SUPPORTING INFORMATION

A versatile approach for site-directed spin labeling and structural EPR studies of RNAs

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I. Supplementary figures

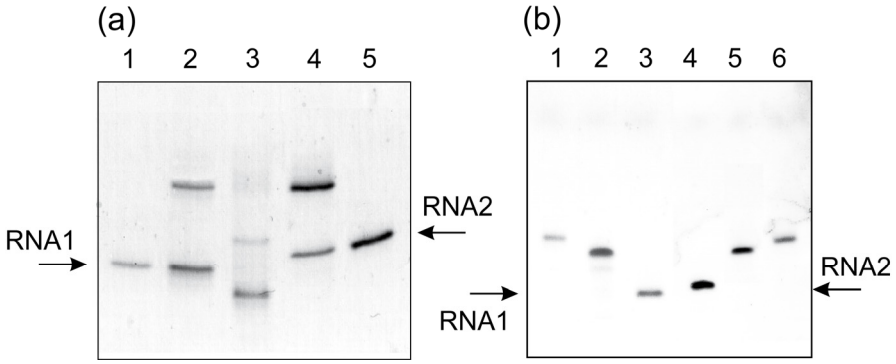

Figure S1. Site-specific introduction of spin labels at the 3'-terminal guanosine residues of RNA1 and RNA2. (a) isolation of the covalent adducts of RNA1 and RNA2 (upper band in lanes 2 and 4, respectively) with complementary DNA oligomers; lane 3, release of RNA1 bearing -RCH_2NH_2 linker (band in the middle part of the gel) from the respective covalent adduct (upper band in lane 2) by hydrolysis of phosphoramide bond, as example; lanes 1 and 5, unmodified RNA1 and RNA2, respectively. (b) in-gel verification of integrities of derivatives RNA1 (lane 2) and RNA2 (lane4) bearing -RCH_2NH_2 linker (after isolation by denaturing PAGE) and derivatives of RNA1 (lane 1) and RNA2 (lane 6) bearing spin label M1 introduced at the linker (after isolation by HPLC); lanes 3 and 4, unmodified RNA1 and RNA2, respectively.

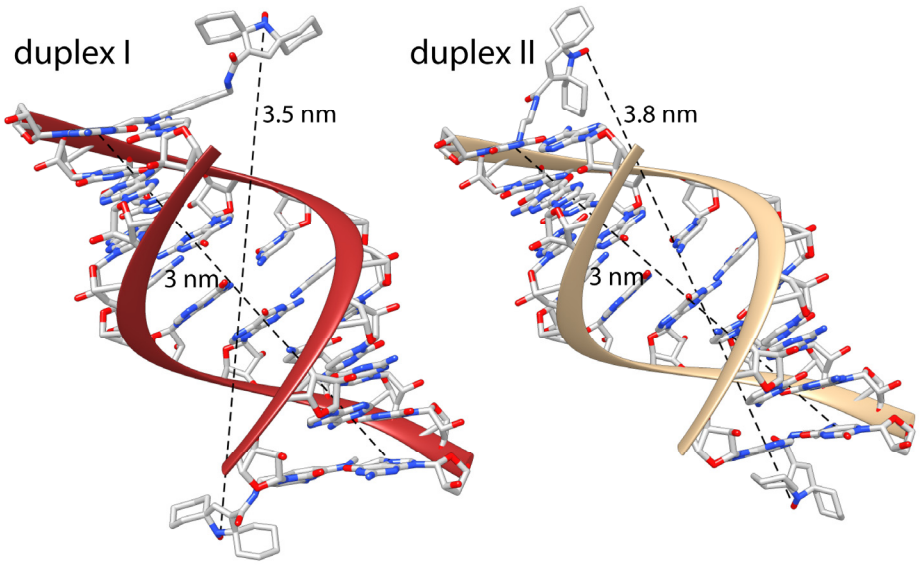

Figure S2. Molecular model of duplex I and duplex II showing the distance between spin-labeled nucleotide residues (3 nm) and measured inter-spin distances (3.5 nm and 3.8 nm for duplexes I and II, respectively). Structures of classical A-form RNA duplexes were generated using NAB.[1] The structure of nitroxide spin labels was generated by Chem3D software and introduced into desired position of RNA using Chimera.[2] Only one of the possible orientations of spin label is shown for each duplex. Spin labels in the duplex are positioned out from the helix major groove, what is in good agreement with CW EPR data shown below in Figure S4.

