

SUPPORTING INFORMATION

Pulsed Dipolar EPR for Self-limited Complexes of Oligonucleotides Studies

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Table of contents

Continuous wave EPR.....	2
Gel shift assay analysis.....	2
Thermal denaturation analysis (Melting analysis).....	3
Data processing of PELDOR data	4
Molecular dynamics simulation and analysis	6
Comparison of PELDOR and molecular dynamics distance distribution for T ₇ -T ₃ [*] , T ₁₀ -T ₃ [*] , T ₂₅ -T ₃ [*] , and T ₃ [*] -T ₂₅ complexes	13

Continuous wave EPR

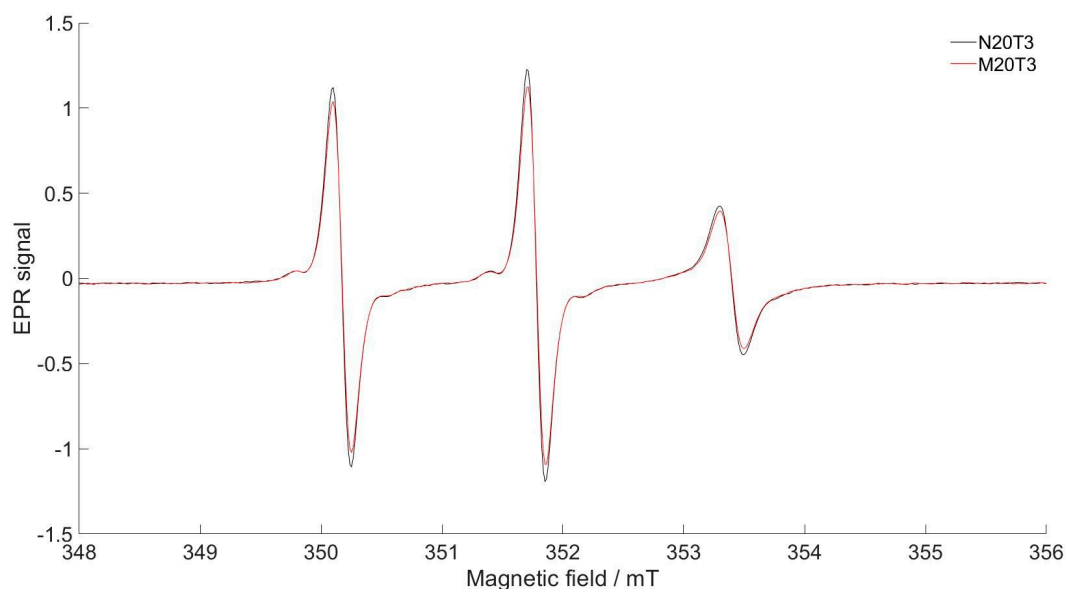


Figure S1. X-band CW EPR spectra of spin-labeled oligonucleotide M20T₃* and N20T₃* samples were measured at room temperature (For parameters, see section 2.6 of the paper).

Gel shift assay analysis

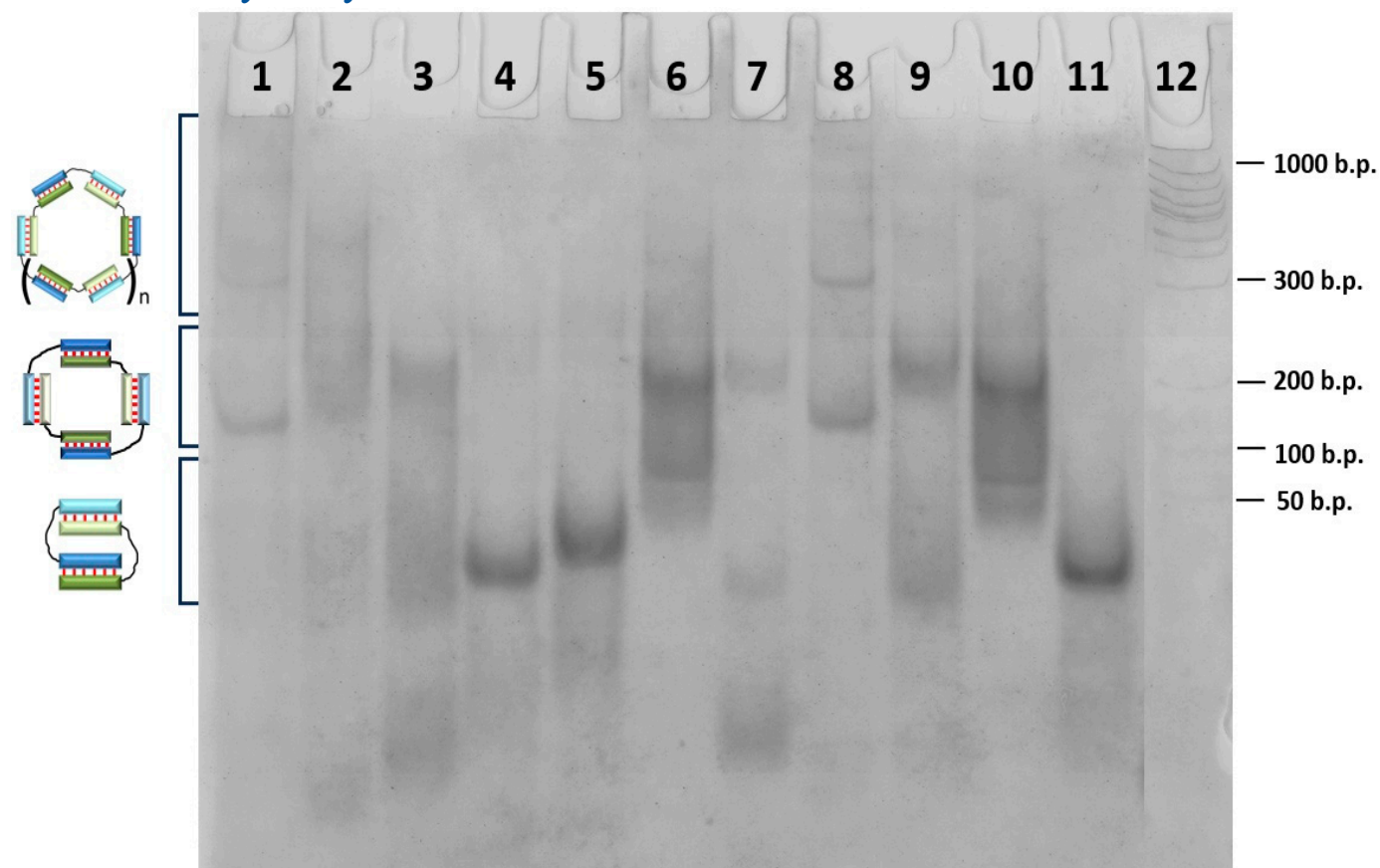


Figure S2. Gel shift assay of oligonucleotide complexes in a ratio of 1:1. Lane 1 T₀_T₃*, lane 2 T₁_T₃*, lane 3 T₃_T₃*, lane 4 T₇_T₃*, lane 5 T₁₀_T₃*, lane 6 T₂₅_T₃*, lane 7 T₃*_T₃*, lane 8 T₃*_T₀, lane 9 T₃*_T₃, lane 10 T₃*_T₂₅, lane 11 T₇_T₃ control, lane 12 ladder (50–1000 bp). A DNA Ladder of 50–1000 bp (SibEnzyme, Russia) was used to evaluate the mobility of complexes. Stains-all (Sigma, USA) was used to stain the gel.

