Supporting Material

A short note on the analysis of distance measurements by electron paramagnetic resonance

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MD Simulation

As an example for the Rice distribution of spin distances, we used molecular dynamics simulations of a double-stranded DNA doubly-labeled with the phosphorothiolate-substituted nitroxide spin label R5 (see Figure S1).

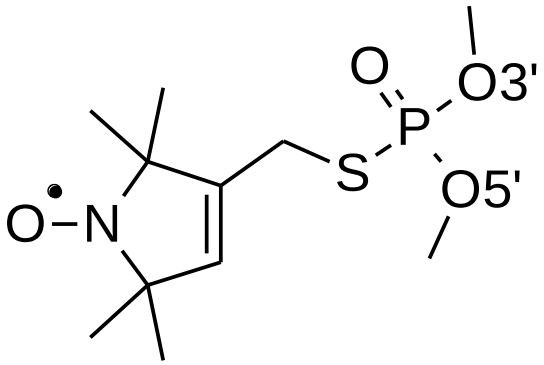

Figure S1: Structure of the phosphorothiolate-substituted nitroxide spin label R5

A DNA dodecamer was generated with the nucgen module of the AMBER 10 molecular dynamics package [1]:

5′AAGCAAAAGCAA
3′TTCGTTTTCGTT

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The first-occurring cytosines of both strands (counted from the 5’ end) were replaced by the spin-labeled form. The spin labels were placed manually to guarantee reasonable S-C distances and P-S-C angles. In this way, a doubly-labeled DNA molecule was generated, in which nucleotide 4 of the first and second strand (underlined in the sequence above) are labeled, resulting in a starting distance between the oxygen atoms of the two nitroxides of 1.7 nm. The starting structure is visualized in Figure 4.

The tleap module [1] was used to generate force-field parameter and coordinate files as input for the simulation. The parameters for the standard nucleotides were taken from the Cornell et al. force field (parm94) [2]. For the spin-labeled cytosines and the preceding guanosine the parameters were obtained from the paper published by Price et al. [3]. The DNA molecule was then placed in a cubic solvent box. The distance between the edges of the water box and the closest atom of the solute was at least 12 Å in each direction. Sodium ions were added to maintain electroneutrality. All simulations were performed with the AMBER 10 program package (sander module) [1]. The system was minimized for 5000 steps with constraints of 5 kcal mol^{-1} $Å^{-2}$ on all nucleotides except the spin-labeled ones and then for another 5000 steps without constraints to relax unfavorable conformations generated by the standard placement of the missing atoms. For equilibration, the system was then first heated from 100 K to 300 K for 100 ps and then relaxed to the density corresponding to 1 bar for 300 ps in a sequence of MD simulations using the canonical (NVT) and the isothermal isobaric (NPT) ensemble, respectively. In these simulations harmonic constraints with force constants of 5 kcal mol^{-1} $Å^{-2}$ were applied to all nucleotides except the spin-labeled ones. These restraints were then gradually reduced to zero during 200 ps of NVT-MD. Only the first and last nucleotide of each strand was restrained by 1 kcal mol^{-1} $Å^{-2}$, to avoid rotations and translations of the complete molecule. Finally, 25 parallel production runs (NVT ensemble) of 5 ns (each with a different random seed for the velocities) were performed, resulting in a total simulation time of 125 ns. These long simulation times were necessary to obtain sufficient sampling of the

very flexible spin labels (see below). The particle mesh Ewald (PME) method [4] was used in all simulations to handle long-range electrostatic interactions, and the SHAKE method [5] to constrain bond lengths of bonds involving hydrogen atoms. The time step for all MD simulations was set to 2 fs with a non-bonded cutoff of 12 Å.

