Chapter 9
Conclusions

9.1 PELDOR and Other Distance Measured Methods

In closing, let us briefly compare the PELDOR technique to other structural methods to define its place among them and to summarize the relative advantages and disadvantages.

X-ray crystallography is the most widely used method for determining interatomic distances. It requires good single crystals of the material. In the case of biomolecules, obtaining single crystals may be problematic through lack of the material or instability, poor solubility, or an inability to find the proper conditions for crystallization. As a rule, these problems are difficult to overcome if intricate biological complexes, e.g., protein–membrane complexes, must be analyzed. A less obvious limitation is that the conformations that crystallize are not always the biologically-relevant conformations. The X-ray diffraction process damages the molecules by breaking chemical bonds and can also change the redox state of redox-active centers. The structure obtained from a crystal may not be the structure of the biomolecule in solution, in a cell, or even the intact biomolecule.

EPR methods and, in particular, PELDOR are free from these limitations. A few picomoles of the biomolecules are sufficient for analysis; they can be investigated in various molecular environments in defined redox and pH states, and in complexes with other biosystems. The most important feature of the PELDOR is the fact that this method offers a chance to analyze chaotically-oriented samples. Even intrinsically-disordered proteins or polymers can be studied.

In chaotically-oriented systems, distances can be determined using the fluorescence resonant energy transfer method (FRET) [1]. This optical method is based on the quantum yield of fluorescence from energy transfer between a donor and an acceptor chromophore, which usually must be introduced in the molecule as labels. The mechanism of this transfer can be attributed to dipole–dipole interaction between the electric transition dipoles of the chromophores. The transfer efficiency is proportional to $\alpha/r^{6,}$ where α is a function defined in auxiliary experiments. This

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method has enjoyed wide application thanks to its high sensitivity, as little as one molecule can be detected in an experiment, and the possibility of making measurements in liquid phase. The range of measurable distances almost coincides with the PELDOR range. Among the drawbacks of FRET are the necessity to independently determine the function α, and the size of the chromophores, which are more rigid and bulkier than most spin labels. These drawbacks lead to considerable problems in interpreting distances, to a lower accuracy for r, and to the impossibility of determining $F(r)$.

NMR methods have been widely used to measure distances of a few nanometers. However, NMR has several limitations. One is that only rather short distances, fractions of a nanometer, can be measured directly. Larger distances are obtained from molecular models that try to satisfy all the measured short-range distances. Portions of a molecule having many coexisting conformations may be uncharacterized. Owing to the smaller magnetic moments of nuclei in comparison with the electron magnetic moment, the range of measurable distances in solid state NMR is usually limited to a few nanometers. However, versatility, the highly sophisticated instrumentation and supporting software tools, and broad availability are undeniable advantages of the NMR methods. NMR also benefits from highly developed methods for labeling biomolecules with specific isotopic labels.

The simplest EPR method for measuring distances by dipole broadening is to analyze the width and shape of the CW EPR spectrum. Methods for simulating the spectrum shape and mathematical deconvolution have been developed and reviewed in [2]. Along with dipole interactions, HFI, exchange and quadrupole interactions that make additional contributions to the inhomogeneous linewidth can also be included in the simulation. Such simulations are typically applied for distances of approximately 1.5–2.5 nm, since, at larger distances, the contribution from the dipole interactions becomes negligible compared to other sources of broadening, and simulations fail to obtain reliable and consistent values for the dipole coupling.

The so-called half-field EPR method is applicable for small distances. The forbidden transition between the levels with $m_s = -1$ and $m_s = +1$ for a spin pair in a triplet state become weakly allowed for spins closer than $r \sim 0.5$ nm. This transition has $g \sim 4$ and lies at a magnetic field $\sim H_0/2$, where H_0 is the resonant EPR field of the main allowed transitions. The intensities $I_{\pm 1}$ of these forbidden lines are quite low compared to the intensity I_0 of the allowed lines; nevertheless, the distance between spins can be determined because $I_{\pm 1}/I_0 \propto 1/r^6$ [3]. Various CW and some pulse EPR methods to determine distance are reviewed in [2].

