Increasing the Modulation Depth of Gd$^{III}$-Based Pulsed Dipolar EPR Spectroscopy (PDS) with Porphyrin–Gd$^{III}$ Laser-Induced Magnetic Dipole Spectroscopy

Andreas Scherer, Xuemei Yao, Mian Qi, Max Wiedmaier, Adelheid Godt,* and Malte Drescher*

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ABSTRACT: Distance determination with pulsed EPR has become an important technique for the structural investigation of biomacromolecules, with double electron–electron resonance spectroscopy (DEER) as the most important method. Gd$^{III}$-based spin labels are one of the most frequently used spin labels for DEER owing to their stability against reduction, high magnetic moment, and absence of orientation selection. A disadvantage of Gd$^{III}$–Gd$^{III}$ DEER is the low modulation depth due to the broad EPR spectrum of Gd$^{III}$. Here, we introduce laser-induced magnetic dipole spectroscopy (LaserIMD) with a spin pair consisting of Gd$^{III}$-PyrMTA and a photoexcited porphyrin as an alternative technique. We show that the excited state of the porphyrin is not disturbed by the presence of the Gd$^{III}$ complex and that herewith modulation depths of almost 40% are possible. This is significantly higher than the value of 7.2% that was achieved with Gd$^{III}$–Gd$^{III}$ DEER.

Pulsed dipolar EPR spectroscopy (PDS) has become an important tool for the structural analysis of biomacromolecules like proteins, DNA, and RNA. PDS measures the magnetic dipolar coupling $\omega_{dd}$ between two paramagnetic centers in frozen solution, from which distance distributions in the nanometer range can be calculated. Among the many techniques that have been developed, double electron–electron resonance spectroscopy (DEER) is the most common technique with which both of these disadvantages for Gd$^{III}$-based PDS can be overcome is relaxation induced dipolar modulation enhancement (RIDME). A different approach for PDS is taken in laser-induced magnetic dipole spectroscopy (LaserIMD). Here, the dipolar coupling between a permanent spin label and a label that is transiently converted into a paramagnetic state through photoexcitation is measured. The $\Delta m_J = 1$ transition required for PDS is achieved through intersystem crossing from its photoexcited diamagnetic singlet state ($S = 0$) to the paramagnetic triplet state ($S = 1$), thereby replacing the microwave pump pulse used in DEER (Figure 1a). The bandwidth of the photoexcitation is not limited by the EPR spectrum or the microwave resonator and is virtually infinite. Thus, even though such a combination of two distinct labels

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requires a more difficult labeling scheme for the attachment to a protein.\(^{39,40}\) LaserIMD on Gd\(^{III}\)-based spin labels is a promising technique, because of its potential for achieving higher modulation depths than with Gd\(^{III}\)−Gd\(^{III}\) DEER. Also, the aforementioned broadening artifacts are avoided in LaserIMD, because only one microwave frequency is used and with a spectral width of 1.5 GHz for the triplet state of the porphyrin, the transient spin label used in this study,\(^{37,41}\) the frequency offset between pump and observer spins is significantly larger. High modulation depths of over 40% with Gd\(^{III}\) spin labels can also be achieved by performing DEER on a nitroxide−Gd\(^{III}\) spin pair.\(^{42}\) However, combining Gd\(^{III}\) with a transient spin label instead of a nitroxide has the advantage that photoexcitable groups are endogenous in many proteins like heme-proteins\(^{43}\) or light-harvesting proteins.\(^{44}\) In such a case, only a single Gd\(^{III}\) label needs to be introduced, which reduces potential disturbances on the protein by the labels.\(^{37}\) Furthermore, as lanthanide tags have already been shown to be applicable as a FRET donor,\(^{35}\) the combination of a photoexcitable label and a Gd\(^{III}\) label also opens the possibility to combine luminescence-based spectroscopic techniques like Förster resonance energy transfer (FRET) with PDS.

