Site-Directed Spin Labeling of RNA with A Gem-Diethylisoindoline Spin Label: PELDOR, Relaxation, and Reduction Stability

Christine Wuebben 1, Simon Blume 1, Dinar Abdullin 1, Dominik Brajtenbach 1, Florian Haege 1, Stephanie Kath-Schorr 2 and Olav Schiemann 1,*

1 Institute of Physical and Theoretical Chemistry, University of Bonn, Wegelerstr. 12, 53115 Bonn, Germany; wuebben@pc.uni-bonn.de (C.W.); s6siblum@uni-bonn.de (S.B.); abdullin@pc.uni-bonn.de (D.A.); s6dobraj@uni-bonn.de (D.B.); Florian-Haege@web.de (F.H.)

2 Life & Medical Sciences Institute Chemical Biology & Medicinal Chemistry Unit, University of Bonn, Gerhard-Domagk-Str. 1, 53121 Bonn, Germany; stephanie.kath-schorr@uni-bonn.de

* Correspondence: schiemann@pc.uni-bonn.de
Supporting Information

Contents
1. Synthesis and Analytics of 2* .................................................................3
2. cw EPR ........................................................................................................3
3. 2-Pulse ESEEM Measurements ...............................................................4
4. PELDOR Measurements .........................................................................5
5. PELDOR Data Analysis ..........................................................................6
6. Stability Measurements under Reducing Conditions ..........................9
   6.1. DNA Sequence ..............................................................................9
   6.2. Spin Labeling Reaction .................................................................9
   6.3. DNA Sample Preparation ............................................................9
1. Synthesis and Analytics of 2•

The azide functionalized *gem*-diethylisoindoline spin label 2• (Figure 1b) was synthesized in six steps starting from N-benzylphthalimide with slight modification according to the synthesis reported by Haugland et al. [47]. In order to avoid handling the explosive diazotransfer reagent trifluoromethanesulfonyl azide, imidazole-1-sulfonyl azide hydrochloride was used following the protocol of Goddard-Borger et al. [59]. This led to a yield of 70% for the diazotransfer reaction as compared to the reported 87% for the trifluoromethanesulfonyl azide reaction [47]. Spin label 2• was obtained as a yellow powder in an overall yield of 7%. Its identity and purity were confirmed by high-performance liquid chromatography, IR and EPR spectroscopy, as well as mass spectrometry (Figure S1). In the experimental high-resolution ESI (+) mass spectrum, a negligible amount of a species at 289.2023 m/z was detectable (Figure S1a), which was assigned to the corresponding hydroxylamine (Figure S1c) formed during the ESI measurement, because there was no additional peak in the HPLC analysis.

2. cw EPR

![Figure S1. Analytics of spin label 2•. (a) Experimental high-resolution ESI (+) mass spectrum. (b) Calculated high-resolution ESI (+) mass spectrum of spin label 2•. (c) Calculated high-resolution ESI (+) mass spectrum of the corresponding hydroxylamine. (d) IR spectrum. (e) HPLC run. (f) Experimental cw X-band EPR spectrum in liquid toluene at 295 K (black line) overlaid with the simulation (red line). (g) Zoom in for the 2nd Peak in (f) and (h) experimental cw X-band EPR spectrum in acetonitrile: methylene chloride 1:1 at 100 K (black line) overlaid with the simulation (red line).]
Figure S2. Experimental cw EPR spectrum of (a) $A_2$ and (b) $B_2$ overlaid with their double integral.

Table S1. Spin counting results of $A_2$ and $B_2$.

| Spin Counting | Concentration of the RNA/μM | Calculated Spin Concentration/μM |
|---------------|-----------------------------|----------------------------------|
| $A_2$         | 35                          | 32                               |
| $B_2$         | 35                          | 31                               |

Table S2. Parameters of the cw EPR simulations using easySpin [3] of spin label $2^\text{*}$.

| Parameter | $2^\text{*}$ | Nitroxide-Biradical | Tempo | $2^\text{*}$ |
|-----------|--------------|----------------------|-------|--------------|
|           | 295 K        | 295 K [61]           | 295 K [62] | 100 K [62] |
| $g$-tensor | 2.0036 $^d$  | 2.0063               | 2.0092, 2.0062, 2.0029 $^e$ |
| $A$-tensor/MHz | 38, 9, 1 $^f$ | 42                   | 44, 0.6/1, 0.5 $^f$ | 12, 12, 94 $^g$ |

$^a$ Experimental values of a Bis-TEMPO-bis-Ketal in d$_8$-toluene. $^b$ Experimental values of TEMPO in CCl$_4$. $^c$ For the simulation in frozen solution, an H-strain of 142,420 MHz was used. $^d$ Isotropic $g$-value. $^e$ $g$-Tensor is given in the form $(g_{xx}, g_{yy}, g_{zz})$. $^f$ The hyperfine coupling constants in liquid solution are given in the form $(A_{14N}, A_{13C}, A_{1H})$. $^g$ The $A(14N)$ tensor at 100 K is given in the form $(A_{xx}, A_{yy}, A_{zz})$.

