

Light-induced Pulsed Dipolar EPR Spectroscopy for distance and orientation analysis

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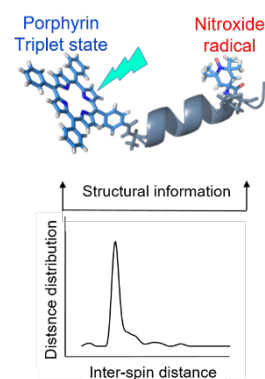
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EPR pulsed dipolar spectroscopy is a well-established technique to determine precise distance distributions between paramagnetic centers with distances ranging from around 1.6 nm up to 16 nm. In combination with site-directed spin labeling, it is ideally suited for structural characterizations of macromolecules and complexes, and has emerged as valuable tool in structural biology.

In recent years, porphyrins have been introduced in the selection of spin labels for dipolar spectroscopy applications. In their ground state, these chromophores are diamagnetic and thus EPR-silent, but, upon laser photoexcitation, their triplet state can be populated via intersystem crossing from the lowest excited singlet state, generating in this way the paramagnetic center.

Here we present various light-induced techniques, which exploit the distinctive properties of the porphyrin triplet state including the electron spin polarization. In combination with nitroxide spin labels or using two porphyrin probes, they enable both the distance and angular distributions between the two paramagnetic moieties to be determined. Pulsed dipolar spectroscopy has been applied on peptide-based spectroscopic rulers in order to test the accuracy, sensitivity and distance limits and it has been extended to paradigmatic proteins, containing an endogenous porphyrin probe.¹⁻⁴ Different chromophores with high triplet yield have also been introduced and the hyperpolarization of the nitroxide radical has been considered for a further increase in sensitivity.⁵

The methodology has a high potential for measuring nanometer distances in more complex biological systems and for future in-cell applications.



References

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