

8.2: ESEEM and HYSCORE

ENDOR or ESEEM?

In ESEEM experiments, polarization transfer from electron spins to nuclear spins and detection of nuclear frequencies on electron spin transitions are based on the forbidden electron-nuclear transitions discussed in Chapter 6. Much of the higher polarization of the electron spin transitions is lost in such experiments, since the angle θ between the quantization axes of the nuclear spin in the two electron spin states is usually small and the depth of nuclear echo modulations is $\sin \theta$. Furthermore, modulations vanish along the principal axes of the hyperfine tensor, where $\theta = 0$ and thus $\sin \theta = 0$. Therefore, lineshape singularities are missing in one-dimensional ESEEM spectra, which significantly complicates lineshape analysis. For this reason, one-dimensional ESEEM experiments are not usually competitive with ENDOR experiments, at least if the ENDOR experiments can be performed at $\nu_{\text{L}} \pm \nu_{\text{H}}$ -band frequencies or even higher frequencies. An exception arises for weakly coupled "remote" nuclei in transition metal complexes where exact cancellation between the nuclear Zeeman and the hyperfine interactions can be achieved for one of the electron spin states at X-band frequencies or slightly below. In this situation, pure nuclear quadrupole frequencies are observed, which leads to narrow lines and easily interpretable spectra. One-dimensional ESEEM data are also useful for determining local proton or deuterium concentrations around a spin label, which can be used as a proxy for water accessibility (Section 10.1.6).

The main advantage of ESEEM compared to ENDOR spectroscopy is the easier extension of ESEEM to a two-dimensional correlation experiment. Hyperfine sublevel correlation (HYSCORE) spectroscopy 8.2.3 resolves overlapping signal from different elements, simplifies peak assignment, and allows for direct determination of hyperfine tensor anisotropy even if the lineshape singularities are not observed.

Three-pulse ESEEM

The HYSCORE experiment is a two-dimensional extension of the three-pulse ESEEM experiment that we will treat first. In this experiment, the amplitude of a stimulated echo after is observed with the pulse sequence $\pi - \tau - \pi - \tau - \pi$ as a function of the variable interpulse delay τ at fixed interpulse delay τ_0 (Fig. 8.4). The block $\pi - \tau - \pi$ serves as a nuclear coherence generator, as discussed in Section 6.3.1 and, simultaneously, creates the polarization grating discussed in the context of the Mims ENDOR experiment (Section 8.1.2). In fact, most of the thermal equilibrium magnetization is converted to the polarization grating whose FID after the final π pulse is the stimulated echo, while only a small fraction is transferred to nuclear coherence. The phase of the nuclear coherence determines how much of it contributes to the stimulated echo after back transfer to electron spin coherence by the last π pulse. For an electron-nuclear spin system $I = 1/2$ this phase evolves with frequencies ν_{H} or $-\nu_{\text{H}}$ if during interpulse delay τ the electron spin is in its $m_S = 1/2$ or $m_S = -1/2$ state, respectively. Hence, the part of the stimulated echo that arises from back transferred nuclear coherence is modulated as a function of τ with frequencies ν_{H} and $-\nu_{\text{H}}$.

An expression for the echo envelope modulation can be derived by product operator formalism using the concepts explained in Section 6.2. Disregarding relaxation, the somewhat lengthy derivation provides

$$M(\tau) = M_0 \left[\cos(\pi \nu_{\text{H}} \tau) + \cos(\pi \nu_{\text{H}} \tau_0) \right]$$

where the terms $\cos(\pi \nu_{\text{H}} \tau)$ and $\cos(\pi \nu_{\text{H}} \tau_0)$ correspond to contributions with the electron spin in its $m_S = 1/2$ or $m_S = -1/2$ state, respectively, during interpulse delay τ . These terms are given by

$$M(\tau) = M_0 \left[\cos(\pi \nu_{\text{H}} \tau) + \cos(\pi \nu_{\text{H}} \tau_0) \right]$$

