

Distance Measurements Between Broadly Distributed Nitroxide Labels in a Series of Synthetic Peptides: The CW/Pulsed EPR Borderline

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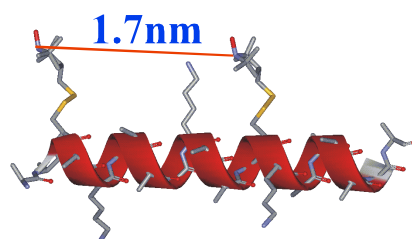
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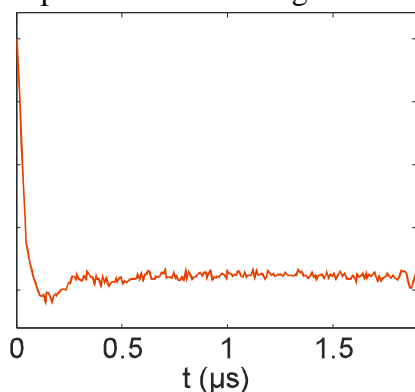
Dipolar coupling between unpaired electron spin centres can be used to measure their separation. The dipolar coupling manifests itself as a broadening of the continuous wave spectrum from the frozen solution but this can only be seen to distances of about 2nm for protonated nitroxide spin labels. Double electron-electron resonance techniques (such as DEER) are useful for longer range couplings, in the region of 1.5 to 8nm⁽ⁱ⁾.

Here, a series of peptides have been synthesised and doubly labelled with methanethiosulfonate spin label (MTSSL) or saturated MTSSL such that the distances between centres are between 0.8 and 4.5nm with the majority between 1.2 and 2.5nm.



Peptide "4K(3,14)": Geometric alpha-helical model with spin labels

The peptides are helical in mixtures of water and trifluoroethanol but not rigid, hence the distances are broadly distributed. Their structures have been characterised using circular dichroism and homonuclear NMR methods. Data obtained from NOESY experiments are being used as distance constraints in structure calculations using molecular dynamics simulations.



4-pulse DEER time trace of 4K(3,14)

The continuous wave results (measurements taken at 50K and with an X-band spectrometer) are interpreted in several ways: simulation (DipFit program⁽ⁱⁱ⁾), second moment analysis⁽ⁱⁱⁱ⁾ and Fourier deconvolution^(iv).

The 4-pulse DEER experiment was performed on all samples with a 12ns pump pulse and DeerAnalysis was used to find the distances and distributions^(v).

References:

(i) Jeschke, G., *Chem Phys Chem* **3** 927-932 (2002)

(ii) Steinhoff, H.J., et al., *Biophys J* **73** 3287-3298 (1997)

(iii) Steinhoff, H.J., *Frontiers in Bioscience* **7** c97-110 (2002).

(iv) Rabenstein, M.D., Shin, Y-K., *Proc Natl Acad Sci USA* **92** 8239-8243 (1995)

(v) <http://www.mpip-mainz.mpg.de/~jeschke/distance.html>