3. 2-Pulse ESEEM Measurements
**Figure S3.** Experimental two-pulse ESEEM spectra of B$_2$ at 50 K in dependence of the magnetic field.

**Figure S4.** Experimental two-pulse ESEEM spectra recorded at 50 K overlaid with their corresponding fits (black line): (a) A$_2$ in deuterated phosphate buffer (red line), (b) B$_2$ in deuterated phosphate buffer (blue line), (c) A$_2$ in deuterated phosphate buffer with additional 17% water (light blue line), (d) B$_2$ in deuterated phosphate buffer with additional 17% water (green line).

4. PELDOR Measurements

**Table S3.** Frequency offsets.

| Notation | Pump Position | Detection Position | Frequency Offset/MHz |
|----------|---------------|--------------------|---------------------|
| $\Delta \nu_{ax}$ | a | x | 40 |
| $\Delta \nu_{bx}$ | b | x | 40 |
| $\Delta \nu_{ab}$ | a | b | 80 |
| $\Delta \nu_{be}$ | b | e | 80 |
| $\Delta \nu_{ac}$ | a | c | 100 |
| $\Delta \nu_{ae}$ | a | e | 160 |
Figure S5. Experimental echo detected field sweep in Q-band of B2 (black line) overlaid with the Simulation (grey line). In dashed lines and depicted in letters are the different pump and detection positions marked.

Table S3. Frequency offsets.

| Notation | Pump Position | Detection Position | Frequency Offset/MHz |
|----------|---------------|--------------------|---------------------|
| Δνax     | a             | x                  | 40                  |
| Δνbx     | b             | x                  | 40                  |
| Δνab     | a             | b                  | 80                  |
| Δνbe     | b             | e                  | 80                  |
| Δνac     | a             | c                  | 100                 |
| Δνae     | a             | e                  | 160                 |

Table S4. Parameters used for simulation of the field sweep spectrum in Figure S5.

| Parameter | 50 K           |
|-----------|----------------|
| g-tensor  | 2.0086, 2.0064, 2.0026 |
| A-tensor/MHz | 18, 18, 102     |
| Line width/MHz | 19.6           |

5. PELDOR Data Analysis

The PeldorFit program [58], which is based on

\[ \nu_{dd} = \frac{g_A g_B \mu_B^2 \mu_0}{4\pi \hbar} \times \frac{1}{r^3} (1 - 3 \cos^2 \theta) \]  \( (1) \)

was used to analyze the orientation selectivity. The configuration file contains three main blocks of information filled in by the user:

1. Instrumental parameters of the PELDOR experiment;
2. Spectroscopic parameters of the involved spins which were obtained by simulating the experimental Q-band spectrum with easySpin [60] (Figure S5). The parameter from the fit are collected in Table S4;
3. The parameters of Table S5 were used as fitting parameters assuming rhombic magnetic tensors for the spins.

The program fits the orientation selective time traces by means of a genetic algorithm and yields the geometric parameters r, ξ, φ, α, β, and γ of a simplified geometric model (Figure
S6) and their distributions. The genetic algorithm was set to a maximal number of generations of 200 and a generation size of 192. The geometric parameters are optimized within the ranges given in Table S5 until the corresponding RSMD reached a minimum. The results are given in Table S6 and S7 including the 16 symmetry-related sets of parameters due to the invariance of the g- and A-tensor towards inversion of their axes.

Table S5. Fitting parameters for PeldorFit.

| Parameter | Mean | Width |
|-----------|------|-------|
| r/nm      | 4–5  | 0–1   |
| ξ/°       | 0–90 | 0–30  |
| ϕ/°       | 0–180| 0–60  |
| α/°       | 0–180| 0–60  |
| β/°       | 0–90 | 0–30  |
| γ/°       | 0–180| 0–60  |

Figure S6. Model of the geometric parameters for PeldorFit.
Table S6. Summary of all sets of angles for A2 of the analysis of the PELDOR time traces using the PeldorFit program.

| Transformation         | ξ/° | φ/° | α/° | β/° | γ/° | RMSD |
|------------------------|-----|-----|-----|-----|-----|------|
| Fitting result         | 37  | 16  | 154 | 56  | 150 | 0.038|
| Inversion of gxxA      | 37  | 16  | 334 | 124 | 30  | 0.038|
| Inversion of gyyA      | 37  | 16  | 334 | 124 | 210 | 0.037|
| Inversion of gzzA      | 37  | 16  | 154 | 56  | 330 | 0.038|
| Inversion of gxxB      | 143 | 344 | 26  | 124 | 330 | 0.038|
| Inversion of gyyB      | 143 | 344 | 206 | 56  | 210 | 0.038|
| Inversion of gzzB      | 143 | 344 | 206 | 56  | 30  | 0.037|
| Inversion of gxxA and gxxB | 143 | 344 | 26  | 124 | 150 | 0.037|
| Inversion of gxxA and gyyB | 143 | 164 | 206 | 124 | 330 | 0.037|
| Inversion of gxxA and gzzB | 143 | 164 | 26  | 56  | 210 | 0.038|
| Inversion of gyyA and gxxB | 143 | 164 | 26  | 56  | 30  | 0.037|
| Inversion of gyyA and gyyB | 143 | 164 | 206 | 124 | 150 | 0.037|
| Inversion of gyyA and gzzB | 143 | 196 | 334 | 56  | 150 | 0.037|
| Inversion of gzzA and gxxB | 143 | 196 | 154 | 124 | 30  | 0.037|
| Inversion of gzzA and gyyB | 143 | 196 | 154 | 124 | 210 | 0.037|
| Inversion of gzzA and gzzB | 143 | 196 | 334 | 56  | 330 | 0.038|

* gxxA, gyyA, and gzzA denote the principal components of the g-tensor of spin A; gxxB, gyyB, and gzzB denote the g-tensor of spin B.

