Room-Temperature Distance Measurements using RIDME and Orthogonal Spin Labels Trityl/Nitroxide

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I. CW EPR spectra at 298 K

CW EPR experiments at room temperature were performed at the X-band using the commercial spectrometer Bruker EMX. Experimental CW EPR settings at room temperature were as follows: sweep width, 10 mT; microwave power, 0.8, 0.2 and 0.02 mW; modulation frequency, 100 kHz; modulation amplitude, 0.1 mT; time constant, 163.84 ms; sweep time, 167.77 s; number of points, 1024; number of scans, 32.

Simulation for trehalose sample was performed using Easyspin tool in MATLAB. Parameters of simulations: \(g_{\text{trityl}} = [2.0022 \ 2.0028 \ 2.0029]\); \(g_{\text{nitroxide}} = [2.0080 \ 2.0060 \ 2.0020]\); \(A_{\text{nitroxide}} = [5.5 \ 5.5 \ 36] \text{ G}\); weight = 1:1.

![Figure S1](image-url) Room temperature CW EPR spectra of studied DNAs at X-band frequency in water/nucleosil and glassy trehalose (indicated in figure). Red line is simulation of CW EPR spectrum for trehalose sample without \(^{13}\text{C}\). N and T indicate EPR lines for nitroxide and trityl radicals, correspondently.

CW EPR spectrum of spin-labeled DNA in trehalose consists of immobilized EPR lines of triarylmethyl (TAM) and nitroxide radicals. Simulation performed in solid state regime revealed that the ratio between amounts of nitroxide/TAM is 1:1 (Figure S1). At the same time, the EPR spectrum of water/nucleosil sample shows the immobilized line of TAM tags and nitroxide lines.
in slow motion and solid state regimes (nitroxide lines indicated in Figure S1). In case of the sample in water/nucleosil, we recorded CW EPR spectra with microwave power in a range of 0.02-0.8 mW, because nitroxide and TAM tags have different spin lattice relaxation times. For example, at high microwave power the line of TAM is saturated, whereas the spectrum of nitroxide is not. At the same time, at small microwave power the lines of the nitroxide are practically invisible. For this reason, we could not perfectly simulate the obtained spectra, but we could undoubtedly indicate the presence of nitroxide lines in CW EPR spectra (Figure S1).
II. Electron spin relaxation times at 298 K

The experimental relaxation curves of studied DNA duplex are depicted in Figure S2, S3 at 10 and 34 GHz frequencies. The obtained values of the electron spin relaxation times are listed in Table 1. The electron spin relaxation times were obtained by mono-exponential fits.

Figure S2. Room-temperature electron spin relaxation time measurements for studied DNA duplexes at 10 GHz (black curve), at 34 GHz (red curve) frequencies and their mono-exponential fits (green line). The measurements were done for trityl tags.

Figure S3. The electron spin relaxation time measurements for studied DNA duplexes at 10 GHz at ambient temperature and their mono-exponential fits (green line). The measurements were done for nitroxide tags.
III. DQC measurements at 298 K

The DQC measurements were carried out at 300 K (X-band) by using six-pulse sequence, $\pi/2 - \tau_1 - \pi - \tau_1 - \pi/2 - \tau_3 - \pi - \tau_3 - \pi/2 - \tau_2 - \pi - \tau_2 - \text{echo}^{3,3}$, with pulse lengths of 10/20 ns for $\pi/2$ and $\pi$ pulses, respectively. The DQC measurements were done at the field position corresponding to the maximum of the EPR spectrum. To filter out the dipolar modulation signal, the 64-step phase-cycling was applied. The initial value for $\tau_1$ was 1000 ns, the initial value for $\tau_2$ was 1300 ns, the delay $\tau_3=30$ ns remained constant. The DQC time trace was recorded by incrementing $\tau_1$ and decrementing $\tau_2$ in 16 ns steps. The number of shots per point was 100, and shot repetition time was 0.2 ms. The total number of scans was 8.

**Figure S4.** Raw data of DQC experiment at room temperature for studied DNA duplex in trehalose.
IV. Room temperature ESEEM measurements

Room temperature ESEEM measurements were done using a three-pulse sequence \((\pi/2-\tau-\pi/2-T-\pi/2-\tau\text{-echo})\) with 10 ns \(\pi/2\) pulses at magnetic field corresponding to the maximum of the TAM spectrum. For suppression of unwanted echoes, a four-step phase cycle was used. The delay \((\tau = 238\text{ ns})\) was adjusted to obtain reasonably strong modulation from \(^{2}\text{H}\) and \(^{1}\text{H}\) nuclei simultaneously. Shot repetition time was 0.5 ms. For the proper comparison of modulation depths, all ESEEM time traces were first normalized to the intensity of unmodulated part of spin echo. For this sake, each ESEEM trace was fitted by 6\(^{th}\)-order polynomial and then divided by the maximum value of this fit (i.e., the value at initial \(T\)). Next, standard ESEEM analysis was employed. The (normalized) polynomial fit was subtracted from the normalized ESEEM time trace, and further processed by Gaussian apodization, zero filling, and Fourier transformation to obtain frequency domain power spectra.