Thermal denaturation analysis (Melting analysis)

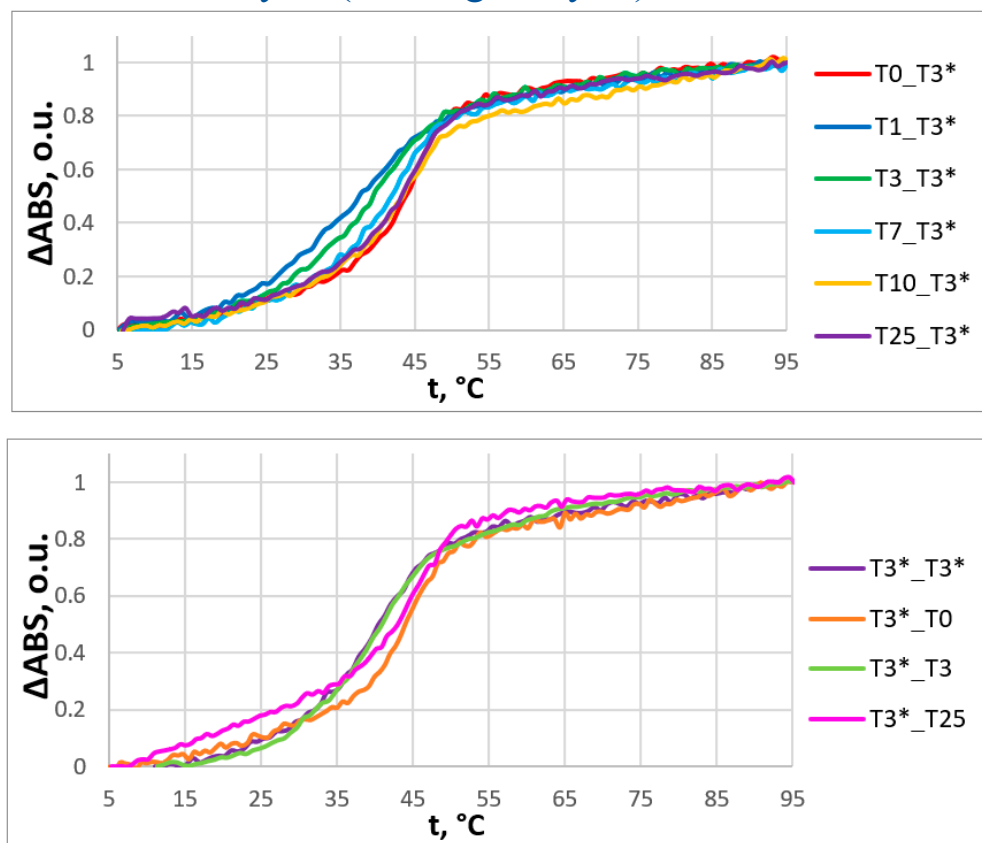


Figure S3. Normalized UV-melting curves for DNA complexes were obtained at 260 nm.

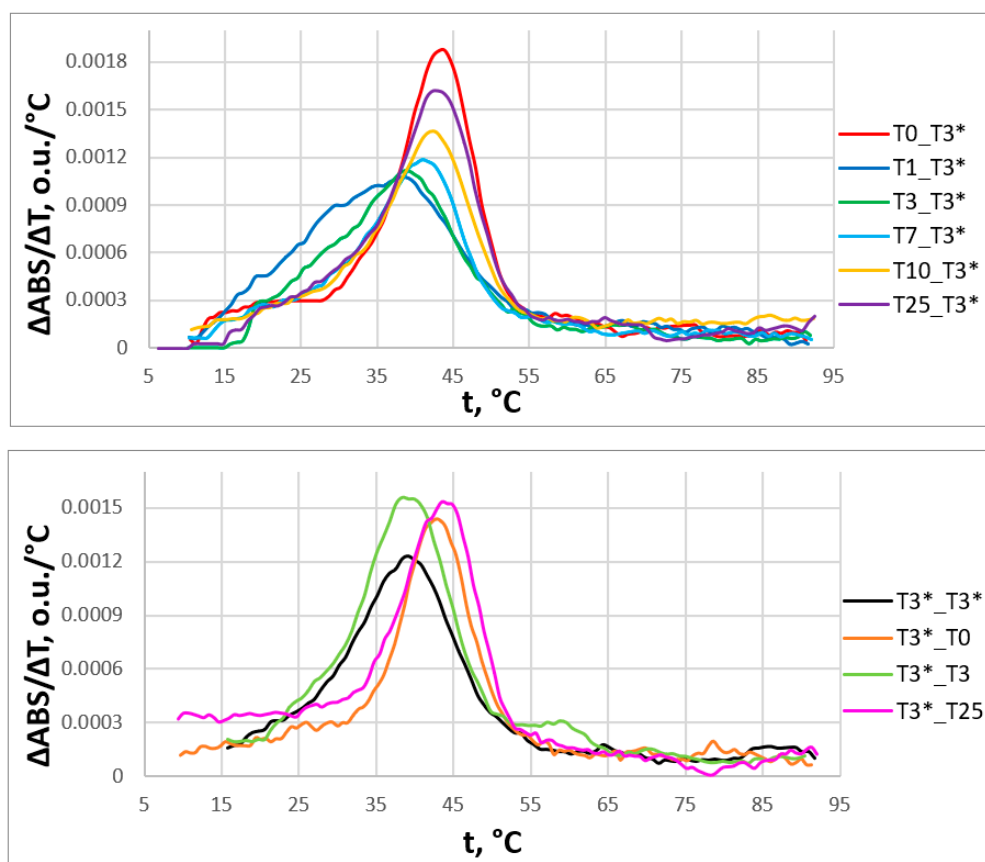


Figure S4. The first derivative of the UV-melting curves, obtained at 260 nm, for DNA complexes.

