exp \left( \frac{-E_k}{kT} \right) \]

for \( k = 0, 1, 2, \ldots \)

(i) \( P_0(T) = g_0 \exp \left( \frac{-E_0}{kT} \right) \)

From Information: \( E_0 = 0 \) and \( g_0 = 1 \)

Thus

\[ P_0(T) = \frac{1}{Q(T)} = \left[ 1 + 2 \exp \left( \frac{-E_0}{kT} \right) + \exp \left( -2 \frac{E_0}{kT} \right) \right]^{-1} \]

\[ \lim_{T \to 0} P_0(T) = 1 \]

\[ P_0(T) = g_1 \exp \left( -\frac{E_0}{kT} \right) = g_1 \frac{\exp \left[ \frac{-E_0}{kT} \right]}{Q(T)} \]

\[ = \frac{1}{2} \exp \left( \frac{-E_0}{kT} \right) \left[ 1 + 2 \exp \left( \frac{-E_0}{kT} \right) + \exp \left( -\frac{2E_0}{kT} \right) \right]^{-1} \]
\[ P_2(T) = 2 \exp(-0) \left(1 + 2 \exp(-0) + \exp(-0)\right)^{-1} \]

\[ \lim_{T \to \infty} P_2(T) = \frac{1}{2} \]

\[ E(T) = E_0 P_0(T) + 2 \varepsilon T \exp\left(-\frac{\Omega^2}{T}\right) + 2 \varepsilon T \exp\left(-\frac{3 \Omega^2}{T}\right) \]

\[ 1 + 2 \exp\left(-\frac{\Omega^2}{T}\right) + \exp\left(-\frac{3 \Omega^2}{T}\right) \]

\[ E(T) = 2 \varepsilon \left[ \exp\left(-\frac{\Omega^2}{T}\right) + \exp\left(-\frac{3 \Omega^2}{T}\right) \right] \]

\[ 1 + 2 \exp\left(-\frac{\Omega^2}{T}\right) + \exp\left(-\frac{3 \Omega^2}{T}\right) \]

(i) \[ \lim_{T \to 0} E(T) = 2 \varepsilon \left[ 0 \times 0 \right] \left[ 1 + 2(0) + 0 \right] = 0 \]

(ii) \[ \lim_{T \to \infty} E(T) = 2 \varepsilon \left[ 1 + 1 \right] \left[ 1 + 2 + 1 \right]^{-1} = \frac{4 \varepsilon}{4} = \varepsilon \]

(iii) \[ \lim_{T \to \infty} E_0(T) = \varepsilon \]
What is $T^*$ such that

$$P_2(T^*) = P_1(T^*)?$$

or at $T^*$

$$Q^{-1}(T^*) = 2Q^{-1}(T^*) \exp \left(-\frac{\Theta}{T^*}\right)$$

or

$$\ln 2 = \Theta \frac{1}{T^*}$$

or

$$\ln 2 = \frac{\Theta}{T^*}$$

Next let's return to part b iii on the next page.

(iii). For $T \ll \Theta$, $E(T) = E(T + \Delta T) = E(T) + \frac{dE(T)}{dT} \Delta T$

so in $\frac{dE(T)}{dT} = 0(T) = 0.$
Therefore

\[ \lim_{T \to 0} C(T) = 0 \]

\[ \lim_{T \to \infty} C(T) = \lim_{T \to \infty} \frac{dE(T)}{dT} = \frac{dE}{dT} = 0. \]

\[ \therefore \lim_{T \to \infty} C^2(T) = 0 \]

\[ \therefore \lim_{T \to \infty} C(T) = 0 \]

because of the gap between

\[ E_0 = 0 \quad \text{and} \quad E = \hbar \omega T \]

adding a small amount will not increase changing \( T \) by a small amount will not increase

the populations in states 1 or 2. These populations

remain zero. So the energy would increase if the System

is heated from \( T \to T + dT \). Hence \( \lim_{T \to \infty} C(T) = 0 \) since for large \( T \), the

probabilities \( p_i(T), i = 0, 1, 2, 3 \) do not change as

\[ T \to T + dT \] since there are no higher energy states.

