

New light-induced Pulsed ESR Dipolar Spectroscopy methodologies for the elucidation of molecular conformation

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The suitability of the photoexcited triplet state of porphyrins for nanometer distance measurements by Pulsed ESR Dipolar Spectroscopy (PDS) was demonstrated in combination with nitroxide radicals. Thanks to its non-Boltzmann population, the photoexcited triplet enhanced signal intensity when used as detection spin in DEER [1] and improved modulation depth when used as pump spin in LaserIMD [2]. Here we exploit the orientation selection effects in these triplet-nitroxide experiments to extract additional conformational information in model peptides with different chromophores, namely tetraphenylporphyrin, diiodo-BODIPY and Erythrosin B (Fig. 1 (a)) [3]. Using simulations and DFT calculations, we extract distance distributions and relative orientations of the two spin-bearing moieties, allowing the dominant conformation of the peptide in frozen solution to be identified. We also present, for the first time, a frequency-correlated version of LaserIMD, which monitors the complete orientation dependence of the system in a single experiment.

Going a step further in light-induced PDS, we present the new technique of Light-Induced Triplet-Triplet Electron Resonance spectroscopy (LITTER) [4], which uses photoexcited triplet states as both detection and pump spins, enabling both the distance and angular distributions between the two triplet moieties to be determined in a model peptide (Fig. 1 (b)). LITTER removes the requirement of current light-induced PDS techniques to have a permanent paramagnetic moiety, becoming more suitable for in-cell applications and potentially giving access to distance determination in unmodified macromolecular systems containing photoexcitable moieties. LITTER also has the potential to enable direct comparison with FRET and combination with microscopy inside cells.

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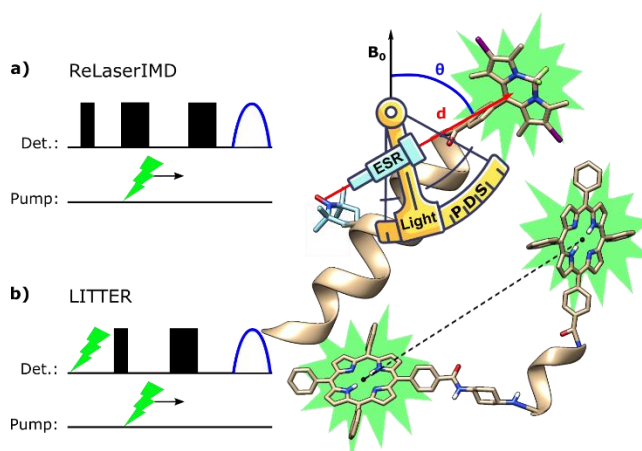


Figure 1. Pulse sequences for the Refocused LaserIMD (a) and LITTER (b) experiments, and structures of model peptides used in the studies of the orientational effects.

