Helical Peptides Bearing Dual Triplet-spin/Fluorescent Labels Enable the First Parallel Distance Measurements by Electron Spin Resonance and Förster Resonance Energy Transfer

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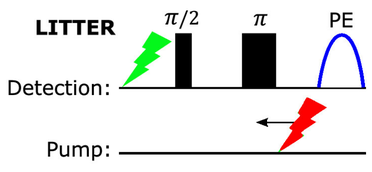
INTRODUCTION

Comparison between different methods in structural biology can be important to identify structural changes or prevent interpretation of structural artifacts related to sample conditions or preparation. Electron spin resonance (ESR) pulsed dipolar spectroscopy (PDS) is a set of techniques for the study of conformational flexibility and disorder in complex biological macromolecular systems.

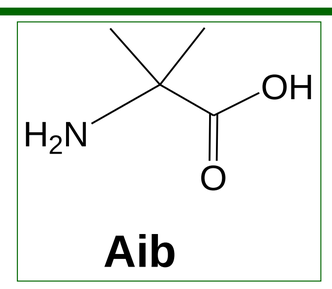


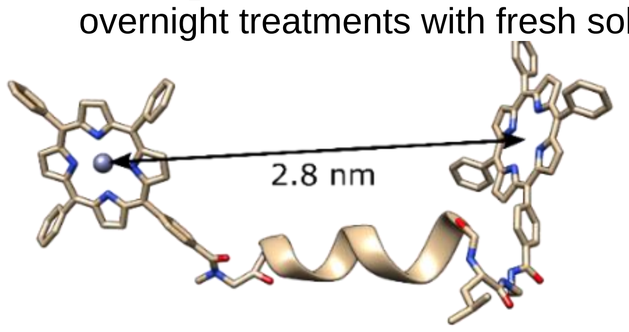
The light-induced triplet–triplet electron resonance (**LITTER**) technique, enables the measurement of the dipolar interaction between two photogenerated triplets, in chromophore-containing molecules, which are ESR silent in their ground state, eliminating the need for permanent spin centers [1]. LITTER pulse sequence: π/2 and π MW detection pulses (**black**) preceded by laser 1 (green) form a primary spin-echo (PE). The intensity is modulated by the formation of the second triplet by time-variant laser 2 (pump, red).

RESULTS & DISCUSSION

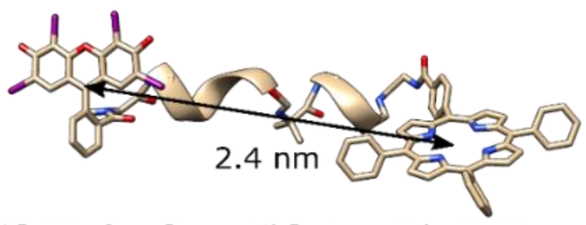
Peptide Synthesis.

1. **Bis-labeled model peptides [1] and [2] containing orthogonal chromophore pairs**, TPP with Zn(II)TPP (ZnTPP) and TPP with erythrosine B (EB), respectively, were synthesized by SPPS to have a rigid α-helical structure resulting from alternating L-leucine-α-aminoisobutyric acid (Leu-**Aib**) residues.
2. **A sarcosine (Sar) linker** was used to attach the labels to the N-terminus, **avoiding the formation of colorless EB spiro-lactam**. This is advantageous over previous strategies as it uses the more affordable non-derivatized chromophore and introduces a shorter linker.
3. **Zn(II) complexation was achieved directly with resin-bound TPP peptide**, by several treatments with a saturated solution of $Zn(Ac)_2$ in a DMF/Ethanol mixture 1:1, thus reducing the number of purification steps. Quantitative complexation on resin required two overnight treatments with fresh solutions of $Zn(Ac)_2$ (Zinc Acetate, 50 equiv.).





