

# Photoexcited triplet states as spin labels: methodology aspects for pulsed dipolar EPR spectroscopy and application to biomolecules

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Photoexcited triplet states of organic molecules are promising spin labels with advanced spectroscopic properties for Pulsed Dipolar Electron Paramagnetic Resonance (PD EPR) spectroscopy. Strong triplet hyperpolarization led to a drastically enhanced sensitivity compared to common DEER with nitroxide observer.

Recently proposed triplet fullerene labels have shown great potential for Double Electron-Electron Resonance (DEER) distance measurements as “observer spins” due to a high quantum yield of the triplet state, hyperpolarization and relatively narrow EPR spectrum [1]. This work [2] demonstrates the applicability of fullerene labels to other PD EPR techniques, such as Relaxation Induced Dipolar Modulation Enhancement (RIDME) and Laser Induced Magnetic Dipolar spectroscopy (LaserIMD). In particular, a specific contaminating signal in LaserIMD experiments was observed, explained and mitigated. Comparative analyses of the signal-to-noise (SNR) ratios were performed for all employed methods. DEER on fullerene-triarylmethyl pair shows best performance, which allows state-of-the-art DEER acquisition at 100 nM with SNR~35 within reasonable 42 hours.

Photoexcited triplet states are also actively used within biological complexes as photosensitizers for photodynamic therapy (PDT) of cancer. In this work, we propose pulsed dipolar EPR spectroscopy in combination with other EPR methods as a tool to determine and characterize binding sites of photosensitizer to biopolymers. The report will show that pulsed dipolar EPR spectroscopy provides valuable information on the structure and function of PDT candidate complexes, exemplified with photosensitizer bound to human serum albumin (HSA)[3] and G-quadruplex DNA.

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[1] *Angewandte Chemie International Edition* **2019**, 58.38, pp. 13271-13275.

[2] *Phys. Chem. Chem. Phys.* **2022**, 24, pp. 4475-4484.

[3] *J. of Photochem. and Photobiol. B: Biology* **2020**, 211, p. 112008.