In initial studies of light-induced PDS, porphyrins were paired with nitroxide labels.\(^{37,41}\) Other label combinations that have been reported since are fullerenes with trityl radicals or nitroxides\(^{46,47}\) the combination of the fluorescent dyes Rose Bengal, Eosin Y, or Atto Thio12 with a nitroxide\(^{40}\) and two porphyrins, both of which are photoexcited.\(^{48}\) We opted for porphyrin as the transient spin label, because of its photo-stability and selected the porphyrin TNPP (Figure 1) because of its water solubility.\(^{49}\) Due to the bulkiness of TNPP, it should be considered that upon attachment to a protein it might interfere with the protein structure. For the persistent spin label, we chose Gd\(^{III}\)(PymiMTA) (Figure 1)\(^{50,51}\) which is structurally similar to the well-known Gd\(^{III}\)(PyMTA).\(^{21,29,52}\)

The substitution of the pyridine ring for a pyrimidine ring has little effect on the EPR-spectroscopic properties like line width

Figure 1. (a) Pulse sequence of LaserIMD. (b) Structural formula of the transient spin label TNPP, the compounds used to install the permanent Gd\(^{III}\)-based spin label with PyMTA or PymiMTA as the ligand, and the model peptides TNPP-pP-Gd\(^{III}\)(PymiMTA) and Gd\(^{III}\)(PymiMTA)-pP-Gd\(^{III}\)(PymiMTA). For the peptide chains, the following letter code is used: A, alanine; G, glycine; P, proline.
and relaxation times, but its bioconversion rate is much higher.  

The combination of a Gd\textsuperscript{III}-based spin label with porphyrin poses potential problems like the incorporation\textsuperscript{53,54} of Gd\textsuperscript{III} into the porphyrin or quenching\textsuperscript{55–57} of the electronically excited porphyrin by the Gd\textsuperscript{III} complex. Here, we set out to explore this spin label combination, establish LaserIMD with this combination as a light-induced PDS technique and compare its performance with that of Gd\textsuperscript{III}–Gd\textsuperscript{III} DEER.

A model system was designed based on a polyproline helix (pP) as spacer\textsuperscript{28,59} between the porphyrin TNPP and Gd\textsuperscript{III}(PymiMTA) (Figure 1b). We attempted to spin label the TNPP-labeled peptide TNPP-GP\textsubscript{3}CP\textsubscript{3}–NH\textsubscript{2} (G, glycine; P, proline; C, cysteine) with the complex Gd\textsuperscript{III}(PymiMTA) by applying a two-step protocol reported for a structurally similar peptide,\textsuperscript{57} with first ligand attachment and second complex formation. However, the attachment of 4-vinyl-PyMTA to the cysteine unit of the peptide TNPP-GP\textsubscript{3}CP\textsubscript{3}–NH\textsubscript{2} failed. Already, in the mentioned previous work, the reaction proceeded rather slowly, even at 40 °C.\textsuperscript{21} Turning to Na[4-vinyl-\{Gd\textsuperscript{III}(PymiMTA)\}] (Figure 1b) as the reaction partner, quantitative spin labeling in a single step was accomplished within 2 h at room temperature. We ascribe the strong increase in reactivity to the increase in electrophility of the vinyl unit after exchanging the pyridine ring for the more electron withdrawing pyrimidine ring. Surprisingly, mass spectrometric analysis of the material, that had been obtained through HPLC, showed a significant amount of sulfoxide linkage (Figure 1, X = SO; Supporting Information, Section S1) alongside the expected sulfide linkage (Figure 1b, X = S). We estimated that the difference between the distance distribution of the sulfide and sulfoxide linkage is negligible and proceeded with the sulfide/sulfoxide mixture. For comparison with Gd\textsuperscript{III}–Gd\textsuperscript{III} DEER, a polyproline helix with two Gd\textsuperscript{III}(PymiMTA) labels (Gd\textsuperscript{III}(PymiMTA)-pP-Gd\textsuperscript{III}(PymiMTA)) was prepared.

Circular dichroism measurements showed that all three peptides adopt a pPII structure in water (Supporting Information, section S2).