Data processing of PELDOR data

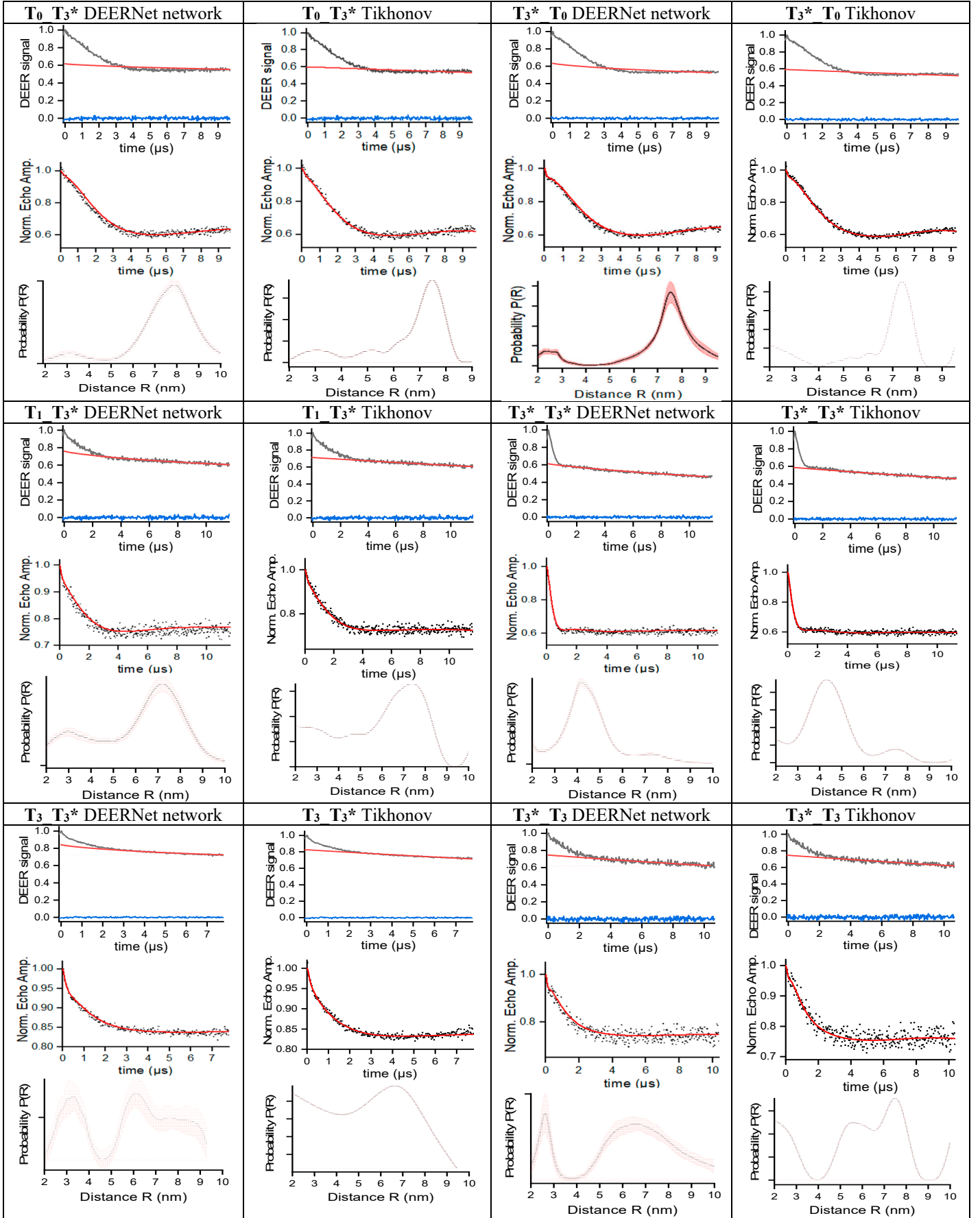
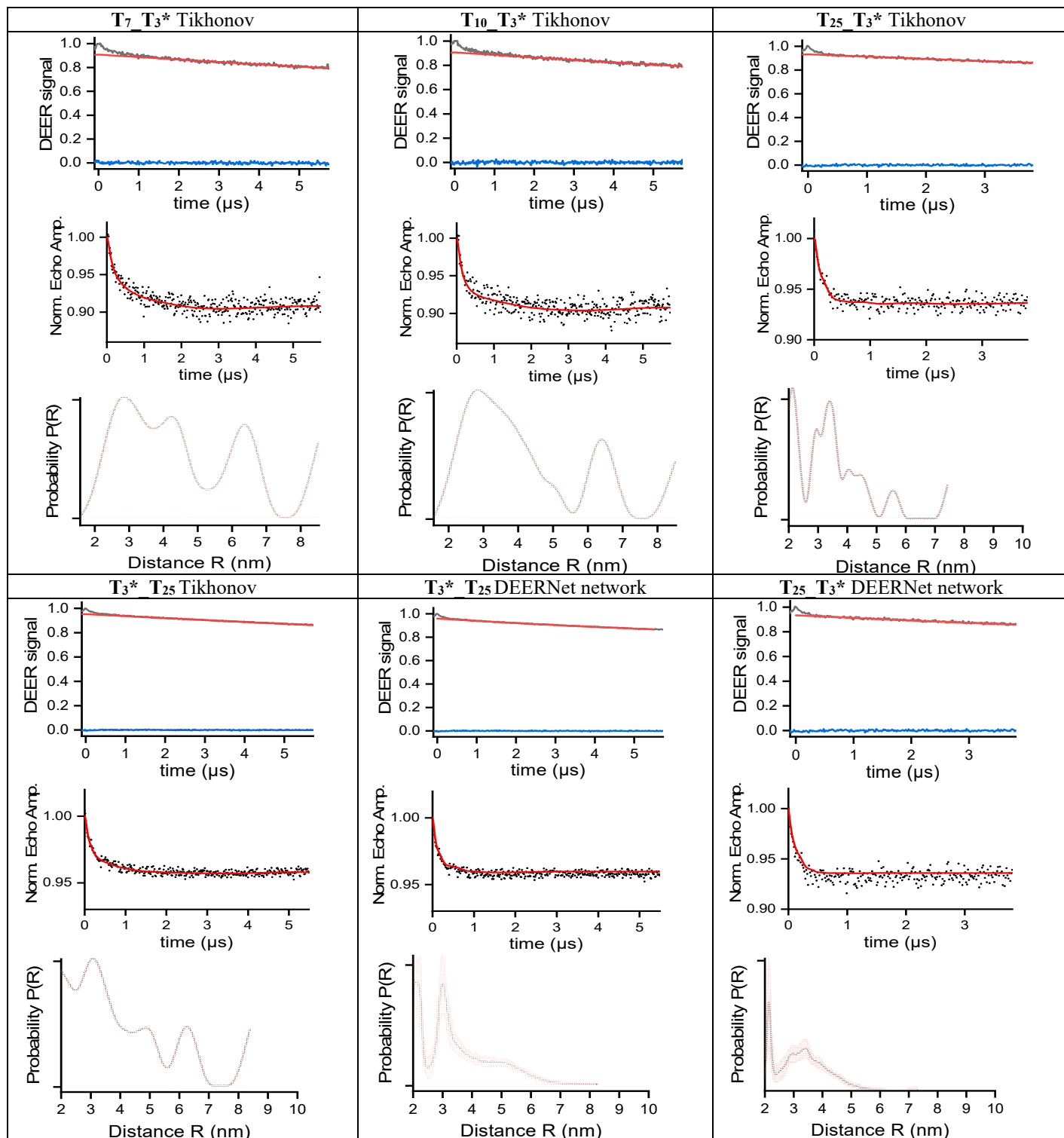
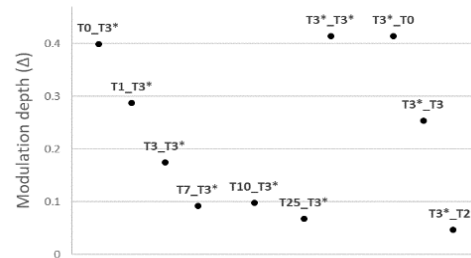


