

## Extra Problems 1 Solutions

1) Complex A will have the lower reduction potential. The phenolate oxygens in complex A will be harder donors than the thiophenolate sulfurs in complex B. Since Cu(I) is a softer metal than Cu(II), it will prefer a softer ligand set. Thus, a relatively hard ligand set will favor a relatively hard metal. As the ligand set becomes harder, the reduction to a relatively soft metal will become more difficult (i.e. at a lower potential).

2a) False – if an organism used calcium to form a precipitate during every nerve signal transduction, that organism would die very quickly. Since the calcium ion concentration outside the cell is several orders of magnitude greater than the concentration inside the cell, there is a corresponding large potential difference across the cell membrane. Allowing the calcium concentration to equilibrate across the membrane causes a depolarization, which is the basis for signal transduction.

b) True.

c) False – blue copper proteins are electron-transfer proteins. A copper transport protein that only carried one copper per protein would be highly inefficient.

3a) EPR would indicate the number of unpaired spins present per molecule (after doubly-integrating the produced spectrum, which is a first-derivative). If the unpaired electron density surrounds or neighbors a nucleus which is spin-active, then splitting will occur in the EPR spectrum (called hyperfine coupling). Based on the splitting pattern observed, an experimenter can deduce the type of nucleus on which the unpaired electron(s) reside and the types of nuclei adjacent to that nucleus. For example, a single unpaired electron residing in a predominately copper-based orbital will display a quartet in the EPR spectrum. If the copper is bound to a sulfur, then there will be additional coupling. Furthermore, EPR is sensitive to the symmetry of the molecule. In summary, EPR will provide information about the magnetic and symmetric properties of the molecule being studied, and could indicate the location of the unpaired electron density, the identity of the nucleus around which it resides, and the identities of the adjacent nuclei.

b) Mössbauer (MB) is most useful for studying iron-containing samples, due to the long half-life of the source isotope. Iron MB observes the transition from the nuclear spin state  $I = 1/2$  to  $I = 3/2$ . Remember that  $m_I = 0, \pm 1/2, \pm 3/2, \dots, \pm I$ . If the electron density around the iron nucleus is spherical or cubic, then the  $m_I$  levels associated with each  $I$  are degenerate (i.e.  $|3/2 \pm 1/2\rangle$  is degenerate with  $|3/2 \pm 3/2\rangle$ ). When this degeneracy is present, the MB spectrum shows a singlet. However, reducing the symmetry of the electron density around the iron nucleus below spherical/cubic causes the  $\pm m_I$  levels to split (i.e.  $|3/2 + 1/2\rangle$  and  $|3/2 - 1/2\rangle$  are degenerate with each other, but not with  $|3/2 \pm 3/2\rangle$ ). This change produces a doublet in the MB spectrum. Consequently, high-spin  $\text{Fe}^{3+}$  and low-spin  $\text{Fe}^{2+}$  will be distinguishable from low-spin  $\text{Fe}^{3+}$  and high-spin  $\text{Fe}^{2+}$ , respectively.

Additionally, the radius of the charge distribution on the nucleus increases going from the  $I = 1/2$  ground state to the  $I = 3/2$  excited state. Since the only orbitals that lack nodes at the nucleus are s-orbitals, the energy of the nuclear excited state (and thus the energy of the nuclear transition)

will vary depending on these orbitals. Nonetheless, p- and d-orbitals can screen the s-orbital electron density and influence the MB spectrum as a result. While the relationships are very complex, empirical observations can be made: for  $^{57}\text{Fe}$ , increasing 4s electron density decreases the center shift and increasing 3d electron density increases the center shift. Thus,  $\text{Fe}^{2+}$  will appear at a higher center shift in an MB spectrum than will  $\text{Fe}^{3+}$ . If there is unpaired, non-averaged, non-integer electron density surrounding the iron, then the effective internal magnetic field will break the degeneracy between the  $\pm m_l$  levels (i.e.  $|^3/2 + 1/2\rangle$  and  $|^3/2 - 1/2\rangle$  are no longer degenerate). While this topic is beyond the scope of this course, a magnetic MB spectrum can be obtained which has multiplets higher than doublets due to this removal of degeneracy. In summary,  $^{57}\text{Fe}$  MB can indicate the oxidation state of the iron in the sample, along with the spin-state and symmetric/magnetic properties.

c) In the context of this problem and the course as a whole, resonance Raman spectroscopy is used to study complexes of dioxygen. Where the  $\nu_{\text{OO}}$  stretch occurs is diagnostic for the binding mode and electron density on the  $\text{O}_2$  moiety. Since the stretching frequency of a bond can be simplified as two masses on a spring in harmonic oscillation, the shift of the frequency based on the change in mass can be calculated. More specifically, substituting  $^{18}\text{O}_2$  for  $^{16}\text{O}_2$  will produce a shift in the  $\nu_{\text{OO}}$  if there is an OO bond. Resonance Raman spectroscopy will provide information on the charge of the oxygen-containing moiety as well as its binding mode.

d) XANES is diagnostic for the type of metal nucleus and oxidation state. Since the X-rays that scatter off of metal nuclei can then scatter off of adjacent nuclei, information about the ligand environment can be determined by looking at EXAFS. The pattern of back-scattered X-rays depends on the masses and locations of the adjacent nuclei. Consequently, EXAFS can elucidate the geometry at the scattering metal and suggest the type of atoms (heavier vs. lighter) to which the metal is bound.