For TPP-Gd\textsuperscript{III} LaserIMD it is important that the Gd\textsuperscript{III} ion is not exchanged between the PymiMTA ligand and TNPP.\textsuperscript{53,54} An indication of that a metal ion has been incorporated into porphyrin is the change in its UV/vis spectrum from four distinct Q-bands of the metal ion free porphyrin to two Q-bands for the Gd\textsuperscript{III} ion loaded porphyrin.\textsuperscript{54} In our case the UV/vis spectrum of TNPP-pP-Gd\textsuperscript{III}(PymiMTA) (Figure 2a, experimental details in Supporting Information, section S3) shows four Q-bands at the same wavelengths as the ion free TNPP, which we take as a strong sign that no Gd\textsuperscript{III} ion exchange took place. This is supported by time-resolved X-band EPR spectra (tEPR) of photoexcited TNPP. Incorporation of a metal ion into porphyrin can significantly change the zero-field splitting and zero-field population of the excited triplet, and hence the tEPR spectrum.\textsuperscript{60,61} As can be seen in parts c and d of Figure 2, TNPP-pP-Gd\textsuperscript{III}(PymiMTA) and ion free TNPP show virtually the same tEPR spectrum with almost identical zero-field splitting values and zero-field populations.

Lanthanide cations and their complexes are known to act as photoquenchers of electronically excited states.\textsuperscript{55–57} Therefore, the close proximity of Gd\textsuperscript{III}(PymiMTA) to TNPP in TNPP-pP-Gd\textsuperscript{III}(PymiMTA) may quench the photoexcited triplet state, which would interfere with the light-induced PDS measurement and might render it impossible. To check whether quenching takes place, the lifetimes of the excited states of TNPP and TNPP-pP-Gd\textsuperscript{III}(PymiMTA) were determined through time-resolved luminescence measurements at room temperature (experimental details in Supporting Information, section S4). The lifetimes, defined as the time up to which the signal has decayed to 1/e of its maximum value, increases from 5.1 \(\mu\)s for TNPP to 5.9 \(\mu\)s for TNPP-pP-
Gd$^{3+}$(PymiMTA) as can be seen in Figure 2b. Additionally, the average lifetimes of the photoexcited triplet state were determined with pulsed EPR spectroscopy at 10 K (details in Supporting Information, sections S5 and S6). Following the same trend as the luminescence lifetimes, the average triplet lifetimes show an increase from 47.7 ms for TNPP to 57.8 ms for TNPP-pP-Gd$^{3+}$(PymiMTA). Based on these results, we conclude that the photoexcited state of TNPP is not quenched by the close-by Gd$^{3+}$(PymiMTA).

