The exam is based on the article by Jeschke and Polyhach “Distance measurements on spin-labeled biomacromolecules by pulsed electron paramagnetic resonance” [Phys. Chem. Chem. Phys. 9, 1895-1910 (2007)]. The article has been distributed to the students 10 days before the exam and the students were urged to familiarize themselves with this particular subject (also by looking up certain key references in the article). The hardcopy of the article was attached to the exam booklet.

The questions are a mix of fairly basic and more challenging. It is only necessary to answer 10 questions out of 15 completely / correctly to earn a perfect total score.

1. Fig. 1 in the paper by Jeschke & Polyhach (in what follows, J&P) shows the signal routed through the integration gate: “The echo intensity $I(t)$ integrated over the full width at half height is recorded”. What is the advantage of integrating the signal (as opposed to simply measuring the maximum amplitude of the signal)? Why only the central portion of the signal is integrated, and not the wings?

Integration improves signal-to-noise ratio, since the signal adds up constructively whereas the noise, by virtue of being random, partially cancels out. Wings are not included since the amplitude of the signal is low at the wings and we would be picking extra noise and doing more harm than good (at least, in this particular application).

2. At p. 1897, J&P state: “For nitroxides, a distance of 2 nm thus corresponds to a dipolar frequency $\Omega_{dd}/(2\pi) = 6.53$ MHz or a splitting of 0.23 mT in magnetic field units.” Why the frequency which is normally expressed in Herz is also expressed in Tesla?

In magnetic resonance, resonant frequency is proportional to the magnetic field sensed by the spin, $\omega \sim B$.

3. Estimate dipolar frequency in Fig. 3 of J&P (express the answer in MHz).

From the graph, it is clear that the period of oscillation is on the order of 0.6 $\mu$s. Hence, the frequency is ca. 1.7 MHz. Note that determining the oscillation period by eye in this situation is not very accurate because the $\cos$ curve is modulated by a steep exponential decay.

4. Estimate dipolar frequency corresponding to the spin-spin distance of 6 nm.

The dependence on distances is cubic, hence from question 2,
\[ \Omega_{dd} / 2\pi = 6.53 \text{ MHz} \cdot (2 \text{ nm} / 6 \text{ nm})^3 = 0.24 \text{ MHz} . \] Similar estimation can be made on the basis of the question 3 (there the distance is 3 nm, as indicated in the plot and mentioned in the figure caption). The result is 0.21 MHz.

5. The method discussed in J&P is based on the ability to selectively excite two spins (A and B, observer and pump, see Figs. 1 and 5). What is the origin of this selectivity, i.e. how is spin A different from spin B? One can come up with a number of explanations. In principle, it is possible to use two different types of nitroxyl labels (e.g. the protein can be labeled using a mixture of A and B, which would produce ca. 50% of AB pairs). However, it is not the case in this study. Alternatively, one can speculate that g-tensors are different for two nitroxide labels because of the different chemical environment. This also turns out to be incorrect (for the labels of the same type, g-tensors are very similar). The actual explanation is different orientation of the two labels. In the frozen solution one nitroxide ring may be oriented perpendicular to the external field, while the other parallel to the field. These spins would resonate at different frequencies (technically, this happens because the g-tensor is anisotropic, i.e. the spin may sense slightly weaker or stronger field depending on the orientation of the ring relative to \( B_0 \) due to the shielding effect by the anisotropic electron environment). Hence, we acquire the ability to selectively excite just one spin out of the two. See the caption of Fig. 2 for the comment on this aspect.

6. According to the commentary in Section 2.2 of J&P (see also Fig. 2), the intensity of the signal can be modeled as \( V(t) = F(t)B(t) \). \( F(t) \) originates from dipolar interaction between the two nitroxide labels residing at the same molecule; it carries the \( \cos \) modulation encoding dipolar frequency. \( B(t) \) originates from dipolar interaction between the nitroxide labels residing at different protein molecules; it is a smooth monotonous decaying curve. Of note, the origin of \( F(t) \) and \( B(t) \) is essentially similar (there is no fundamental difference between intra- and inter-molecular dipolar interactions). Yet, one of the curves is oscillatory and the other is smooth. Why?