The factors $\cos(\pi \nu_{\text{H}} \tau)$ for the $\cos(\pi \nu_{\text{H}} \tau)$ term and $\cos(\pi \nu_{\text{H}} \tau_0)$ for the $\cos(\pi \nu_{\text{H}} \tau_0)$ term describe the blind spot behavior of three-pulse ESEEM. The modulation depth $M(\tau)$ is given by

$$M(\tau) = M_0 \left[\cos(\pi \nu_{\text{H}} \tau) + \cos(\pi \nu_{\text{H}} \tau_0) \right]$$

For small hyperfine couplings, $\nu_{\text{H}} \tau \ll 1$, we have $\cos(\pi \nu_{\text{H}} \tau) \approx 1$, so that Eq. (8.5) reduces to

$$M(\tau) = M_0 \left[\cos(\pi \nu_{\text{H}} \tau) + \cos(\pi \nu_{\text{H}} \tau_0) \right]$$

i.e., the modulation depth is inversely proportional to the square of the magnetic field. Using Eqs. (4.10) and (4.11) we find for protons not too close to a well localized unpaired electron

[Math Processing Error]

where *[Math Processing Error]* is the angle between the electron-proton axis and the static magnetic field *[Math Processing Error]*.

Because of the star topology of electron-nuclear spin systems (Fig. 4.4(a)), Eq. (8.3) can be easily extended by a product rule to multiple nuclei with spins *[Math Processing Error]*, where *[Math Processing Error]* is an index that runs over all nuclei. One finds

[Math Processing Error]

In the weak modulation limit, where all modulation depths *[Math Processing Error]* fulfill the condition *[Math Processing Error]*, the ESEEM spectrum due to several coupled nuclei is the sum of the spectra of the individual nuclei.

Copy and Paste
Image here.
Delete this
placeholder image

Figure 8.4: Pulse sequences for three-pulse ESEEM (a) and HYSCORE (b). In three-pulse ESEEM, time *[Math Processing Error]* is varied and time *[Math Processing Error]* is fixed. In HYSCORE, times *[Math Processing Error]* and *[Math Processing Error]* are varied independently in order to obtain a two-dimensional data set.

HYSCORE

The HYSCORE experiment is derived from the three-pulse ESEEM experiment by inserting a microwave *[Math Processing Error]* pulse midway through the evolution of nuclear coherence. This splits the interpulse delay *[Math Processing Error]* into two interpulse delays *[Math Processing Error]* and *[Math Processing Error]* (Fig. 8.4(b)), which are varied independently to provide a two-dimensional data set *[Math Processing Error]* that depends parametrically on fixed interpulse delay *[Math Processing Error]*. The inserted *[Math Processing Error]* pulse inverts the electron spin state. Hence, coherence that has evolved with frequency *[Math Processing Error]* during interpulse delay *[Math Processing Error]* evolves with frequency *[Math Processing Error]* during interpulse delay *[Math Processing Error]* and vice versa. In the weak modulation limit, the HYSCORE experiment correlates only frequencies *[Math Processing Error]* and *[Math Processing Error]* of the same nuclear spin. The full modulation expression for the HYSCORE experiment contains a constant contribution and contributions that vary only with respect to either *[Math Processing Error]* or *[Math Processing Error]*. These contributions can be removed by background correction with low-order polynomial functions along both dimensions. The remaining modulation corresponds to only cross peaks and can be expressed as

[Math Processing Error]

with

[Math Processing Error]