For a fair comparison of TNPP-Gd$^{3+}$ LaserIMD and Gd$^{3+}$–Gd$^{3+}$ DEER, we first optimized the signal-to-noise ratio (SNR) of the latter by using broadband shaped pulses as pump pulses and fine-tuned the pulse shapes, frequency widths, pulse lengths and observer and pump pulse frequencies. The SNR is calculated as the ratio of the modulation depth and the experimental noise, normalized to the square-root of the measurement time. With the applied optimization heuristic as described in detail in Supporting Information, section S7, the best SNR was obtained when observing at the maximum of the Gd$^{3+}$ EPR spectrum at 34 GHz with a Gaussian pulse and pumping with a 200 ns WURST pulse with $n = 12$ and a sweep width of 300 MHz. Gd$^{3+}$–Gd$^{3+}$ DEER with these settings on Gd$^{3+}$(PymiMTA)-pP-Gd$^{3+}$(PymiMTA), resulted in a modulation depth of 7.2% and an SNR of 530 h$^{-1/2}$ (Figure 3a and Table 1). Although this is a significantly larger modulation depth than the 2.0% that were reached with a rectangular pump pulse, the modulation depth stays behind what can be achieved with LaserIMD. To prove this, TNPP-Gd$^{3+}$ LaserIMD data were recorded at 10 K for TNPP-pP-Gd$^{3+}$(PymiMTA) (results in Figure 4; experimental details in Supporting Information, section S5). The SRT was optimized to 100 ms and the laser energy per pulse to 1.4 mJ (Supporting Information, section S6). If available, temperatures below 10 K can also be used as they are known to give a higher spin-polarization for Gd$^{3+}$ in the Q-band. To analyze the dipolar trace, its zero-time needs to be determined precisely. As this cannot always be reliably done for LaserIMD, a LaserIMD experiment with an additional refocusing pulse, termed reLaserIMD, has been suggested as it allows an accurate zero-time determination. However, the introduction of the additional pulse increases the overall trace length which decreases the SNR. To combine the best of both worlds, we tried a different approach where the data were recorded with LaserIMD and then shifted by a zero-time determined with reLaserIMD. As the zero-time does not depend on the dipolar evolution time, the latter can be chosen as short as possible in order to maximize the echo intensity, which allows to record a reLaserIMD trace with a high sensitivity in a short time. Furthermore, for laser systems with a constant delay of the laser flash, the zero-time depends only on the microwave pulse lengths. In such a case, once the zero-time for a given pulse length has been determined, it can be reused for future measurements and it is not necessary to measure reLaserIMD every time. The combination of reLaserIMD and LaserIMD was found to give a reliable zero-time (Supporting Information, section S9) and an SNR increase from 150 h$^{-1/2}$ for TNPP-Gd$^{3+}$ reLaserIMD to 190 h$^{-1/2}$ for TNPP-Gd$^{3+}$ LaserIMD (Figure 4). The thus optimized TNPP-Gd$^{3+}$ LaserIMD gave a modulation depth of 39.4% (Table 1). Even though this stays behind the modulation depth of 50% that has been reported for Gd$^{3+}$–Gd$^{3+}$ RIDME, TNPP-Gd$^{3+}$ LaserIMD has the advantage, that the laser excites no transitions with $|m_{J}| > 1$ and therefore no overtones coefficients are needed for data analysis.

The modulation depth of 39% for TNPP-Gd$^{3+}$ LaserIMD might be significantly higher than the 7.2% for Gd$^{3+}$–Gd$^{3+}$ DEER, but the crucial parameter for the determination of distribution distances is the SNR. Here, TNPP-Gd$^{3+}$ LaserIMD has a lower SNR of 190 h$^{-1/2}$ compared to 530 h$^{-1/2}$ for Gd$^{3+}$–Gd$^{3+}$ DEER. Due to the short longitudinal relaxation time of Gd$^{3+}$(PymiMTA)-pP-Gd$^{3+}$(PymiMTA) of 35.6 μs (Supporting Information, section S10), it was possible to use a fast shot repetition time (SRT) of 100 μs (Supporting Information, section S7). In contrast, TNPP requires a much longer SRT of 100 ms, because with a triplet relaxation time of 57.8 ms, the signal would saturate otherwise. Therefore, in a given measurement time, Gd$^{3+}$–Gd$^{3+}$ DEER benefits from much more scans being accumulated than for TNPP-Gd$^{3+}$ LaserIMD, which explains the better SNR of the former. As most transient labels used so far for LaserIMD require an SRT in the millisecond range, the continuation of the development of transient spin labels with faster triplet relaxation times is necessary to open up the full possibilities of LaserIMD with Gd$^{3+}$-based spin labels.

