Electron Nuclear Cross Polarization (eNCP) in ENDOR Spectroscopy

T. Orlando, R. Rizzato, I. Bejenke, I. Tkach, M. Bennati

EPR Spectroscopy Research Group, Max Planck Institute for Biophysical Chemistry, D-37077 Göttingen, Germany

Electron nuclear cross polarization (eNCP) is a method that we developed to transfer electronic polarization to nuclear spins in a more efficient way with respect to conventional sequences. It requires concomitant microwave (MW) and radiofrequency (rf) irradiation under the fulfillment of specific conditions of rf field strengths and irradiation offsets. Recently, the validity of the method was demonstrated in combination with Electron Nuclear Double Resonance (ENDOR) spectroscopy, using both model system and proteins\(^1\).

In analogy to CP in NMR, the polarization transfer in eNCP is achieved by locking the electron spin magnetization with MW irradiation in the \((x, y)\) plane, perpendicular to the external magnetic field. During this process, the electron spin can be described in a tilted frame\(^2\), where the quantization axis is along an effective field resulting from the combination of hyperfine coupling \(A\) and MW field strength \(\omega_{1,e}\). The simultaneous rf irradiation at the frequency \(\omega_{rf}\) at an offset \(\Delta\omega_n = \omega_n - \omega_{rf}\) (being \(\omega_n\) the nuclear Larmor frequency) tunes the energy of the states in the tilted frame and induces the polarization transfer if one of the four matching condition is fulfilled\(^3\).

A malonic acid single crystal, having a well defined strong hf coupling, was used as model system. The CP-ENDOR spectra, obtained working at W-band (94 GHz) at \(T = 30\), display a polarized pattern, where the relative intensities of the two lines match the theoretical predictions. The same experiments were carried out on a protein, the \(\beta_2\) subunit of \textit{E.coli} ribonucleotide reductase (RNR), which contains an essential tyrosyl radical. Notably, a larger signal-to-noise (S/N) ratio (factor of about 3.5) was observed for CP-ENDOR with respect to the standard Davies-ENDOR technique, although both experiments were individually optimized for best performance. This demonstrates that CP-ENDOR provides an attractive and efficient new method to record ENDOR spectra on biological samples.

![Figure 1: Schematic representation of the CP-ENDOR experiment in the case of an electron spin \(S = 1/2\) coupled with a nuclear spin \(I = 1/2\) with hyperfine coupling \(A < 0\), as in the malonic acid case. The predicted spectrum matches the experimental results.]

References