In this representation with unsigned nuclear frequencies, one has *[Math Processing Error]* for the weak coupling case *[Math Processing Error]* and *[Math Processing Error]* for the strong coupling case *[Math Processing Error]*, as can be inferred from Fig. 6.1. Hence, *[Math Processing Error]* in the weak coupling case and *[Math Processing Error]* in the strong coupling case. In the weak coupling case, the cross peaks that correlate nuclear frequencies with the same sign (*[Math Processing Error]* terms) are much stronger than those that correlate frequencies with opposite *[Math Processing Error]* terms) whereas it is the other way around in the strong coupling case. Therefore, the two cases can be easily distinguished in HYSCORE spectra, since the cross peaks appear in different quadrants (Fig. 8.5). Furthermore, disregarding a small shift that arises from the pseudo-secular part *[Math Processing Error]* of the hyperfine coupling (see below), the cross peaks of a given isotope with spin *[Math Processing Error]* are situated on parallels to the anti-diagonal that corresponds to the nuclear Zeeman frequency *[Math Processing Error]*. This frequency in turn can be computed from the nuclear *[Math Processing Error]* value (or gyromagnetic ratio *[Math Processing Error]*) and the static magnetic field *[Math Processing Error]*. Peak assignment for *[Math Processing Error]* nuclei is thus straightforward. For nuclei with *[Math Processing Error]* the peaks are further split by the nuclear quadrupole interaction. Unless this splitting is much smaller than both the hyperfine interaction and the nuclear Zeeman interaction *[Math Processing Error]*, numerical simulations are required to assign the peaks and extract the hyperfine and nuclear quadrupole coupling.

Copy and Paste
Image here.
Delete this
placeholder image

Figure 8.5: Schematic HSCORE spectrum for the phenyl radical (compare Fig. 4.6). Note that hyperfine couplings are given here in frequency units, not angular frequency units. Signals from weakly coupled nuclei appear in the right

[Math Processing Error]

quadrant. To first order, these peaks are situated on a line parallel to the anti-diagonal that intersects the

[Math Processing Error]

axis at

[Math Processing Error]

. The doublets are centered at

[Math Processing Error]

and split by the respective hyperfine couplings. Signals from strongly coupled nuclei appear in the $(-, +)$ quadrant. To first order, these peaks are situated on two lines parallel to the anti-diagonal that intersect the

[Math Processing Error]

axis at

[Math Processing Error]

and

[Math Processing Error]

. The doublets are centered at half the hyperfine coupling and split by

[Math Processing Error]

.

The small pseudo-secular shift of the correlation peaks with respect to the anti-diagonal contains information on the anisotropy *[Math Processing Error]* of the hyperfine interaction (Fig. 8.5). In the solid state, the cross peaks from different orientations *[Math Processing Error]* form curved ridges. For a hyperfine tensor with axial symmetry, as it is encountered for protons not too close to a well-localized unpaired electron, the maximum shift in the diagonal direction corresponds to *[Math Processing Error]* and is given by *[Math Processing Error]*. Since *[Math Processing Error]* is known, *[Math Processing Error]*, and thus the electron-proton distance *[Math Processing Error]* can be computed from this maximum shift. If *[Math Processing Error]*, which is usually the case, the orientation with maximum shift is at the same time the orientation with maximum modulation depth.

The curved ridges end at their intersection with the parallel to the anti-diagonal. These points correspond to the principal values of the hyperfine tensor and modulation depth is zero at these points. However, it is usually possible to fit the theoretical ridge to the experimentally observed ridge, as the curvature near *[Math Processing Error]* together with the position of the *[Math Processing Error]* point fully determines the problem.

Copy and Paste
Image here.
Delete this
placeholder image

Figure 8.6: Schematic HYSCORE spectrum for a proton with an axial hyperfine tensor with anisotropy $[Math Processing Error]$ and isotropic component $[Math Processing Error]$. The correlation peaks from different orientations form curved ridges (red). Curvature is the stronger the larger the anisotropy is and the ratio of squared anisotropy to the nuclear Zeeman frequency determines the maximum shift with respect to the $[Math Processing Error]$ anti-diagonal.

Analysis of HYSCORE spectra requires some precaution due to the blind-spot behavior (factor $[Math Processing Error]$ in Eq. (8.9)) and due to orientation selection by the limited bandwidth of the microwave pulses that is much smaller than spectral width for transition metal complexes. It is therefore prudent to measure HYSCORE spectra at several values of $[Math Processing Error]$ and at several observer positions within the EPR spectrum.

This page titled [8.2: ESEEM and HYSCORE](#) is shared under a [CC BY-NC 4.0](#) license and was authored, remixed, and/or curated by [Gunnar Jeschke](#) via [source content](#) that was edited to the style and standards of the LibreTexts platform.