| Modulation Depths and SNR for Gd$^{3+}$–Gd$^{3+}$ DEER, TNPP-Gd$^{3+}$ (re)LaserIMD and TNPP-Gd$^{3+}$ reLaserIMD from Figures 3 and 4 |
|---------------------------------|------------|--------|
| Gd$^{3+}$–Gd$^{3+}$ DEER (WURST, pump pulse) | 7.2 (6.9, 7.6) | 530 (500, 610) |
| TNPP-Gd$^{3+}$ LaserIMD | 39.4 (38.9, 40.1) | 190 (180, 210) |
| TNPP-Gd$^{3+}$ reLaserIMD | 35.8 (35.3, 36.5) | 150 (140, 170) |
In conclusion, we have shown that TNPP and Gd\textsuperscript{III}(PymiMTA) are a suitable label pair for light-induced EPR measurements and PDS in particular. UV/vis measurements, time-resolved EPR spectroscopy as well as lifetime measurements of the excited TNPP gave neither an indication of a Gd\textsuperscript{III} ion exchange between the labels nor an indication of quenching of the photoexcited triplet state of the TNPP by Gd\textsuperscript{III}-PymiMTA. LaserIMD is an interesting technique, which can be used not just for structural investigations on biological systems with endogenous photoexcitable moieties; the presence of a photoexcitable label also makes it a suitable technique to combine PDS and luminescence measurements. Furthermore, the increase in modulation depth from 7.2\% for Gd\textsuperscript{III}-Gd\textsuperscript{III} DEER to 39.7\% for TNPP-Gd\textsuperscript{III} LaserIMD promises a significant increase in SNR, once sorter SRTs become applicable.

**ASSOCIATED CONTENT**

**Data Availability Statement**
The raw data are available at [https://doi.org/10.5281/zenodo.7051255](https://doi.org/10.5281/zenodo.7051255).

**Supporting Information**
The Supporting Information is available free of charge at [https://pubs.acs.org/doi/10.1021/acs.jpcllett.2c02138](https://pubs.acs.org/doi/10.1021/acs.jpcllett.2c02138).

Synthesis of the model peptides, details on sulfoxide formation and Gd\textsuperscript{III}−Fe\textsuperscript{II} ion exchange, details on CD, UV/vis, fluorescence lifetime, and time-resolved and pumped EPR measurements, MNR optimization of DEER and LaserIMD, zero-time determination for LaserIMD, and longitudinal and transversal relaxation of Gd\textsuperscript{III} ([PDF](#)).

**AUTHOR INFORMATION**

**Corresponding Authors**

Malte Drescher — Department of Chemistry and Konstanz Research School Chemical Biology, University of Konstanz, 78457 Konstanz, Germany; [orcid.org/0000-0002-3571-3452](https://orcid.org/0000-0002-3571-3452); Email: malte.drescher@uni-konstanz.de

Adelheid Godt — Faculty of Chemistry and Center of Molecular Materials (CM\textsubscript{Z}), Bielefeld University, 33615 Bielefeld, Germany; [orcid.org/0000-0001-8453-1439](https://orcid.org/0000-0001-8453-1439); Email: godt@uni-bielefeld.de

**Authors**

Andreas Scherer — Department of Chemistry and Konstanz Research School Chemical Biology, University of Konstanz, 78457 Konstanz, Germany; [orcid.org/0000-0002-0708-0686](https://orcid.org/0000-0002-0708-0686)

Xuemei Yao — Faculty of Chemistry and Center of Molecular Materials (CM\textsubscript{Z}), Bielefeld University, 33615 Bielefeld, Germany

Mian Qi — Faculty of Chemistry and Center of Molecular Materials (CM\textsubscript{Z}), Bielefeld University, 33615 Bielefeld, Germany

Max Wiedmaier — Department of Chemistry and Konstanz Research School Chemical Biology, University of Konstanz, 78457 Konstanz, Germany

Complete contact information is available at: [https://pubs.acs.org/doi/10.1021/acs.jpcllett.2c02138](https://pubs.acs.org/doi/10.1021/acs.jpcllett.2c02138)

**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

(1) Pannier, M.; Veit, S.; Godt, A.; Jeschke, G.; Spiess, H. W. Dead-Time Free Measurement of Dipole–Dipole Interactions between Electron Spins. *J. Magn. Reson.* **2000**, *142*, 331–340.

(2) Yee, E. F.; Diensthuber, R. P.; Vaidya, A. T.; Borbat, P. P.; Engelhard, C.; Freed, J. H.; Bittl, R.; Möglisch, A.; Crane, B. R. Signal Transduction in Light–Oxygen–Voltage Receptors Lacking the Adduct-Forming Cysteine Residue. *Nat. Commun.* **2015**, *6*, 10079.