The difference is that in the case of intramolecular interaction the distance between the spins is fixed (more precisely one should speak about a narrow distance distribution). As a result, \( F(t) \) oscillations occur at one well-defined frequency. Conversely, in the case of intermolecular interactions, the spin-spin distances vary widely (recall that we are talking about the frozen solution which is somewhat dilute with respect to the protein). Consequently, \( B(t) \) is a superposition of many oscillatory curves with different frequencies. Finally, a superposition of many oscillatory curves with different (random) frequencies looks like a smooth decay (dephasing effect).

7. Pulses depicted in Fig. 1 of J&P are marked "\( \pi/2 \)" and "\( \pi \)." Explain the meaning of
these labels.

This is called pulse flip angle. The magnetization rotates on a cone (or a circle) through 90° or 180°.

8. Referring to the dipolar oscillations, J&P point out that “good fits are obtained if ... observation time (is) at least two periods of the dipolar oscillation for the mean distance.” Rationalize this observation.

This is necessary to obtain a sufficiently good sampling. For instance, if only the initial portion of the response function is available (e.g. a quarter of a period) it is impossible to recover the correct oscillation frequency and impossible to separate $F(t)$ from $B(t)$.

9. Consider two rectangular pulses:

(1) short duration $t_p^{(1)}$; high power $\omega_t^{(1)}$

(2) long duration $t_p^{(2)}$; low power $\omega_t^{(2)}$

which are matched in terms of area, $\omega_t^{(1)}t_p^{(1)} = \omega_t^{(2)}t_p^{(2)}$. One of these pulses can be described as ‘broadband’, and the other as ‘selective’. Which is which? Please explain.

The short high-power pulse is broadband. Indeed, the large rf field $\omega_t^{(1)}$ overrides the offset (difference in resonance frequencies) $\Omega$. This can be also seen from Eq. (3.10)

10. At p.1900, J&P state: “The excitation profile of this subsequence is thus

$p_A(\Omega) = p_{\pi/2}(\Omega)p_2^2(\Omega)$ (3.13)"

What is the origin of the exponent 2 in $p_2^2(\Omega)$?

There are two $\pi$ pulses with the flip angle $\Omega$ in the subsequence (upper line in Fig. 1). Each pulse incurs losses because of incomplete excitation and the losses hence build up. For instance, if the efficiency of each pulse is 0.5 then after the pair of pulses is applied the net efficiency becomes 0.25.

11. What is the point of distance determination by pulsed EPR when high-resolution structure determination techniques such x-ray crystallography and NMR spectroscopy are available?

Many systems do not crystallize well or not at all (membrane proteins, disordered proteins, amyloids, etc.). At the same time, NMR can target only relatively small biomolecules.

12. What is the meaning of the last factor, $\sqrt{1/T_i(T)}$, in Eq. (3.18). Why signal-to-noise ratio is proportional to this factor?
As we acquire the signal, we should allow for a delay between every two consecutive scans to let magnetization recover (recycling delay). This delay should be approximately equal to the relaxation time $T_1$ (if we wait much longer than that, we are wasting valuable measurement time; if we do not wait long enough, magnetization does not recover and we lose signal). The number of scans $N_S$ that are collected during the experiment is inversely proportional to the recycling delay, $N_S \sim 1/ T_1$. On the other hand, signal to noise increases as $\sqrt{N_S}$ (signal adds up as $N_S$, noise adds up as $\sqrt{N_S}$), hence $S/N$ varies as $(N_S/\sqrt{N_S}) = \sqrt{N_S} = \sqrt{1/T_1}$.

13. Deuteration of the matrix (water + glycerol) may or may not improve the outcome of the experiment, depending on the positions of nitroxide labels in the protein. Why does it happen that sometimes the improvement is observed and sometimes not?