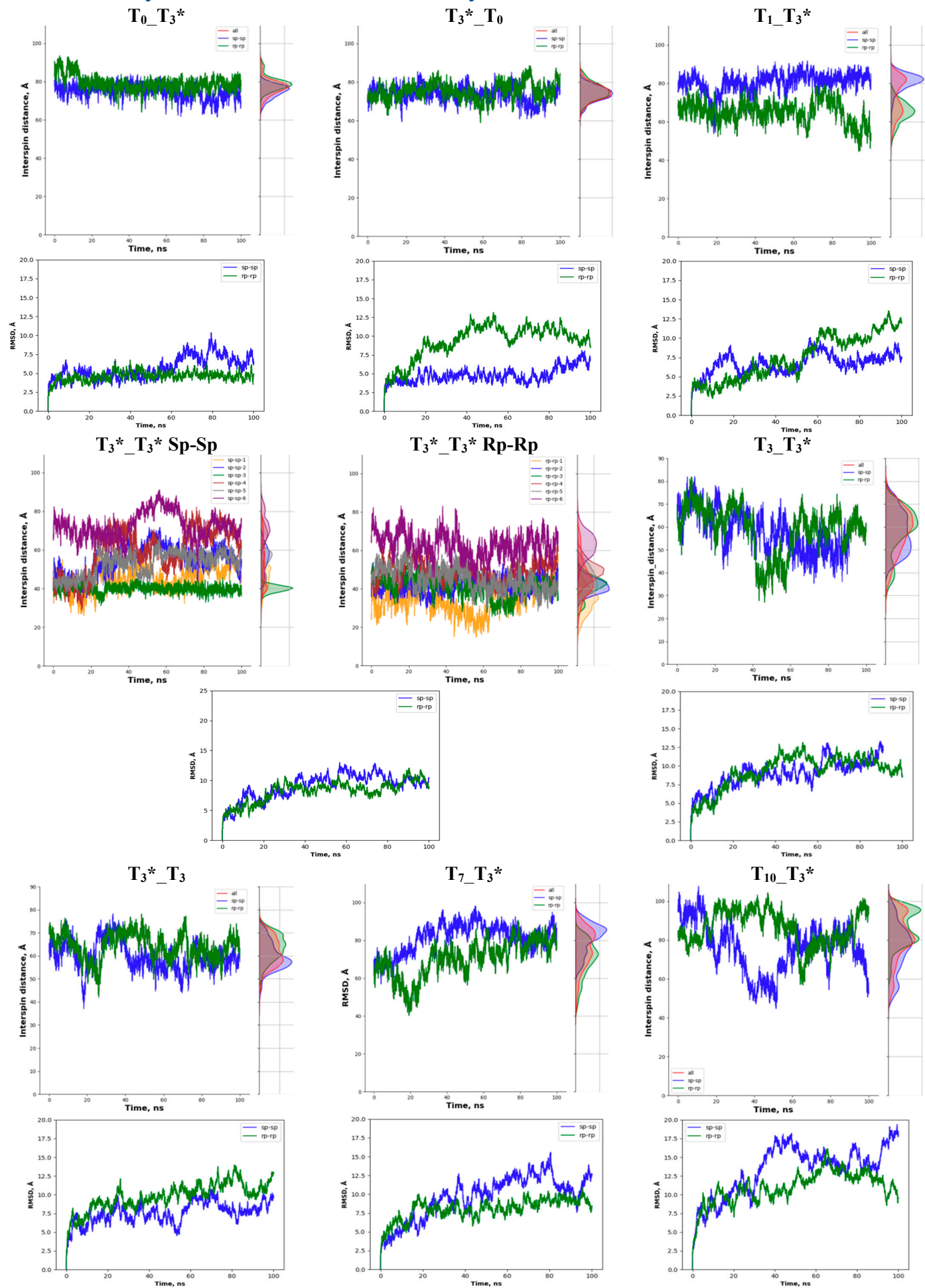
Figure S5. Q-band PELDOR measurements of $T_0_T_3^*$, $T_3^*_T_0$, $T_1_T_3^*$, $T_3_T_3^*$, $T_3^*_T_3$, and $T_3^*_T_3^*$. PELDOR time trace (black) with the background fits (red) and an imaginary part of the signal (blue) is shown on the top. Middle background-corrected PELDOR time trace (black dots) and DEERNet or Tikhonov fit (red). The distance distributions on the bottom for each sample are calculated using the DEERNet network or Tikhonov regularization. Shaded areas show the uncertainty of distance determination.

Table S1. Modulation depth extracted by DeerAnalysis software for self-assembled oligonucleotide complexes.

$T_0_T3^*$	$T_1_T3^*$	$T_3_T3^*$	$T_3^*_T3^*$	$T_7_T3^*$	$T_{10}_T3^*$	$T_{25}_T3^*$
0.399	0.288	0.175	0.414	0.092	0.098	0.068
$T_3^*_T0$	$T_3^*_T3$		$T_3^*_T_{25}$			
0.409	0.254		0.047			

**Figure S6.** Q-band PELDOR measurements of $T_7_T3^*$, $T_{10}_T3^*$, $T_3^*_T_{25}$, and $T_{25}_T3^*$. PELDOR time trace (black) with the background fits (red) and an imaginary part of the signal (blue) is shown on the top. Middle background-corrected PELDOR time trace (black dots) and DEERNet or Tikhonov fit (red). The distance distributions on the bottom for each sample are calculated using the DEERNet network or Tikhonov regularization. Shaded areas show the uncertainty of distance determination.

Molecular dynamics simulation and analysis



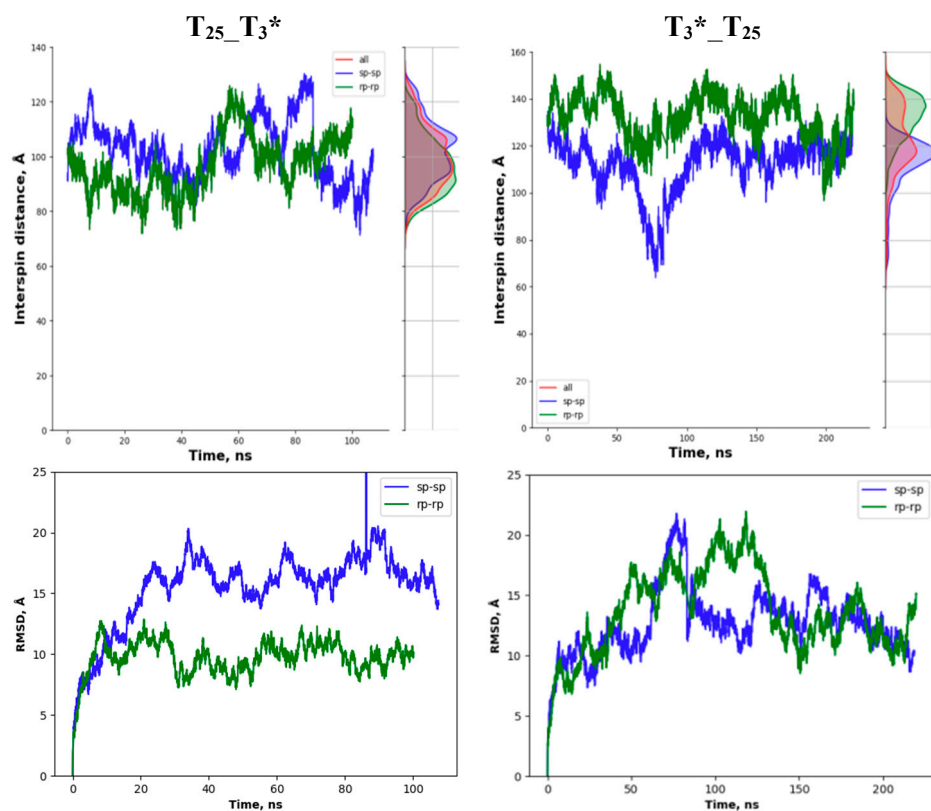
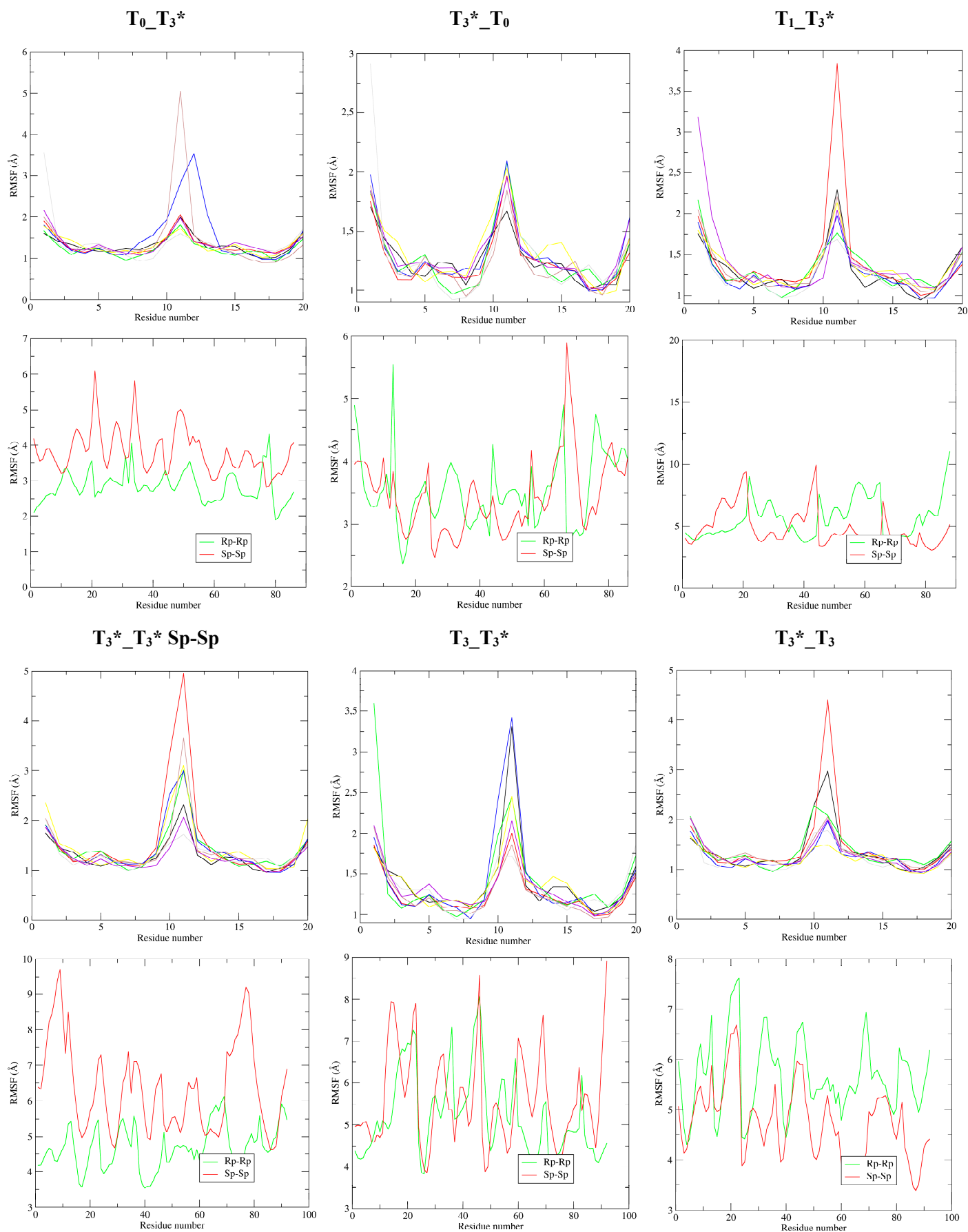