(3) Yang, Y.; Chen, S.-N.; Yang, F.; Li, X.-Y.; Feintuch, A.; Su, X.-C.; Goldfarb, D. In-Cell Destabilization of a Homodimeric Protein Complex Detected by DEER Spectroscopy. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117*, 20566.

(4) Giannoulis, A.; Feintuch, A.; Barak, Y.; Mazal, H.; Albeck, S.; Unger, T.; Yang, F.; Su, X.-C.; Goldfarb, D. Two Closed ATP- and ADP-Dependent Conformations in Yeast Hsp90 Chaperone Detected by Mn(II) EPR Spectroscopic Techniques. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117*, 395.
for Light-Induced Pulsed Dipolar Spectroscopy. Chem. Commun. 2020, 56, 14669–14672.
(41) Dal Farra, M. G.; Richert, S.; Martin, C.; Larminie, C.; Gobbo, M.; Bergantino, E.; Timmel, C. R.; Bowen, A. M.; Di Valentin, M. Light-Induced Pulsed EPR Dipolar Spectroscopy on a Paradigmatic Hemeprotein. ChemPhysChem 2019, 20, 931.
(42) Garbuio, L.; Bordignon, E.; Brooks, E. K.; Hubbell, W. L.; Jeschke, G.; Yulikov, M. Orthogonal Spin Labeling and Gd(III)−Nitroxide Distance Measurements on Bacteriophage T4-Lysozyme. J. Phys. Chem. B 2013, 117, 3145–3153.
(43) Reedy, C. J.; Gibney, B. R. Heme Protein Assemblies. Chem. Rev. 2004, 104, 617–660.
(44) Büchel, C. Evolution and Function of Light Harvesting Proteins. Journal of Plant Physiology 2015, 172, 62–75.
(45) Herath, I.; Breen, C.; Hewitt, S.; Berki, T.; Kassir, A.; Dodson, C.; Judd, M.; Jabar, S.; Cox, N.; Otting, G.; Butler, S. J. A Chiral Lanthanide Tag for Stable and Rigid Attachment to Single Cysteine Residues in Proteins for NMR, EPR and Time-Resolved Luminescence Studies. Chemistry A European Journal 2021, 27, 13009.
(46) Timofeev, I. O.; Politanskaia, L. V.; Tretyakov, E. V.; Polienko, Y. F.; Tormyshev, V. M.; Bagryanskaya, E.; Krumkacheva, O. A.; Fedin, M. V. Fullerene-Based Triplet Spin Labels: Methodology Aspects for Pulsed Dipolar EPR Spectroscopy. Phys. Chem. Chem. Phys. 2022, 24, 4475–4484.
(47) Krumkacheva, O. A.; Timofeev, I. O.; Politanskaia, L. V.; Polienko, Y. F.; Tretyakov, E. V.; Rogozhnikova, O. Yu.; Trukhin, D. V.; Tormyshev, V. M.; Chubarov, A. S.; Bagryanskaya, E. G.; Fedin, M. V. Triplet Fullerene as Prospective Spin Label for Nanoscale Distance Measurements by Pulsed Dipolar EPR. Angew. Chem. 2019, 131, 13405.
(48) Beltran, A.; Henbest, K. B.; De Zotti, M.; Gobbo, M.; Timmel, C. R.; Di Valentin, M.; Bowen, A. M. Light-Induced Triplet−Triplet Electron Resonance Spectroscopy. J. Phys. Chem. Lett. 2021, 12, 80−85.
(49) Sannikova, N.; Timofeev, I.; Bagryanskaya, E.; Bowman, M.; Fedin, M.; Krumkacheva, O. Electron Spin Relaxation of Photoexcited Porphyrin in Water−Glycerol Glass. Molecules 2020, 25, 2677.
(50) Keller, K. Metal Centres in Pulsed Dipolar Spectroscopy—From Methodology to Application. 2019.
(51) Yao, X. Promotionsarbeit Universität Bielefeld. 2019.