II. Original PELDOR/DEER time traces of the data shown in Figure 2

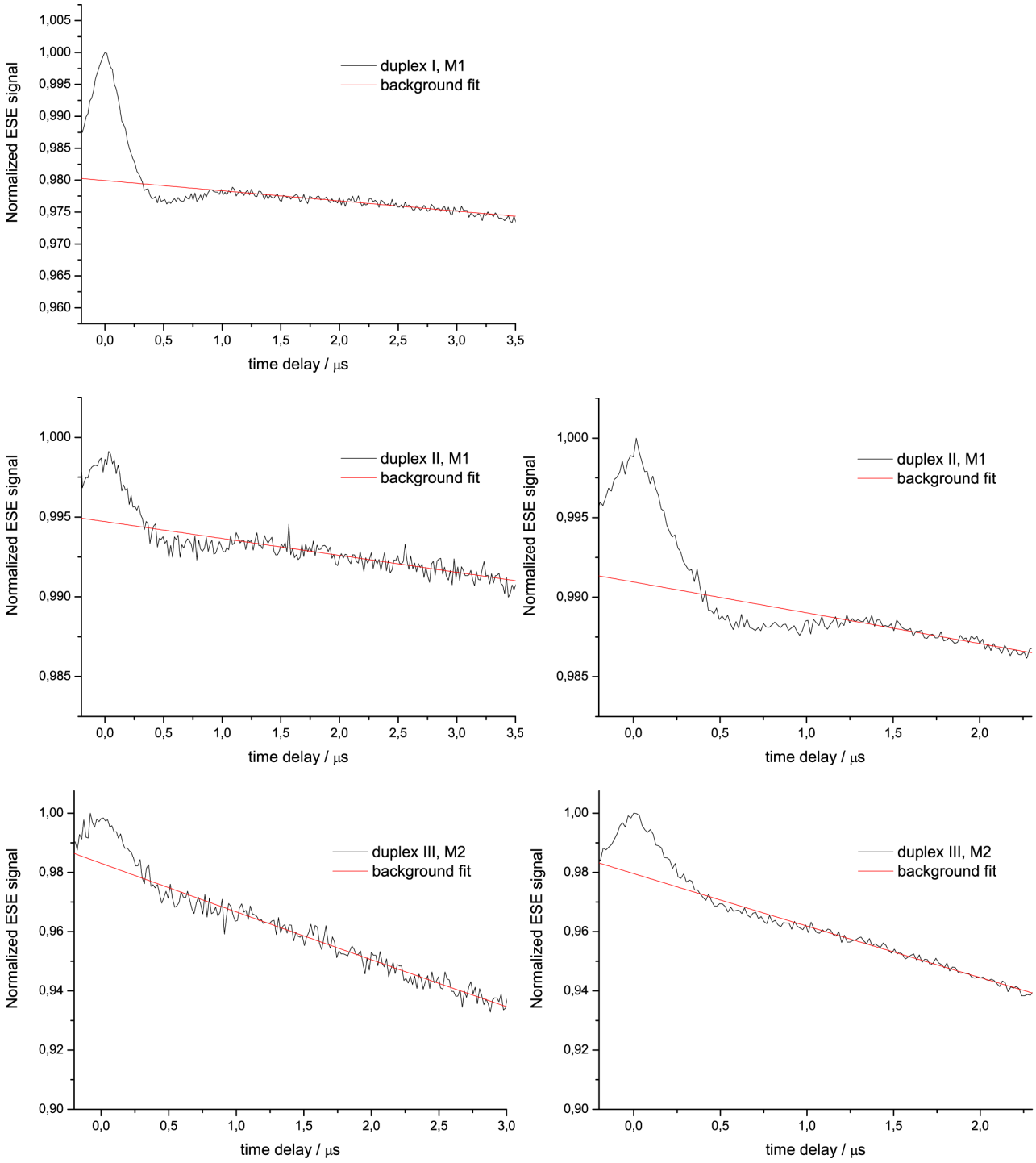

Figure S3. Original PELDOR/DEER time traces (before background correction) corresponding to those presented in Figure 2 of the main text. Two sets of traces for duplexes II and III correspond to the two values of dipolar evolution time in the four-pulse DEER sequence.

III. CW EPR data for spin-labeled oligonucleotides and duplex I

Figure S4 shows continuous wave (CW) EPR spectra of duplex I and spin-labeled oligonucleotides (RNA1-RCH_2NH-M1 and RNA2-RCH_2NH-M1) which constitute it. The data was collected at concentrations ca. 10 μM of spin-labeled oligonucleotides in aqueous solution (without addition of glycerol) at room temperature. Bruker Elexsys E580 spectrometer (resonator ER 4118X-MD5) has been used. Simulations have been done using EasySpin 4.5.0 toolbox for Matlab.[3]