Figure S7. Interspin distances and interspin distance distributions along the molecular dynamics (MD) trajectories for oligonucleotide complexes are on the top. The RMSD values along the MD trajectories are at the bottom for each sample.



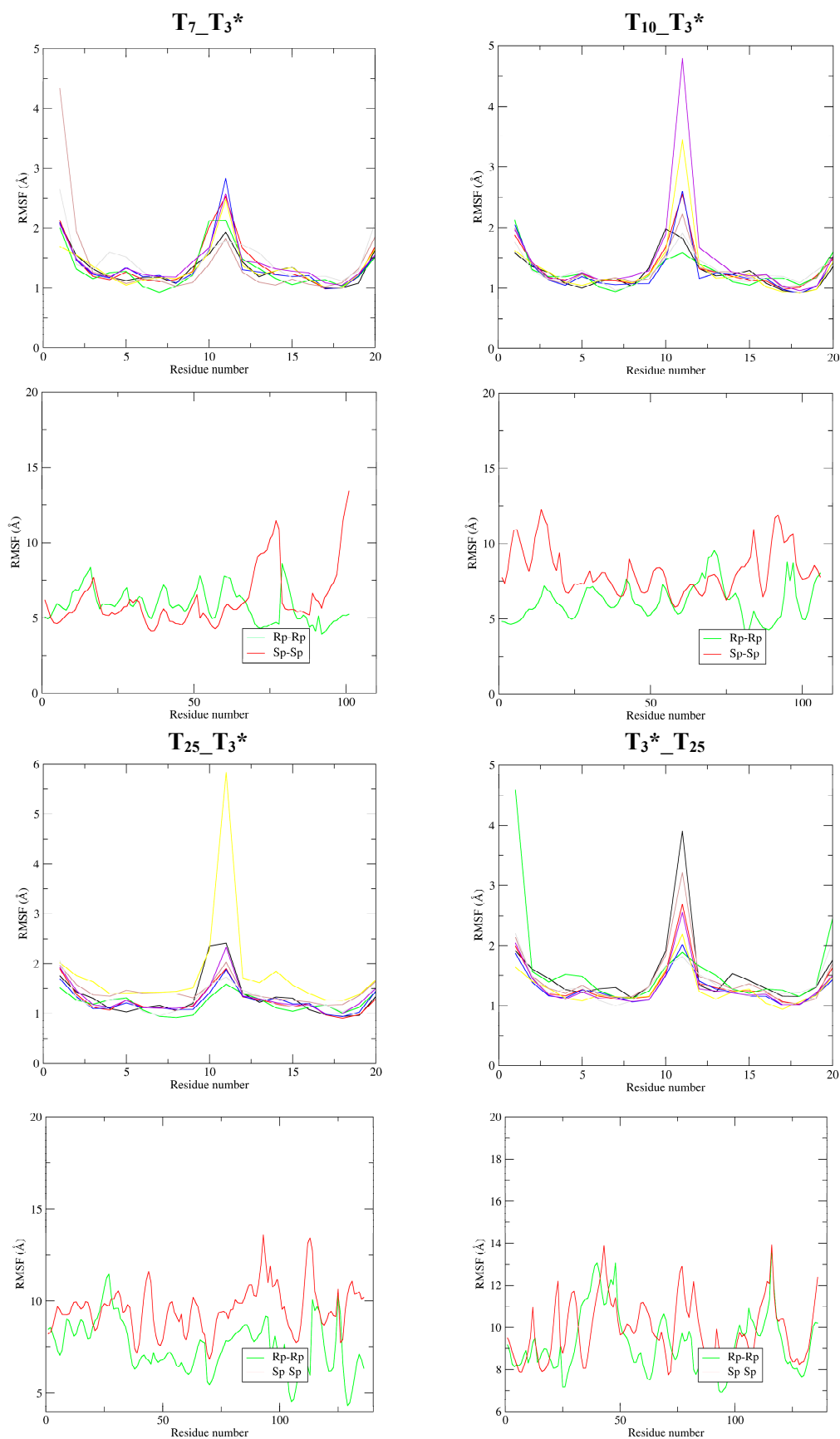
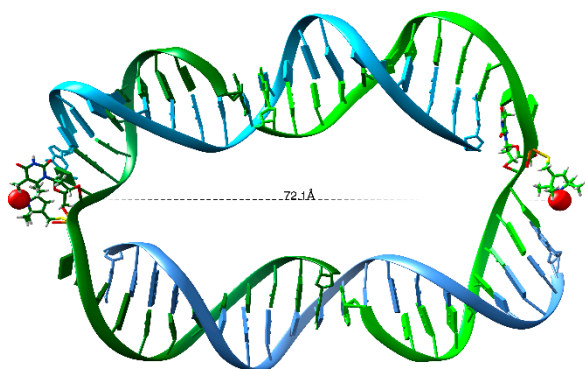
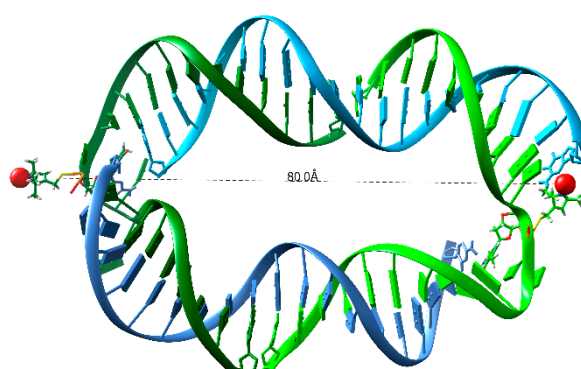


Figure S8. RMSF per residue values for the complex and duplexes in the complex are indicated at the top and bottom for each sample, respectively.