If there were an infinite number of excited state, no

matter how high \( T \) is, an increase in \( T \) will increase

the populations of the higher energy states. This

implies that \( E(T + dT) > E(T) \) and so \( E(T) \leq E(T + dT) \)

and, hence \( \lim_{T \to \infty} C^2(T) \to 0 \).
From Thermodynamics

\[ A(T) = E(T) - T S(T) \]

So

\[ S(T) = \frac{E(T) - A(T)}{T} \]

Also from Problem 2b1 since \( E = k_b \Theta \)

\[ E(T) = 2 \Theta \frac{\left[ \exp \left( \frac{\Theta}{T} \right) + \exp \left( -\frac{2 \Theta}{T} \right) \right]}{1 + 2 \exp \left( \frac{\Theta}{T} \right) + \exp \left( -\frac{2 \Theta}{T} \right)} \]

Also from Statistical Thermodynamics

\[ A(T) = -k_b T \ln Q(T) = -k_b T \ln \left[ \frac{1 + 2 \exp \left( -\frac{\Theta}{T} \right)}{1 + 2 \exp \left( \frac{\Theta}{T} \right) + \exp \left( -\frac{2 \Theta}{T} \right)} \right] \]

\[ S(T) = 2 \Theta \frac{\left[ \exp \left( -\frac{\Theta}{T} \right) + \exp \left( -\frac{2 \Theta}{T} \right) \right]}{1 + 2 \exp \left( -\frac{\Theta}{T} \right) + \exp \left( -\frac{2 \Theta}{T} \right)} \]

\[ = k_b \Theta \ln \left[ \frac{1 + 2 \exp \left( -\frac{\Theta}{T} \right)}{1 + 2 \exp \left( \frac{\Theta}{T} \right) + \exp \left( -\frac{2 \Theta}{T} \right)} \right] \]

As noted before

\[ \lim_{T \to 0} Q(T) = \lim_{T \to 0} \left[ 1 + 2 \exp \left( -\frac{\Theta}{T} \right) + \exp \left( -\frac{2 \Theta}{T} \right) \right] \]

\[ \therefore \lim_{T \to 0} S(T) = 2 \Theta \lim_{T \to 0} \left\{ \frac{1}{T} \left[ \exp \left( -\frac{\Theta}{T} \right) + \exp \left( -\frac{2 \Theta}{T} \right) \right] \right\} \]
Next let's define \( u = T^{-1} \). Then from the previous equation

\[
\lim_{T \to 0} S(T) = 2k \lim_{u \to 0} \left\{ u \exp\left[-u(\frac{8a}{9})\right] + u \exp\left[-2u(\frac{3}{4})\right]\right\}
\]

but the exponentials in \( \{ \} \) go faster than \( u \to \infty \).

So
\[
\lim_{T \to 0} S(T) = 0
\]

This result is expected because the system can only in the ground state. Thus from Boltzmann's formula \( S = \beta \ln W \),

\( S = 2k \ln 1 = 0 \), so the reason that \( S \to 0 \) as \( T \to 0 \) is that there is perfect order at \( T = 0 \).

(ii) \( \lim_{T \to 0} S(T) \)

As \( T \to 0 \), there isn't perfect order since all three states are populated. Thus we expect \( \lim_{T \to 0} S(T) > 0 \) (since \( S \) cannot be negative).

We work out \( \lim_{T \to 0} S(T) \) from the formula given just before part (i) in the limit \( T \to 0 \)

\[
\lim_{T \to 0} S(T) = 2k \left\{ \frac{1}{T} \left( \frac{4a}{9} \right) + 2 \ln 4 \right\},
\]

The term proportional to \( T^{-1} \) vanishes as \( T \to \infty \). Thus
\[
\lim_{T \to 0} S(T) = 2k \ln 2
\]