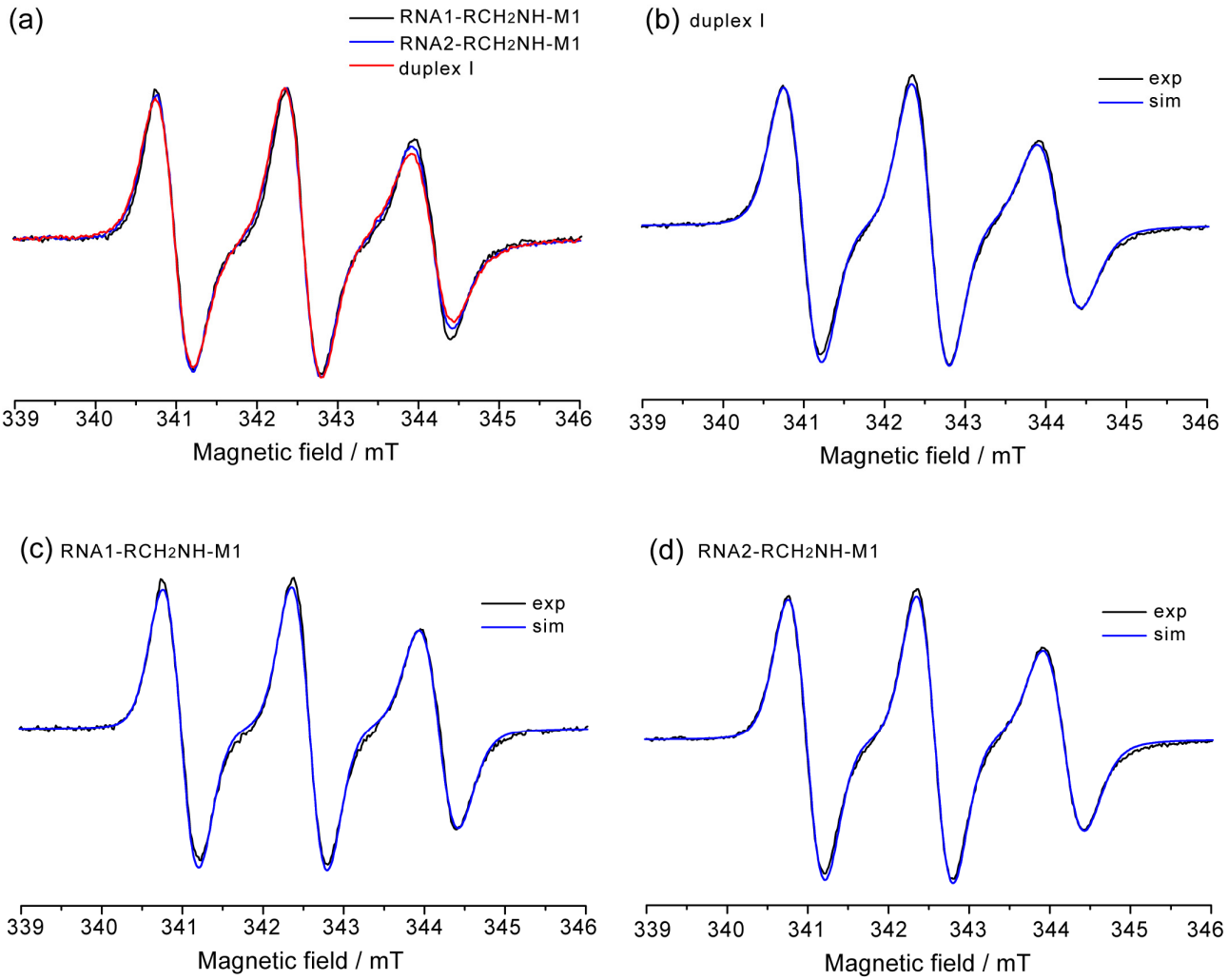

Figure S4. CW EPR spectra of spin-labeled oligonucleotides (RNA1-RCH_2NH-M1 and RNA2-RCH_2NH-M1) and duplex I at 293 K. (a) Comparison of experimental spectra of I, RNA1-RCH_2NH-M1 and RNA2-RCH_2NH-M1. (b) Experimental spectrum of duplex I (exp) and simulation (sim). (c) Experimental spectrum of spin-labeled oligonucleotide RNA1-RCH_2NH-M1 (exp) and simulation (sim). (d) Experimental spectrum of spin-labeled oligonucleotide RNA2-RCH_2NH-M1 (exp) and simulation (sim). Parameters of simulations are given in Table S1.

The differences in the shape of the spectrum for duplex I, RNA1-RCH_2NH-M1 and RNA2-RCH_2NH-M1 (and corresponding rotational correlation times, see Table 1) are not significant. It is reasonable, since if spin labels in the duplex are positioned out from the helix major groove (Figure S2), their rotational motion should not differ a lot from that in single stranded oligonucleotides. Thus, the CW EPR data agrees well with the other conclusions of the paper.

Table S1. Parameters of simulations shown in Figure S4. A_{ii} are the components of hyperfine interaction (HFI) tensor, τ_c is the rotational correlation time, Γ_{FWHM} is the linewidth (Gaussian) accounting for unresolved residual HFI constants.

	g-tensor	$A_{xx} = A_{yy}$ / mT	A_{zz} / mT	τ_c / ns	Γ_{FWHM} / mT
RNA1-RCH_2NH-M1	[2.0091 2.0061 2.0022]	0.5	3.80	0.32	0.49
RNA2-RCH_2NH-M1	[2.0091 2.0061 2.0022]	0.5	3.79	0.44	0.49
Duplex I	[2.0091 2.0061 2.0022]	0.5	3.77	0.54	0.49

References

1. J. Macke Thomas and A. Case David, in *Molecular Modeling of Nucleic Acids*, American Chemical Society, 1997, vol. 682, pp. 379-393.
2. E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng and T. E. Ferrin, *J. Comput. Chem.*, 2004, **25**, 1605-1612.
3. S. Stoll, A. Schweiger, *J. Magn. Reson.,* 2006, **178**, 42-55.