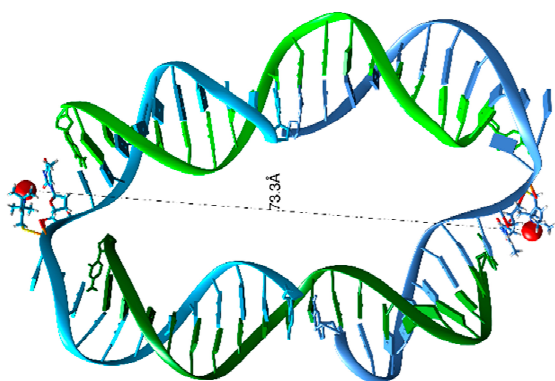
T₀_T₃* Sp-Sp



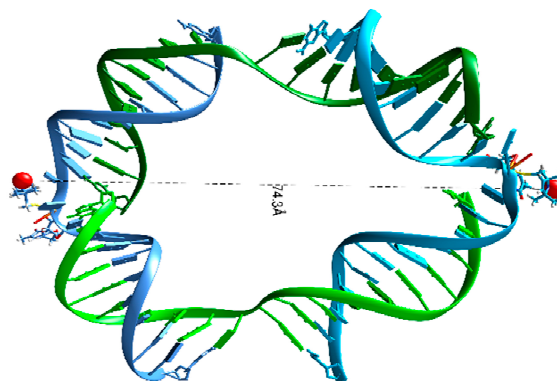
T₀_T₃* Rp-Rp



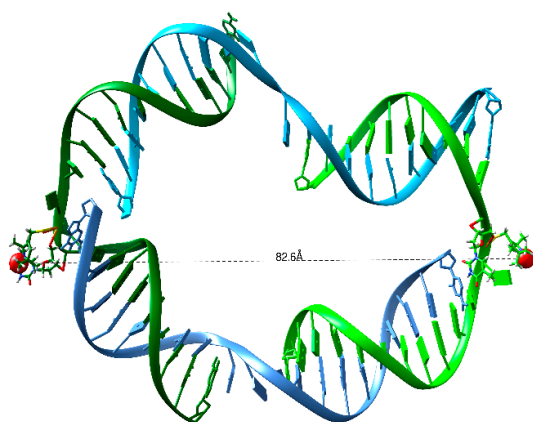
T₃*_T₀ Sp-Sp



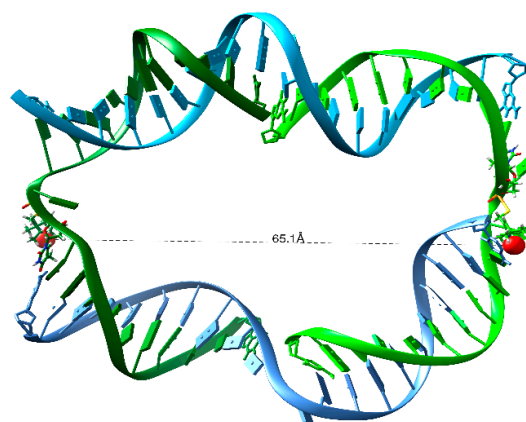
T₃*_T₀ Rp-Rp



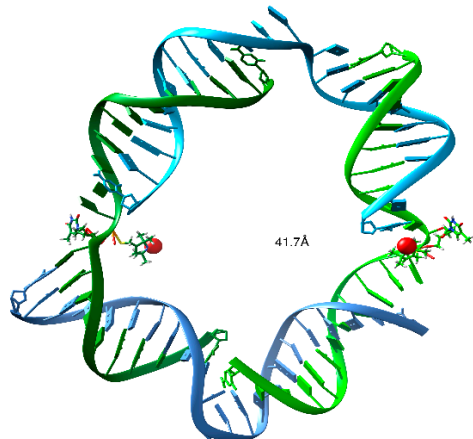
T₁_T₃* Sp-Sp



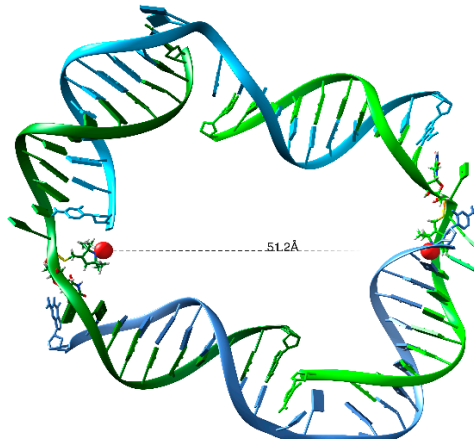
T₁_T₃* Rp-Rp



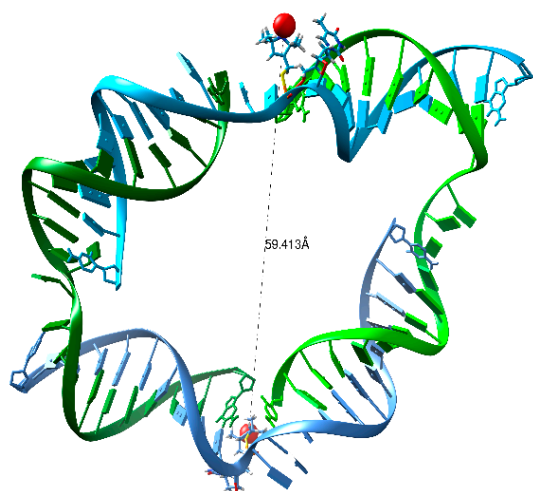
T₃_T₃* Sp-Sp



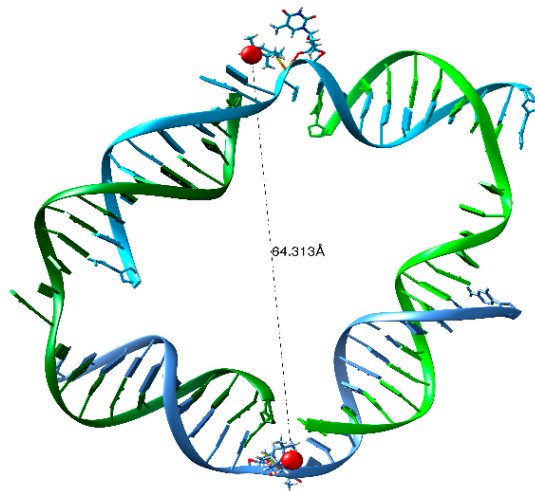
T₃_T₃* Rp-Rp



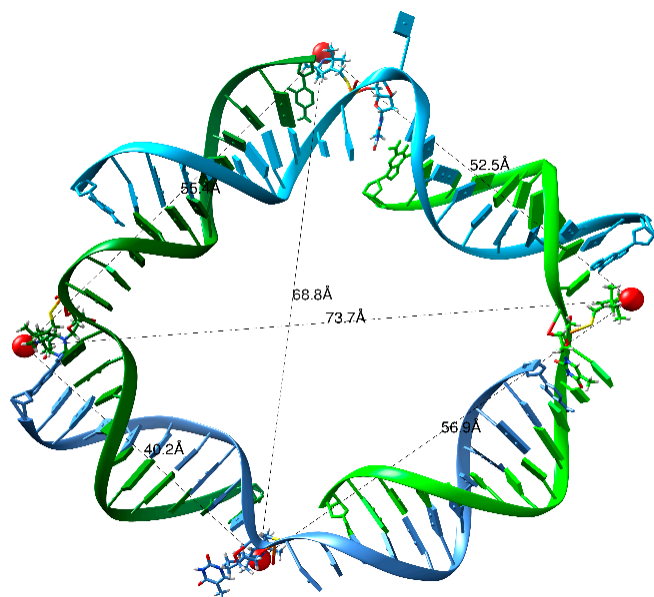
T₃*_T₃ Sp-Sp



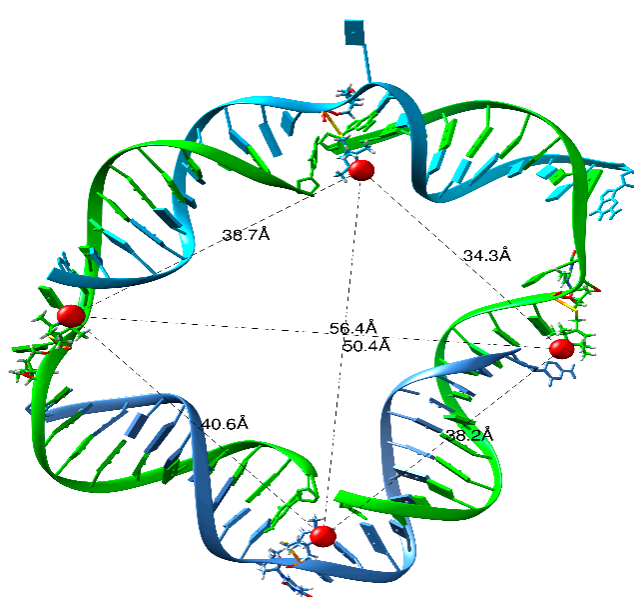