[1] **ZnTPP**-Sar-[Leu-Aib]$_4$-Leu-Eda-**TPP**



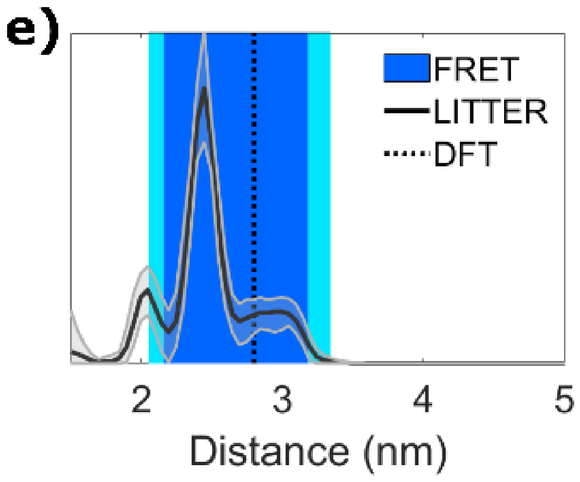
[2] **EB**-Sar-[Leu-Aib]$_4$-Leu-Eda-**TPP**

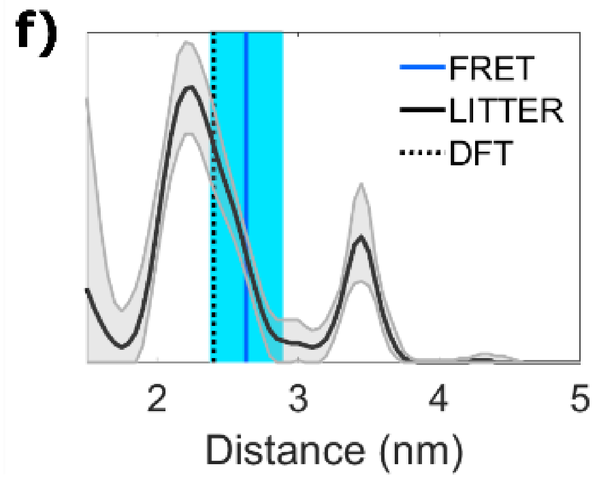
Either 5-(4-Carboxyphenyl)-10,15,20-(triphenyl)porphyrin (**TPP**-COOH) or Erythrosin B (**EB**) was successfully inserted at the N-terminus by coupling reactions repeated three times (1h, 1h, and overnight, respectively) with three equivalents of the chosen chromophore.

1,2-Diaminoethane-trityl resin [thus preloaded with the C-terminal linker ethylenediamine (Eda)] was used, and the C-terminal **TPP** group was added in solution.

Light-induced pulsed dipolar spectroscopy methods have been developed based on the photogenerated triplet state of a **TPP** moiety as photoswitchable spin-label.

Chromophore center-to-center distances determined by in vacuo DFT optimization.





The first LITTER experiments with optically orthogonal chromophores and 2-color photoexcitation resulted in larger modulation depths corresponding to a **4-fold shortening of the acquisition time** with respect to previous 1-color measurements are reported.

Comparison of the distances determined for [**1**] (**e**) and [**2**] (**f**) by FRET (blue) with error bounds (cyan), LITTER (solid black line) with 95% confidence intervals (gray), which include analysis of the uncertainty in the background correction, and DFT (dotted black line).

CONCLUSIONS

The 2-color LITTER represents a dramatic step forward in biological structural determination in systems where FRET labels can be added or innate chromophores used. The choice of orthogonal chromophores provides a significant improvement in data quality compared to the initial homo-LITTER experiments. Furthermore, the selective optical addressability of each chromophore has the potential to enable **unambiguous determination of the PDS distance in systems with more than two labels**, without the interference of multi-spin effects. Optical orthogonality is easier to achieve and allows a wider choice of labels than spin-label orthogonality. There is also potential for increasing the modulation depth by further reducing the spectral overlap and unwanted excitations by choosing different chromophore pairs and excitation wavelengths. LITTER requires low concentrations and is likely suitable for in-cell ESR studies, where conventional nitroxide labels degrade rapidly. When combined with fluorescence microscopy, LITTER could provide location-specific structural infor-mation for labelled biomolecules inside cells.

REFERENCES

[1] Bertran A., Henbest K.B., De Zotti M., Gobbo M., Timmel C. R., Di Valentin M., Bowen A. M. J. Phys. Chem. Lett. **2021**, 12, 80-85.
[2] Bertran A., Morbiato L., Sawyer J., Dalla Torre C., Heyes D.J., Hay S., Timmel C.R., Di Valentin M., De Zotti M., Bowen A.M. J. Am. Chem. Soc. **2023**, 145, 22859−22865.

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