Research Highlight #148

Long Range Inter-spin Distance Determination Under Near-Physiological Conditions Using L-band Non-Adiabatic Rapid Scan Electron Paramagnetic Resonance (NARS EPR) Spectroscopy

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Introduction: Rabenstein and Shin showed that inter-spin distances (*r*) can be determined by measuring the extent of spectral broadening that results from the introduction of a second spin to the system [1]. If several inter-spin distances are obtained, they can be used as rigorous constraints in protein structure calculations. In this highlight, we show that by going to L-band, nitroxide linewidths are 4-6 times narrower than at the more commonly used X-band. As a consequence, the upper limit of the method can be increased from 18Å to 32Å. The extension of the technique to L-band was previously unfeasible because of the factor of ten reduction in sensitivity due the decrease in frequency. The decreased linewidth as well as the application of NARS, which was recently developed at the National Biomedical EPR Center, provided the means to overcome these losses and has since opened doors to studies that were previously impossible.

Methods: L-band NARS EPR spectra of a series of helical peptides in a 50% d8-glycerol v/v solution were collected at -20°C to ensure no averaging of the dipolar interaction. A detailed description of the sample preparation process, sample requirements, L-band NARS acquisition method, and dipolar analysis can be found in our recent publication on this study [2].

Results:.The inhomogeneous linewidth of the single-labeled helical peptide shown in Fig.1 was 2.3 G, allowing for inter-spin measurements of up to 28 Å. Dipolar broadening was present in all doubly-labelled mutants and the calculated distances are displayed in Table 1 with the expected distances determined by a helical model. Comparison of the two data sets shows the measurements to be in excellent agreement, and validates both the NARS and L-band distance determination methods. Narrower lines (1.9 G, data not shown) have been achieved at room temperature in the soluble protein, T4 lysozyme. Higher temperatures could be used due to the larger size of the protein compared to the 22 amino acid peptide, and distance measurements up to 32 Å are feasible provided that the sensitivity is sufficient.

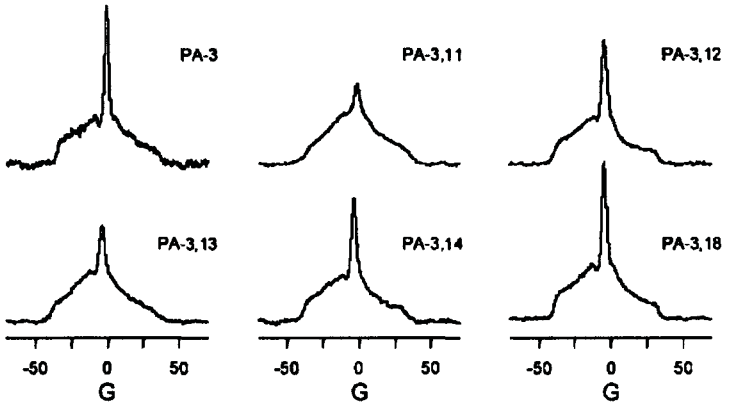

Figure 1: L-band NARS EPR spectra normalized to spin

Table 1: Experimentally measured values as determined by L-band 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

Implications: It has become common practice to measure inter-spin distances in EPR using a four-pulse dead time free technique known as Double Electron-Electron Resonance (DEER). Although the range of application is wide (~20-80 Å), DEER requires cryogenic temperatures and cryoprotectants, such as glycerol, which can affect the native protein structure. The technique presented in this highlight makes measurements under near-physiological conditions feasible in the 8-32 Å range. Our contribution provides the means to do thorough comparative studies on the effects of temperature and/or cryoprotectants on protein structure.

Discussion: An investigation into the effects of cryoprotectants and temperature is already underway at the National Biomedical EPR Center. We are currently studying how measurements in the cytoplasmic domain of the Band 3 protein change in the presence of Ficoll, glycerol and sucrose. Preliminary results suggest that cryoprotectants play a critical role in the rotameric equilibrium of the spin-label. This suggests that they can also affect localized protein structure. These experiments are the first of their kind, and, to the authors' knowledge, are the first to be able to *directly* measure the effect.

[1] Rabenstein MD, Shin YK. Determination of the distance between two spin labels attached to a macromolecule. Proc.Natl. Acad. Sci. USA (1995)92:8239-8243. [PMC41132]

[2] Kittell AW, Hustedt EJ, Hyde JS. Inter-spin distance determination using L-band (1-2 GHz) non-adiabatic rapid sweep electron paramagnetic resonance (NARS EPR). J. Magn. Reson. (2012) 221:51-56. [NIHMS552879]