Table S7. Summary of all sets of angles for B2 of the analysis of the PELDOR time traces using the PeldorFit program.

| Transformation         | ξ/° | φ/° | α/° | β/° | γ/° | RMSD |
|------------------------|-----|-----|-----|-----|-----|------|
| Fitting result         | 44  | 155 | 175 | 34  | 19  | 0.048|
| Inversion of gxxA      | 44  | 155 | 355 | 146 | 161 | 0.048|
| Inversion of gyyA      | 44  | 155 | 355 | 146 | 341 | 0.048|
| Inversion of gzzA      | 44  | 155 | 175 | 34  | 199 | 0.048|
| Inversion of gxxB      | 136 | 205 | 5   | 146 | 199 | 0.048|
| Inversion of gyyB      | 136 | 205 | 185 | 34  | 341 | 0.048|
| Inversion of gzzB      | 136 | 205 | 185 | 34  | 161 | 0.048|
| Inversion of gxxA and gxxB | 136 | 205 | 5   | 146 | 341 | 0.048|
| Inversion of gxxA and gyyB | 136 | 25  | 185 | 146 | 199 | 0.048|
| Inversion of gxxA and gzzB | 136 | 25  | 5   | 34  | 341 | 0.048|
| Inversion of gyyA and gxxB | 136 | 25  | 5   | 146 | 199 | 0.048|
| Inversion of gyyA and gyyB | 136 | 25  | 185 | 34  | 161 | 0.048|
| Inversion of gyyA and gzzB | 44  | 335 | 355 | 34  | 19  | 0.048|
| Inversion of gzzA and gxxB | 44  | 335 | 175 | 146 | 161 | 0.048|
| Inversion of gzzA and gyyB | 44  | 335 | 175 | 146 | 341 | 0.048|
| Inversion of gzzA and gzzB | 44  | 335 | 355 | 34  | 199 | 0.048|

* gxxA, gyyA, and gzzA denote the principal components of the g-tensor of spin A; gxxB, gyyB, and gzzB denote the g-tensor of spin B.
Figure S7. Pymol illustration of the critical range of protons in the vicinity of the spin labels labeled within MtsslWizard [63–65].

6. Stability Measurements under Reducing Conditions

6.1. DNA Sequence

The DNA strands 5′ GGG TGX CTG GTA CCC 3′ and 5′ A GGG TAC CAG ACA CCC A 3′ were purchased from metabion.

6.2. Spin Labeling Reaction

The spin labeling was conducted in the same way as the labeling of the RNA (see Section 3.1.2.). Afterwards, the DNA was purified through reverse-phase high-performance liquid chromatography with an Agilent 1200 Series HPLC System (Agilent Technology, Santa Clara, CA, USA) in combination with a Zorbax 300SB-C18 (4.6 mm × 150 mm) column (Agilent Technologies, Santa Clara, CA, USA). As the eluent was a 0.1 M aqueous solution of triethylammonium acetate (VWR Applichem), an increasing percentage of acetonitrile (VWR Chemicals) (8% to 20% over 14 min, then up to 80% acetonitrile for 15 min) was used.

Figure S8. (a) Chromatogram at 260 nm and (b) deconvoluted mass of 2′ DNA (calculated mass 4897.925, found mass 4897.37). (c) Overlay of the HPLC runs of the unlabeled (black line) and labeled DNA (red line).

6.3. DNA Sample Preparation

The labeled single DNA strand was mixed 1:1 with the unmodified DNA strand in phosphate-buffered saline solution (PBS; 137 mM sodium chloride (Carl Roth), 10 mM sodium hydrogen phosphate (Carl Roth), 2.7 mM potassium chloride (Carl Roth), 1.8 mM potassium dihydrogen phosphate (Carl Roth), 1.0 mM magnesium chloride (Carl Roth), and 10% dimethyl sulfoxide (VWR Chemicals)).
phosphate (Carl Roth) pH 7.4). The mixture was denaturated for 5 min at 70 °C and incubated for at least 15 min at room temperature.

Table S8. Power settings, Q-value and time laps before EPR measurement.

|          | Power / mW | Dead Time / min | Q-Value |
|----------|------------|-----------------|---------|
| 2'-DNA/Asc | 5.529      | 11              | 6500    |
| 2'-DNA/HeLa | 5.529     | 6               | 6200    |
| MTSL/Asc  | 5.568      | 9               | 6500    |
| MTSL/HeLa | 5.537      | 5               | 6200    |