T₃*_T₃ Rp-Rp



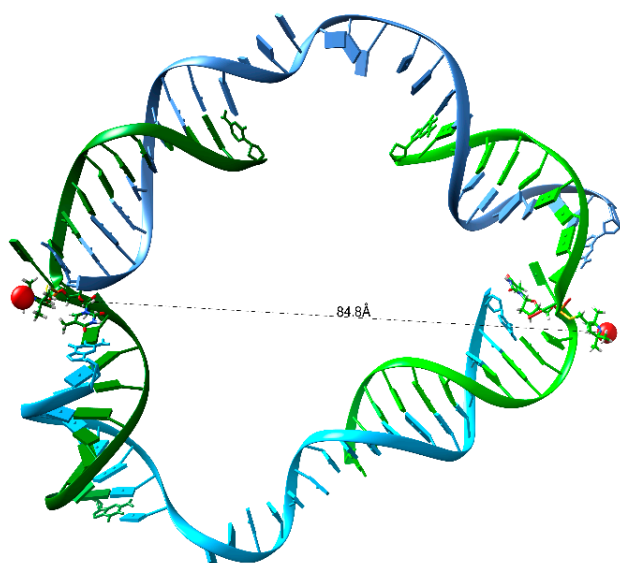
T₃*_T₃* Sp-Sp



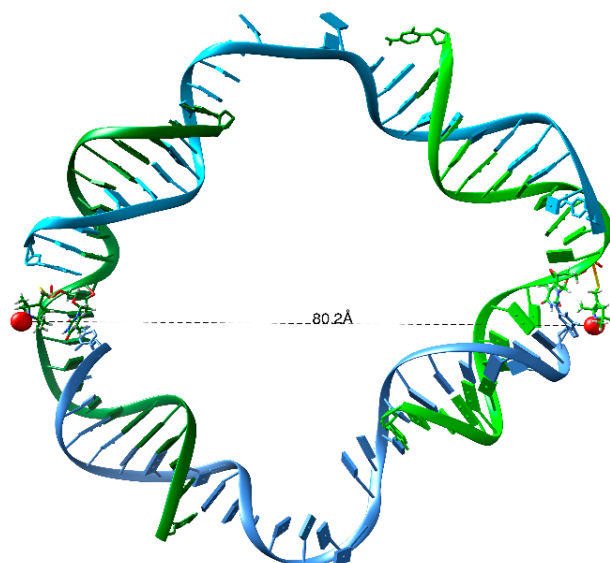
T₃*_T₃* Rp-Rp



T₇_T₃* Sp-Sp

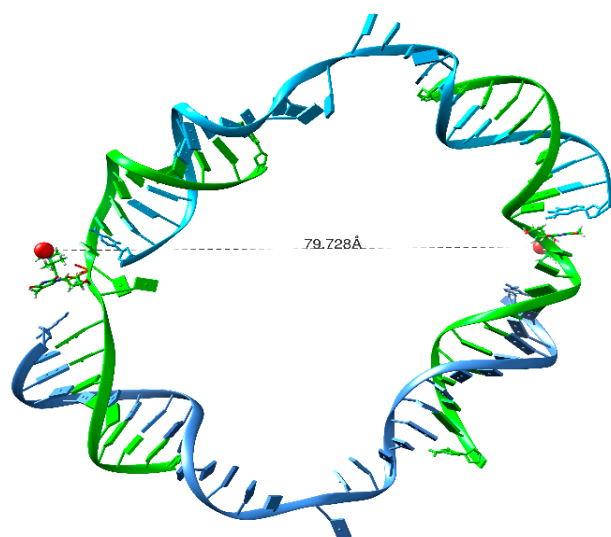


T₇_T₃* Rp-Rp

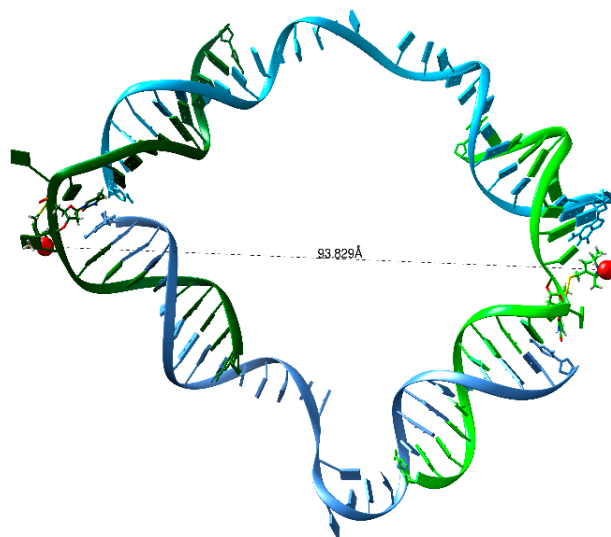


T₁₀_T₃* Sp-Sp

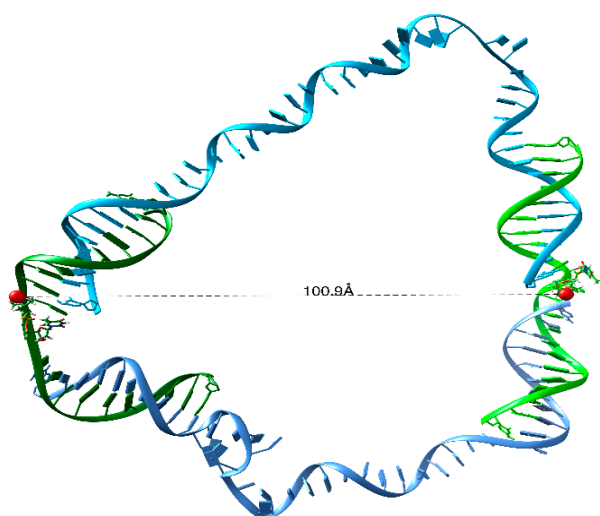
T₁₀_T₃* Rp-Rp



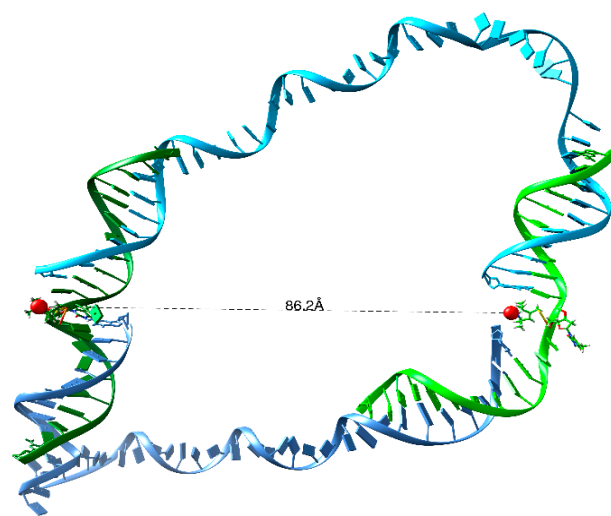
T₂₅_T₃* Sp-Sp



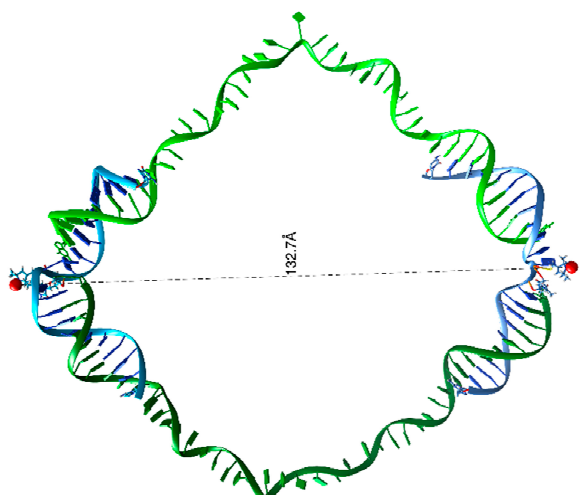
T₂₅_T₃* Rp-Rp



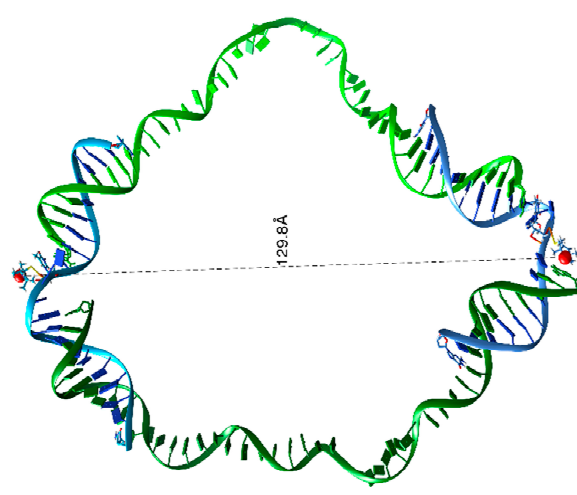
T₃*_T₂₅ Sp-Sp



T₃*_T₂₅ Rp-Rp



132.7Å



129.8Å

