Research Highlight #143

Measuring Inter-Spin Distances in the Borderline Region Using Very Low Frequency Non-Adiabatic Rapid Sweep Electron Paramagnetic Resonance (VLF NARS-EPR)

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Introduction: When a system is not motionally averaged, a pair of spins is coupled by a through-space dipolar interaction. The energy of the interaction is inversely related to the cube of the distance, *r*, between the two spins. In continuous wave (CW) EPR, this manifests itself as a spectral broadening resulting from convolution of the dipolar Pake function [1,2]. However, if the broadening is small relative to the inhomogeneous linewidth, the dipolar interaction is not apparent. At X-band, g-anisotropy dominates the rigid-limit spectrum and the upper limit for distance determination ranges from 16-18 Å. Electron double resonance (ELDOR) experiments such as DEER allow for measurements up to 80 Å, but the lower limit hovers in the 18-20 Å range because the bandwidths of the pump and observe pulses must be smaller than the dipolar coupling frequency to avoid spurious signals [3,4]. As a result, there exists a region between 16-20 Å where both techniques are necessary to accurately calculate a distance. In the present work, we show that by going to very low frequencies (VLF), this so-called borderline region no longer exists because linewidths are four to seven times narrower than those at X-band. This was previously not possible because the factor of ten loss in sensitivity due to the frequency dependence of the Boltzmann factor could not be overcome. The recent development of non-adiabatic rapid sweep (NARS) EPR at the National Biomedical EPR Center improves sensitivity by factor of four overcoming this loss [5].

Methods: A series of 11 helical peptides with cysteine mutations strategically placed throughout were synthesized and labeled with perdeuterated MTSL (pdMTSL). EPR spectra were collected at -20°C in the presence of 50% d^8 glycerol using a Varian E-9 spectrometer equipped with a B-H15 Bruker field controller, L-band bridge, and a one-loop-one-gap resonator operating at 1.9 GHz. NARS was performed as described previously by Kittell et al. with one minor adjustment [5]. A new voltage controlled amplifier was implemented, allowing for field excursions of 50 G peak-to-peak on the triangular waveform operating at 2.6 kHz (260 kG/s). To avoid potential broadenings arising from non-linearities in the triangular sweep, spectra were collected in 5 G increments over 140 G spanning the nitroxide spin envelope. Each segment was averaged 100 k times (38.5 s/segment, 18 min/spectrum). Spectra were pieced together by isolating the central 5 G of each segment and concatenating them end to end. Baseline variability was removed using a third-degree polynomial fit, and each spectrum was further processed using a Fourier filter to remove high frequency noise.

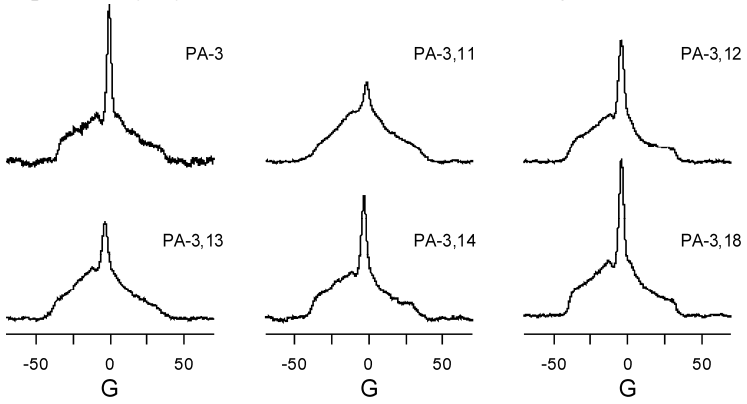

Figure 1: VLF NARS EPR spectra normalized to spin concentration.

Table 1: Experimentally measured values as determined by VLF NARS-EPR compared to the expected values calculated from a helical model in Å

	Exp. *r*	Exp. σ	Mod. *r*	Mod. σ
PA-3,11	15.6	3.4	17.0	4.3
PA-3,12	22.4	3.8	22.2	2.9
PA-3,13	20.4	>4.0	20.3	5.3
PA-3,14	18.9	>3.5	18.9	4.8
PA-3,18	25.9	5.9	26.4	5.5

Results: The inhomogeneous linewidth of the single cysteine mutant shown in Fig. 1 was 2.3 G, allowing inter-spin measurements up to 28 Å. Dipolar broadening was present in all double mutants, also shown in Fig. 1. The calculated distances and distributions are presented in Table 1 with the expected distances determined by a helical model. The model and experimental measurements are in excellent agreement, with only minor differences present at the shortest and longest distances due to poor SNR in broad signals and small changes in the narrow signals, respectively.

In addition, narrower lines have been achieved at room temperature using the soluble protein T4 lysozyme. As a result, inter-spin distances of up to 30 Å have been measured (data not shown). It is concluded that a new general method for inter-spin distance determination has been developed.

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