The three and four pulse versions of PELDOR are currently the most widely applied pulsed dipole spectroscopy methods. Single frequency pulse methods, such as “2 + 1” [4] or the single frequency technique for refocusing dipolar couplings (SIFTER) [5], are used much less often. However, the single-frequency double quantum coherence (DQC) method is frequently used [6, 7]. In DQC, the EPR spectrum is fully excited by a sequence of six pulses, and the decay of one of the spin echo signals, which is modulated by the dipolar frequencies, is measured.

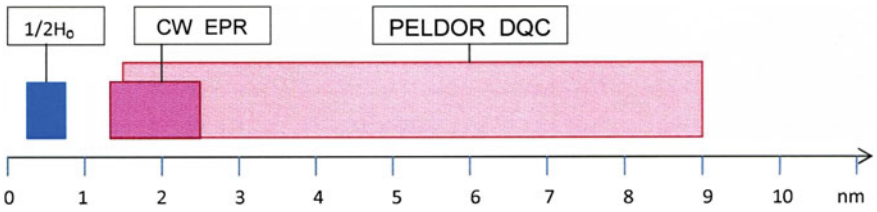

Fig. 9.1 Approximate range of distances measured by CW and pulse EPR spectroscopy

This special pulse sequence provides a way to extract the pairwise dipolar interactions as in PELDOR. DQC places stringent requirements on the hardware and requires more sophisticated theoretical treatments than does PELDOR. Investigations into the composition and properties of various biomolecular structures using the DQC method are reviewed in detail in [8]. As noted above, DQC is efficient in measuring distances up to $r \sim 7.0$–8.0 nm and has greater sensitivity because the full EPR spectrum is excited and contributes to the signal instead of just a portion, as is the case of PELDOR. On the other hand, DQC does not seem capable of giving information about relative orientations of the spins that are available from orientation selection in PELDOR. Like the other single-frequency pulse techniques, DQC is used only in a few laboratories. The approximate range of distances measured by CW and pulse EPR spectroscopy are shown in Fig. 9.1.

The PELDOR technique holds an important place among other structurally-oriented methods of radiospectroscopy, such as CW and pulse EPR, NMR, and NQR. The simplicity of the measurement and the modest instrumental requirements make it available for many laboratories, together with the established methods and software for interpreting its results, are important features of the PELDOR technique. The necessity to introduce paramagnetic centers, e.g., spin labels, is partially compensated for by the large arsenal of special methods, e.g., SDSL, for precisely introducing labels into simple molecules as well as complex biological structures.

9.2 Summary

In conclusion, let us review the main features of the PELDOR technique.

- PELDOR eliminates the inhomogeneous broadening in EPR spectral lines and obtains a direct measurement of the magnetic dipole–dipole interactions in non-oriented systems;
- PELDOR routinely measures distances between paramagnetic centers with a high accuracy in the range of $\sim$ 1.5–8.0 nm, and in some systems, to even twice that distance;

- PELDOR provides the distance distribution function $F(r)$ from the time trace $V(t)$ and can determine other features of the spatial distribution of paramagnetic centers, e.g., their mutual orientation; and
- PELDOR provides an estimation of the number N of centers coupled by dipolar interactions in spatially distinct groups, i.e., complexes, aggregates, clusters, etc.

The PELDOR method is available to a wide circle of researchers having access to pulse EPR spectrometers because of its modest hardware requirements. The theory supporting PELDOR has been developed and is expressed in software tools that support analysis of experimental results to obtain structural information.

PELDOR spectroscopy allows one to go beyond the measurement of distances. One can begin to investigate aggregation; the formation of supramolecular complexes; the interaction of various biologically-important structures with membranes; and even dynamic processes involving paramagnetic particles. PELDOR spectroscopy, together with other high-resolution EPR methods, has a rich future with many more applications in physics, chemistry, and biology.

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