Figure S9. Comparison of the most representative structures in MD trajectories obtained by cluster analysis for oligonucleotide complexes for Sp-Sp and Rp-Rp stereoisomers.

Comparison of PELDOR and molecular dynamics distance distribution for $T_7_T_3^*$, $T_{10}_T_3^*$, $T_{25}_T_3^*$, and $T_3^*_T_{25}$ complexes

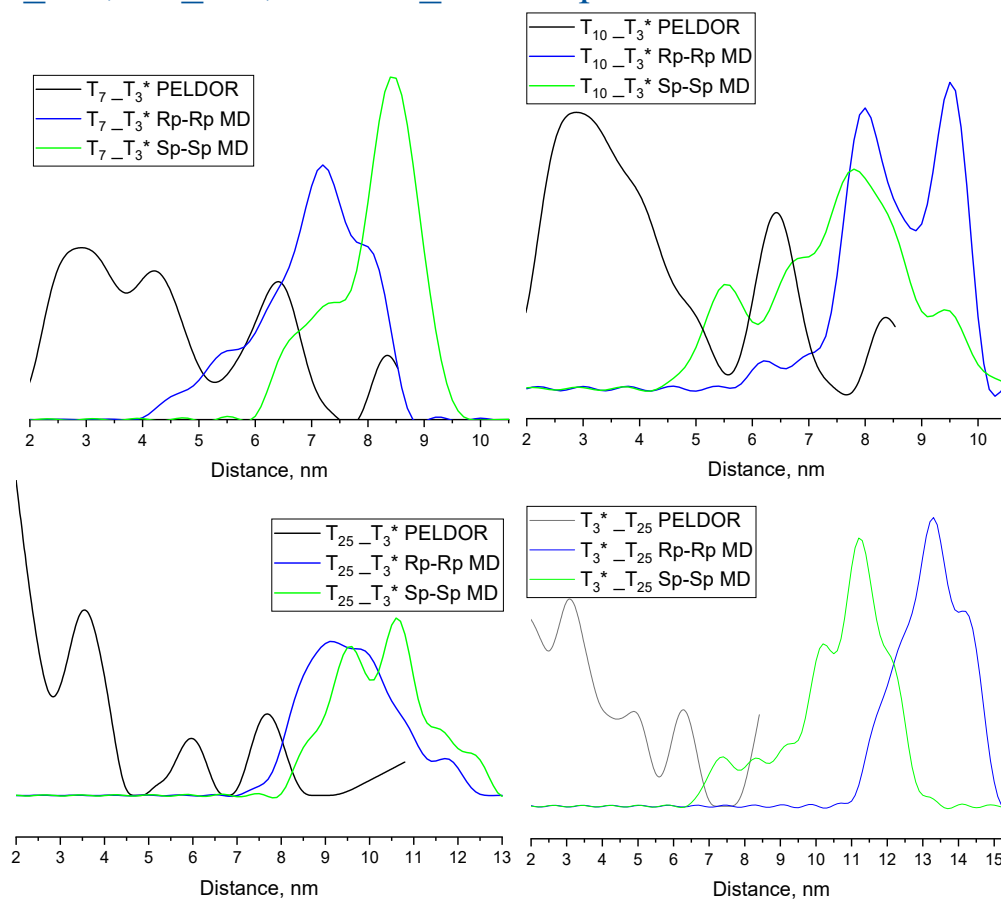


Figure S10. Spin-spin distance distributions for $T_7_T_3^*$, $T_{10}_T_3^*$, $T_{25}_T_3^*$, and $T_3^*_T_{25}$ complexes from Tikhonov regularization of PELDOR time traces and MD trajectory analysis.