(52) Yang, Y.; Wang, J.-T.; Pei, Y.-Y.; Su, X.-C. Site-Specific Tagging Proteins via a Rigid, Stable and Short Thiolether Tether for Paramagnetic Spectroscopic Analysis. Chem. Commun. 2015, 51, 2824–2827.
(53) Zang, L.; Zhao, H.; Zheng, Y.; Qin, F.; Yao, J.; Tian, Y.; Zhang, Z.; Cao, W. Twenty-Fold Enhancement of Gadolinium-Porphyrin Phosphorescence at Room Temperature by Free Gadolinium Ion in Liquid Phase. J. Phys. Chem. C 2015, 119, 28111–28116.
(54) Zang, L.; Zhao, H.; Hua, J.; Qin, F.; Zheng, Y.; Zhang, Z.; Cao, W. Water-Soluble Gadolinium Porphyrin as a Multifunctional Theranostic Agent: Phosphorescence-Based Oxygen Sensing and Photosensitivities. Dyes Pigm. 2017, 142, 465–471.
(55) Buono-core, G. E.; Li, H.; Marciniak, B. Quenching of Excited States by Lanthanide Ions and Chelates in Solution. Coord. Chem. Rev. 1990, 99, 55–87.
(56) Lis, S.; Elbanowski, M.; Mąkowska, B.; Hnatejko, Z. Energy Transfer in Solution of Lanthanide Complexes. J. Photochem. Photobiol., A 2002, 150, 233–247.
(57) Li, J.; Wang, Y.; Jiang, X.; Wu, P. An Aqueous Room-Temperature Phosphorescence Probe for Gd3+. Chem. Commun. 2022, 58, 2686−2689.
(58) Stryer, L.; Haugland, R. P. Energy Transfer: A Spectroscopic Ruler. Proc. Natl. Acad. Sci. U. S. A. 1967, 58, 719–726.
(59) Garbuio, L.; Lewandowski, B.; Wilhelm, P.; Ziegler, L.; Yulikov, M.; Wenneners, H.; Jeschke, G. Shape Persistence of Polypeptide II Helical Oligoprolines. Chemistry A European Journal 2015, 21, 10747−10753.
(60) Ishii, K.; Fujisawa, J.; Ohba, Y.; Yamauchi, S. A Time-Resolved Electron Paramagnetic Resonance Study on the Excited States of Tetraphenylporphins(tin)(II) Coordinated by p-Pyridyl Nitronyl Nitroxide. J. Am. Chem. Soc. 1996, 118, 13079.
(61) Tait, C. E.; Neuhaus, P.; Peaks, M. D.; Anderson, H. L.; Timmel, C. R. Transient EPR Reveals Triplet State Delocalization in a Series of Cyclic and Linear α-Conjugated Porphyrin Oligomers. J. Am. Chem. Soc. 2015, 137, 8284–8293.
(62) Hintze, C.; Morgen, T. O.; Drescher, M. Heavy-Atom Effect on Optically Excited Triplet State Kinetics. PLoS One 2017, 12, No. e0184239.
(63) Biefer, A.; Bückner, D.; Drescher, M. Light-Induced Dipolar Spectroscopy — A Quantitative Comparison with LiDEER and LaserMD. J. Magn. Reson. 2018, 296, 29–35.
(64) Fabregas Ibáñez, L.; Jeschke, G.; Stoll, S. DeerLab: A Comprehensive Toolbox for Analyzing Dipolar EPR Spectroscopy Data. Magn. Reson. 2020, 1, 209.
(65) Di Valentin, M.; Albertini, M.; Zurlo, E.; Gobbo, M.; Carbonera, D. Porphyrin Triplet State as a Potential Spin Label for Nanometer Distance Measurements by PELDOR Spectroscopy. J. Am. Chem. Soc. 2014, 136, 6582–6585.
(66) Scherer, A.; Yao, X.; Qi, M.; Wiedmaier, M.; Godt, A.; Drescher, M. Raw Data for "Increasing the Modulation Depth of Gd(III)-Based Pulsed Dipolar EPR Spectroscopy (PDS) with Porphyrin-GdIII Laser Induced Magnetic Dipole Spectroscopy. Zenodo 2022. DOI: 10.5281/zenodo.7051255.