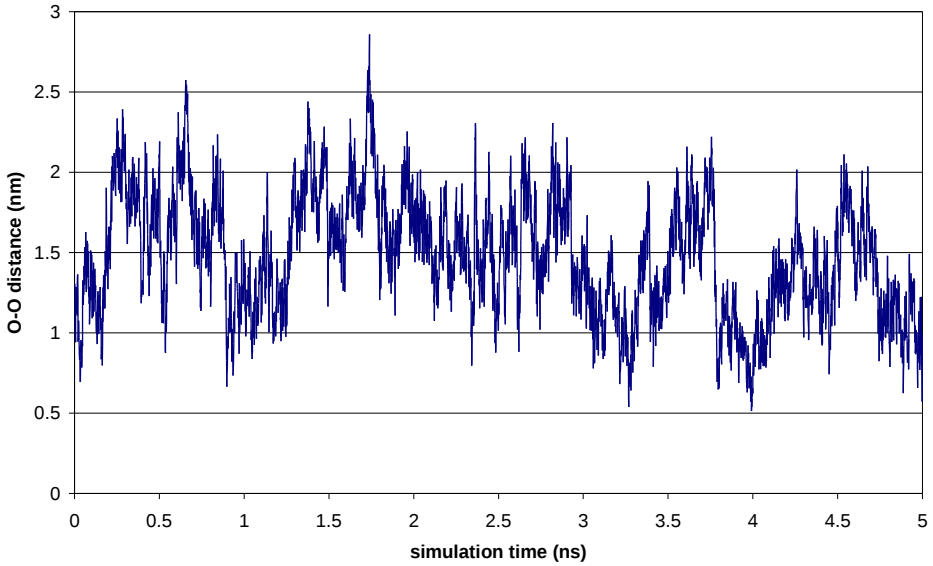

Figure S2: Time series of the oxygen-oxygen distance of the spin labels over 5 ns of one MD simulation.

For the analysis of the MD data, snapshots were taken every 2 ps and the distance between the oxygen atoms of the spin labels was measured. The time series of this distance for one of the independent MD runs is shown in Figure S2. Distances from 0.5 nm and up to almost 3 nm can be observed, demonstrating the high flexibility of the spin labels. The distances of all 25 simulations were binned into 0.01 nm intervals to obtain the distance distribution shown in Figure 5, which was then fitted by a Rice as well as a Gauss distribution. Additionally, the structures of the snapshots of all simulations were matched onto the first structure of the first simulation and average coordinates for all atoms were calculated. From this average structure, the distance between the most probable oxygen-oxygen positions of the two spin labels and the standard deviation of the atomic positions are determined.

The model system

A polyproline helix II (PPII) is a left-handed helix with an axial translation of 0.31 nm per residue, with dihedral angles ϕ = -75°, ψ= 145°, ω = 180°.

Herein a synthesized peptide Ac-Cys-[Pro]13-Cys-NH2 was used, which forms a PP II helix in water [6].

The peptide was diluted in a Tris-HCl buffer (10mM, pH 7.2, 150 mM NaCl). The MTSL spin label [(1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl)-methanethiosulfonate)] was added at 5x molar excess per cysteine from a 3.4 mM stock solution in DMSO and incubated overnight at room temperature. Following this, the free spin label was removed by dialysation with deuterated water. Approx. 50% vol. glycerol was added giving a final peptide concentration of 150-200 µM. Samples were transferred into 3mm (outer diameter) quartz glass sample tubes, shock frozen in liquid nitrogen, and placed in the spectrometer.

DEER experiments were performed at T = 50 K at X-band frequencies using a Bruker Elexsys E580 spectrometer equipped with a split-ring resonator. The magnetic field and pump frequency were adjusted such that the pump π-pulse (length 12 ns) was applied to the maximum intensity band of the nitroxide spectrum and 35 MHz below the resonator mode. The observer frequency was increased by 70 MHz with respect to the pump frequency. Pulse lengths of the observer channel were 16 ns and 32 ns, for π/2- and π-pulse, respectively. A phase cycle (+x)-(–x) was applied to the first observer pulse. The complete pulse sequence is given by: $\pi/2_{obs}$-τ_1-π_{obs} -t-π_{pump}-(τ_1+τ_2-t)-π_{obs}-τ_2-echo. The DEER time-traces for ten different τ_1-values spaced by 8 ns starting at τ_1 = 400 ns were added in order to suppress deuterium modulations.