If labels are attached to the surface of the protein and are solvent exposed, then significant improvement is obtained. Otherwise if labels are located in the protein core, or otherwise shielded from solvent (e.g. by bound ligand), there is no improvement (see p. 1903).

14. The Tikhonov regularization method, described in Section 4.1 of J&P, seeks to minimize the roughness of the distance distribution function $P(r)$. Why distribution function cannot be rough?

$P(r)$ results from averaging over many degrees of freedom (all sort of fluctuations and motions in the molecule), hence the roughness is averaged out. Also, we simply do not care for roughness – we do not mind seeing a smoothened function $P(r)$ so long as the shape is properly reproduced and the number of degrees of freedom is effectively restricted (smooth function can be modeled using a relatively small number of parameters). Rugged $P(r)$ can lead to underdetermined optimization problem and false minima.

15. A paramagnetic label can be attached to cystein residue using long and flexible linker or, alternatively, short and rigid linker. Please, discuss advantages and disadvantages of these two approaches.

Long linker is not good, because we no longer measure the distance distribution between the two cystein residues in a biomolecule, but between the two nitrooxyls that start to live their own life. Also, broadened distance distribution $P(r)$ is obtained in this situation, which is not good as it tends to smear out the oscillations (superposition of many oscillatory functions tends to be a smooth function, see question 6). Thus short and rigid linker is preferable. The only reservation is that under some circumstance rigid linker may prove to be more disruptive to the protein native structure.
No Biochemistry crib available
April 02, 2011
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Ph# 45478

No Organic crib available
April 02, 2011
Written by Professor Mao
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Question 1
Chaperone proteins take specific metal ions from specific transmembrane proteins that bring specific metal ions into the cells. The metallochaperone then passes the metal ion to a specific apoprotein, thereby making the required metalloprotein.

Question 2
A) Carbonic anhydrase
B) Zn$^{2+}$
C) One zinc, 3 histidines, 1 hydroxide
D) 5 steps: CO$_2$ binds the protein (not the metal), hydroxide attacks CO$_2$ to make a bound bicarbonate, isomerization of the bicarbonate, release of HCO$_3^-$ with water binding in its place, proton loss to regenerate the Zn-OH.

Question 3
Marine phytoplankton.

Question 4
Iron-sulfur clusters, hemes, and blue copper centers.

Question 5
A) MRI is based upon NMR relaxation of protons in water. The water in different tissues can look pretty similar, thus providing an image that is lacking in clarity.
B) Gd$^{3+}$
C) It has 7 unpaired electrons, the most of any metal ion. More electrons means better relaxation of the water protons.
D) The Gd$^{3+}$ is wrapped in a large ligand, but still leaves space for a water to bind.
\( \psi(x=0) = 0, \quad \psi(x=\infty) = 0, \quad E_n = \hbar \omega (2n + \frac{3}{2}) \)

Degeneracy = 2

Tunneling and Energy Shift

Two non-interacting half harmonic oscillators

\( A \quad B \quad C \quad D \quad E \quad F \quad G \quad H \quad I \quad J \quad K \quad L \quad M \quad N \quad O \quad P \quad Q \quad R \quad S \quad T \quad U \quad V \quad W \quad X \quad Y \quad Z \)

1. For \( A(a, b) \), \( \psi(x=a) = \psi(x=b) = 0 \)
2. \( E(A) < E(A) + E(B) \)
3. \( \frac{d}{dr} \rightarrow \frac{d}{da} \quad \text{rdr} \rightarrow r^2 \text{dr} \quad \frac{e^2}{r} = -\frac{e^2}{r} \)
4. \( 15(1) \alpha(\gamma) 25(2) \beta(2) = 15(2) \alpha(1) 23(1) \beta(1) \)
5. \( \psi(1,2) = -\psi(2,1) \)
6. \( E = -\frac{g}{2} \left( 2 + \frac{1}{4} + 1 \right) = -\frac{g}{2} (2 + \frac{1}{4}) \)