4) The reduced form of HiPIP is  $\text{Fe}^{\text{II}}_2\text{Fe}^{\text{III}}_2$  and the oxidized form is  $\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}_3$ . Since the iron atoms are bridged by sulfides, the MB spectrum will show that there is only one iron oxidation state on the MB timescale.  $\text{HiPIP}_{\text{red}}$  will appear to contain  $\text{Fe}^{2.5+}$  and  $\text{HiPIP}_{\text{ox}}$  will appear to contain  $\text{Fe}^{2.75+}$ . Table 5.2 from L&B shows that  $4\text{Fe}-4\text{S}$  clusters with two ferrous and two ferric irons have center shifts around 0.42 mm/s, but those with one ferrous and three ferric irons have center shifts around 0.31 mm/s. Thus, the reduced and oxidized forms of HiPIP should be distinguishable. However, only  $^{57}\text{Fe}$ , which is 2.2% abundant, will be detectable by MB. To study the self-exchange reaction of HiPIP, one could use  $^{57}\text{Fe}$ -enriched  $\text{HiPIP}_{\text{ox}}$  and unenriched  $\text{HiPIP}_{\text{red}}$  (or vice versa). Starting with a known concentration of the enriched  $\text{HiPIP}_{\text{ox}}$ , one could add varying concentrations of unenriched  $\text{HiPIP}_{\text{red}}$  and freeze the sample at varying times after its addition. Also, an experimenter could use different starting concentrations of  $\text{HiPIP}_{\text{ox}}$ . Measuring the  $[\text{HiPIP}_{\text{ox}}]$ -, time- and  $[\text{HiPIP}_{\text{red}}]$ -dependence of the decay of the  $\text{HiPIP}_{\text{ox}}$  MB signal will allow the determination of the self-exchange rate. The advantage of MB is that it can distinguish magnetically different nuclei with similar electronic properties. One can substitute natural abundance iron for  $^{57}\text{Fe}$ , without perturbing the chemical reactivity of the substrate, to allow MB visualization. Furthermore, iron oxidation and spin states are well-known and have been studied extensively by MB. However, MB requires the sample be frozen in a matrix, which can be experimentally time-consuming. Additionally, MB can require a great deal of or  $^{57}\text{Fe}$ -enriched sample, both of which can be difficult and expensive. Furthermore, the timescale and

averaging nature of the MB experiment can obscure more detailed information. Since the  $\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}_2$  form of the 4Fe-4S cluster has characteristic UV-vis absorptions and the  $\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}_3$  form does not, one could use stopped-flow optical spectroscopy as an alternative. Using a known concentration of  $\text{HiPIP}_{\text{red}}$  and adding in varying concentrations of  $\text{HiPIP}_{\text{ox}}$ , allowing varying mixing times, and varying the temperature would allow elucidation of the self-exchange rate. Also, the starting concentration of the  $\text{HiPIP}_{\text{red}}$  could be changed. The measurable would be the decrease in the  $\text{HiPIP}_{\text{red}}$  optical absorption intensities.

5a)  $Z$  is the atomic number of an atom, and corresponds to the number of protons in the nucleus. X-rays interact with and are scattered by electrons. Since the number of electrons surrounding a nucleus increases with increasing  $Z$ , there will be more scattering with a heavier atom. Since S ( $Z=16$ ) is much heavier than C ( $Z=6$ ), it will have much more electron density than C, and the scattering patterns will be markedly different. N ( $Z=7$ ) and C ( $Z=6$ ) have similar  $Z$  values and, thus, similar amounts of electron density. As a result, the pattern of scattered X-rays produced by carbon will be similar to that by nitrogen.

b) The energy of an X-ray photon can be computed as follows:

$$E = (h \cdot c) / \lambda = [(6.62 \cdot 10^{-27} \text{ erg} \cdot \text{s}) \cdot (1 \text{ kcal} / 2.4 \cdot 10^{11} \text{ erg}) \cdot (3 \cdot 10^{10} \text{ cm/s})] / (1.54 \cdot 10^{-8} \text{ cm}) \\ = 537 \text{ kcal}$$

One would not expect a typical sample to survive when the energy of *each* photon is this great. However, this energy is only relevant to sample destruction when it is absorbed or scattered inelastically (partial absorption). Since the X-rays are emitted from a copper source, only copper atoms can absorb the X-rays. The overwhelming majority of the X-rays will pass through the sample or be scattered.

c) While XAS cannot distinguish oxygen ( $Z=8$ ) from nitrogen ( $Z=7$ ), it can distinguish geometric differences. A bidentate glutamate will have a different E-M-E angle than two cis histidine ligands, but this difference will be small. Basically, XAS will not be useful.