**Figure S5.** ESEEM analyses at room temperature for TAM tags in studied DNA in glassy trehalose (black line) and water/nucleosil (red line).
V. Background functions for X-band RIDME experiments

We used DeerAnalysis program\textsuperscript{4} for the analysis of background decay. The background function in RIDME experiments can be simulated by a stretched exponential function:

\[ B(t) = S_0 \cdot \exp\left(-\left(\frac{\tau}{\tau_0}\right)^{D/3}\right), \]

where \( S_0 \) is a vertical scaling factor, \( \tau_0 \) is the 1/e decay time, \( D/3 \) is the stretching exponent. We varied: (1) starting time for background fitting in the range of 600-800 ns for glassy trehalose and 400-1000 ns for water/nucleosil; (2) stretching exponent \( D \) from 5 to 6 for glassy trehalose and from 3 to 6 for water/nucleosil. The modulation depth and position of distance at 6.5 nm are changed noticeably by a variation of the baseline function. We chose a background function at which the narrowest distance distribution with mean distance of four is achieved.

\textbf{Figure S6.} The room temperature X-band RIDME of studied DNA duplex in water/nucleosil and in trehalose (indicated in figure) (A) The experimental time traces (black) with different stretched exponential background functions (color line); (B) The background-divided time traces (color line) with corresponding fits based on Tikhonov regularization analysis with regularization parameters of 1000; (C) The distance distributions obtained using DeerAnalysis toolbox corresponding to background function.
VI. X-band RIDME experiments with different $T_{\text{mix}}$ at 298 K

Figure S7. The room temperature X-band RIDME of studied DNA duplex in trehalose and in water/nucleosil (indicated in figure) (A) The experimental time traces (black and green) with different mixing times (indicated in figure) and corresponding stretched exponential background functions (red); (B) The background-divided time traces (black and green) and corresponding fits (red) based on Tikhonov regularization analysis with regularization parameters of 1000 for trehalose and water/nucleosil; (C) The distance distributions obtained using DeerAnalysis toolbox."
VII. Q-band RIDME at 298 K

Room temperature RIDME experiments at Q-band were performed with a dead time free five pulse sequence: $\pi/2 - \tau_1 - \pi - \tau_2 - \pi/2 - T_{\text{mix}} - \pi/2 - \tau_3 - \pi - \tau_4 - \text{echo}$. The maximum available power was limited to 1 W; therefore, the RIDME experiment was conducted with 100/200 ns for $\pi/2$ and $\pi$ pulses, $\tau_1 = 400$ ns, $\tau_2 = 350$ ns, $\tau_3 = 3.2$ $\mu$s, $\tau_4 = \tau_3 + \tau_2 - \tau_1$. The delay $\tau_2$ was incremented in steps of 16 ns, while $\tau_3$ was decremented by the same interval. The mixing time $T_{\text{mix}}$ was 20 $\mu$s. To remove echo crossings and phase offsets, an eight-step phase cycle was applied. The nuclear modulation suppression cycle for deuterium was applied, in which $\tau_1$ was incremented 8 times by 16 ns. A shot repetition time was 550 $\mu$s and the number of shots per point was 200. 137 scans were taken corresponding to a total accumulation time of about 55 hours.

Figure S8. The room temperature Q-band RIDME experiments of studied DNA duplex in trehalose: (A) the experimental time traces (black) with corresponding stretched exponential background functions (green); (B) the background-divided time traces (black) and corresponding fits (red) based on Tikhonov regularization analysis with regularization parameters of 1000; (C) the distance distributions (red line) obtained using DeerAnalysis4 toolbox. Blue line shows the distance distribution obtained by X-band RIDME in trehalose at room temperature.
VIII. Q-band PELDOR at 50 K

The PELDOR/DEER measurements were done at 50 K using a standard four-pulse DEER sequence with pulse lengths 34/68 ns for probe, 90 ns for pump and 70 MHz of offset frequency at Q-band for trehalose sample. We used the main line of TAM for pumping and the nitroxide (\(M_S = +1\)) for observing. A shot repetition time was 8 ms and the number of shots per point was 100. 65 scans were taken corresponding to a total accumulation time of about 14 hours.

Figure S9. The Q-band PELDOR experiments of studied DNA duplex in trehalose at 50 K: (left) the background-divided time traces (black) and corresponding fits (red) based on Tikhonov regularization analysis with regularization parameters of 100; (right) the distance distributions (red line) obtained using DeerAnalysis4 toolbox. Blue line shows the distance distribution obtained by X-band PELDOR in trehalose at 80 K.

References