

9.2: DEER

The four-pulse DEER experiment

The most commonly used experiment for distance distribution measurements in the nanometer range is the four-pulse **double electron electron resonance** (DEER) experiment (Figure 9.2), which is sometimes also referred to as **pulsed electron electron double resonance** (PELDOR) experiment. All interactions of the observer spin are refocused twice by two π pulses at times $2\tau_1$ and $2\tau_1 + 2\tau_2$ after the initial $\pi/2$ pulse. Repeated refocusing is necessary since all spin packets must be in phase at $t = 0$ and overlap of the pump π pulse with the $\pi/2$ observer pulse would lead to signal distortion. The first refocusing with interpulse delay τ_1 restores the situation (1) immediately after the $\pi/2$ pulse with phase x , where the magnetization vectors of all spin packets are aligned with the $-y$ axis. $\{\{ \}^{\wedge\{1\}}\}$ In practice, coherence is excited on both observer spin transitions (blue in the energy level panels), but for clarity we consider only observer spin coherence that is on the upper transition and is symbolized by a wavy line in panel (1).

During time t after the first refocusing, magnetization vectors of spin packets with different resonance offset dephase (panel (2)). Only the on-resonant spin packet, marked dark blue, is still aligned with the $-y$ direction. The pump pulse flips the coupling partner and thus transfers the coherence to the lower observer spin transition. The resonance frequency of this transition is shifted by the dipole-dipole coupling d in all spin packets. Observer spin magnetization further dephases until the time just before application of the second observer π pulse (3)) and, in addition, the whole bundle of spin packet magnetization vectors precesses counterclockwise with the frequency shift d . The originally on-resonant spin packet thus gains phase $d(\tau_2 - t)$ before the second observer π pulse is applied. The second observer π pulse with phase x corresponds to a 180° rotation about the x axis. This mirrors the bundle of magnetization vectors with respect to the y axis, inverting phase of the observer spin coherence (panel (4)). The bundle, which still precesses counterclockwise with angular frequency d now lags the $+y$ axis by phase $d(\tau_2 - t)$. During the final interpulse delay of length τ_2 the bundle as a whole gains phase $d\tau_2$ (grey arrow in panel (4) and simultaneously realigns along its center due to echo refocusing. However, the center corresponding to the originally on-resonant spin packet does not end up along $+y$, as it would have in the absence of the pump pulse. Rather, this spin packet has gained phase dt with respect to the $+y$ direction (panel (5)). The magnetization vector component along $+y$, which corresponds to the echo signal, is given by $\cos(dt)$.

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Figure 9.2: Four-pulse DEER sequence, coherence transfers, and evolution of the observer spin magnetization. Pulses shown in blue are applied to the observer spin, the pump pulse shown in red is applied to its coupling partner. The echo at time $2\tau_1$ (dashed blue line) is not observed. Interpulse delays τ_1 and τ_2 are fixed, time t is varied, and the echo amplitude is observed as a function of t .

The distance range of the DEER experiment is limited towards short distances by the requirement that, for echo refocusing, the observer pulses must excite both observer transitions, which are split by d and, for coherence transfer, the pump pulse must excite both transitions of the coupling partner, which are also split by d . In other words, both the observer refocused echo subsequence and the pump pulse must have an excitation bandwidth that exceeds d . This requirement sets a lower distance bound of about 1.8 nm at X-band frequencies and of about 1.5 nm at Q-band frequencies. A limit towards long distances arises, since several dipolar oscillations need to be observed for inferring the width or even shape of a distance distribution and at least one oscillation needs to be observed for determining the mean distance. This requires $t > 2\pi/d$. On the other hand, we have $t < \tau_2$ and the fixed interpulse delay τ_2 cannot be much longer than the transverse relaxation time T_2 , since otherwise coherence has completely relaxed and no echos is observed. Electron spin transverse relaxation times are of the order of a few microseconds. Depending on sample type (see Section 9.1.2), τ_2 can be chosen between 1.5 and 20 μs , corresponding to maximum observable distances between 5 and 12 nm.

Sample requirements

In the wanted coherence transfer pathway of the DEER experiment, observer pulses exclusively excite observer spins and the pump pulse exclusively excites the coupling partner. The excitation bandwidth must be sufficiently large to cover the dipole-dipole coupling d at all orientations, i.e., larger than $\omega_{\parallel} - 2\omega_{\perp}$. If the two coupled spins have the same EPR spectrum, this spectrum must

be broader than twice this minimum excitation bandwidth. This condition is fulfilled for nitroxide spin labels (Chapter 10) and transition metal ions at all EPR frequencies, whereas some organic radicals, such as trityl radicals, have spectra that are too narrow at X-band or even Q-band frequencies. Furthermore, T_2 must be sufficiently long for at least the observer spins. This condition can be fulfilled for almost all $S = 1/2$ species at temperatures of 10 K (transition metal complexes) or $50 \cdots 80$ K (organic radicals), but may require cooling below 4.2 K for some high-spin species. For high-spin species with a half-filled valence shell, such as Mn(II) ($S = 5/2$) or Gd(III) ($S = 7/2$) measurement temperatures of 10 K are also sufficient.

Sample concentration should be sufficiently low for intermolecular distances to be much longer than intramolecular distances. For short distances, concentrations up to $200\mu\text{M}$ are possible, but concentrations of $10 \cdots 50\mu\text{M}$ provide better results, if a spectrometer with sufficient sensitivity is available. Depending on distance and T_2 , measurements can be performed down to concentrations of $10 \cdots 1\mu\text{M}$. For membrane proteins reconstituted into liposomes, data quality is not only a function of bulk spin concentration, but also of lipid-to-protein ratio. This parameter needs to be optimized for each new protein. Required sample volume varies between a few microliters (W-band frequencies) and $150\mu\text{L}$ with $50\mu\text{L}$ at Q-band frequencies usually being optimal.

If concentration is not too high and the low-temperature limit of transverse relaxation can be attained, T_2 depends on the concentration and type of protons around the observer spin. Deuteration of the solvent and cryoprotectant (usually glycerol) usually dramatically improve data quality. If the matrix can be perdeuterated, deuteration of the protein or nuclei acid may further prolong T_2 and extend distance range or improve signal-to-noise ratio.

Complications arise if more than two unpaired electrons are found in the same molecule, but these complications can usually be solved. However, none of the spin pairs should have a distance shorter than the lower limit of the accessible distance range.

¹ This assumes that the observer π pulse has phase y . If it has phase x , the magnetization vector is along $\backslash(+y\backslash)$ after the first refocusing and all following magnetization panels are mirrored with respect to the $\backslash(x\backslash)$ axis.

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