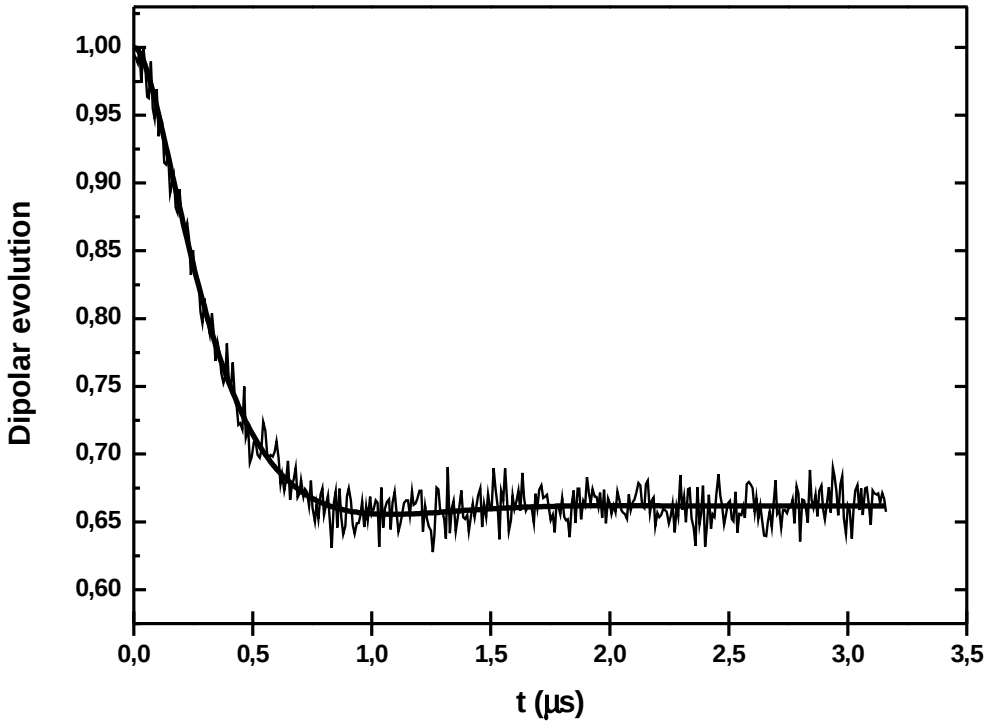

Figure S3: DEER trace. Background corrected dipolar evolution data from four-pulse DEER experiment for the model system. Thick solid line: fit corresponding to the distance distribution shown in Fig. S4 derived by Tikhonov regularization.

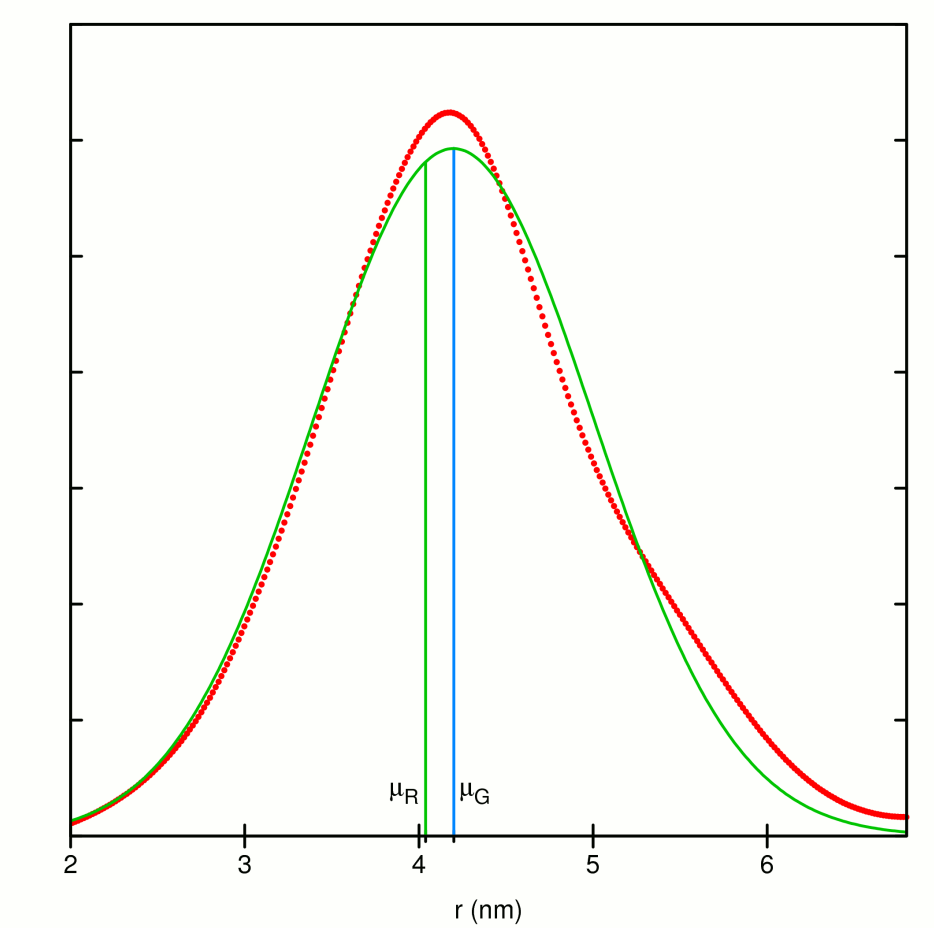

Figure S4: Experimental distance distribution (red dots) of the model system obtained from the dipolar evolution (Fig. S3) using Tikhonov regularization, Rice (green line) and Gauss (blue line, lies exactly under the green line) fit with their resulting distances μ_G= 4.20 nm and μ_R= 4.04 nm.

References

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Ref Type: Computer Program

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