

Mn²⁺ - nitroxide W-band DEER as tool to measure nm scale distances in RNA and protein RNA complexes.

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The pulse DEER (Double Electron-Electron Resonance) technique is frequently applied for nanometer scale distances measurements in biomolecules. It's most common application is to measure distances between two nitroxide spin labels attached at specific positions in the macromolecules of interest and it has been successfully applied to both proteins and nucleic acids. Nevertheless, the DEER experiment is not limited to distance measurements between nitroxide spin labels and it has been successfully used for distance measurements between other types of paramagnetic centers, such as pairs of organic radicals, Cu²⁺ and Gd³⁺ ions and metal ion-nitroxide pairs.

High field pulse EPR measurements offers a number of advantages over conventional X-band (9.5GHz) frequencies, particularly for half integer high spin ions with an isotropic g, such as Mn²⁺ (S=5/2) because of the narrow linewidth of their central $| -1/2 \rangle \rightarrow | 1/2 \rangle$ transition at high fields that leads to high sensitivity.

In this work we demonstrate W-band (95GHz) DEER distance measurements between nitroxide spin-labeled RNA and Mn²⁺ that binds either to the same RNA molecule, or to the RNA helicase DbpA.. In the latter it substitutes for the Mg²⁺ in the ATPase active site of the enzyme. Optimal experimental parameters such as the selection of the pump and observer spins, microwave (MW) power, pulse length and frequency separation between the two MW channels are discussed and evaluated.

Paramagnetic Mn²⁺ occurs naturally as an intrinsic cofactor in nucleic acids and metalloenzymes or it can be introduced artificially into many systems as a substitute for the diamagnetic Mg²⁺. Thus our results pave the way for many new applications of DEER in the systems where nitroxide spin labeling is problematic or where introduction of the additional, orthogonal, paramagnetic probe provides additional information.