Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

All-atom Molecular Dynamics simulations of spin labelled double and single-strand DNA for EPR studies

Supporting Information

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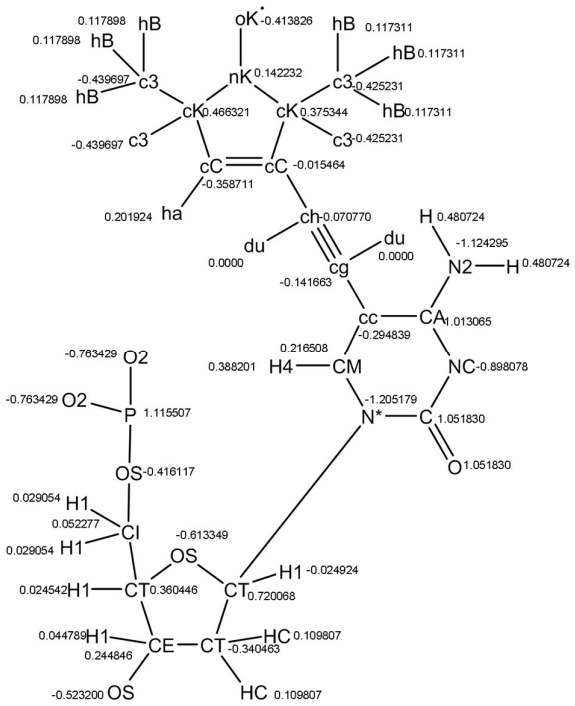

Figure S1: Atom types and partial charges for C* spin label.

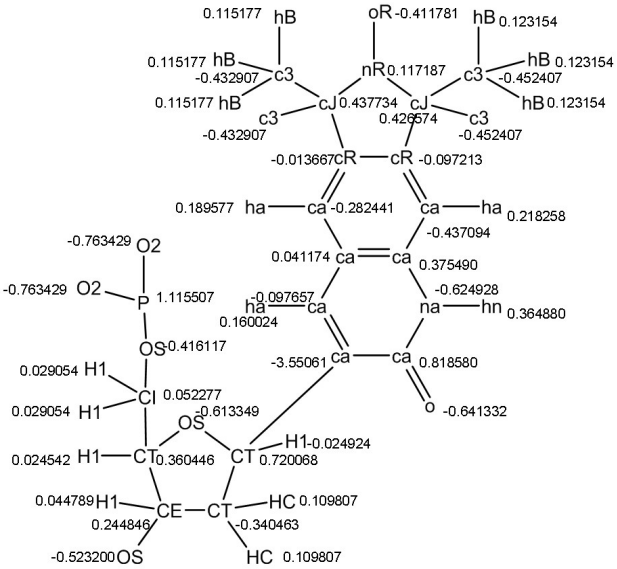

Figure S2: Atom types and partial charges for Q spin label.

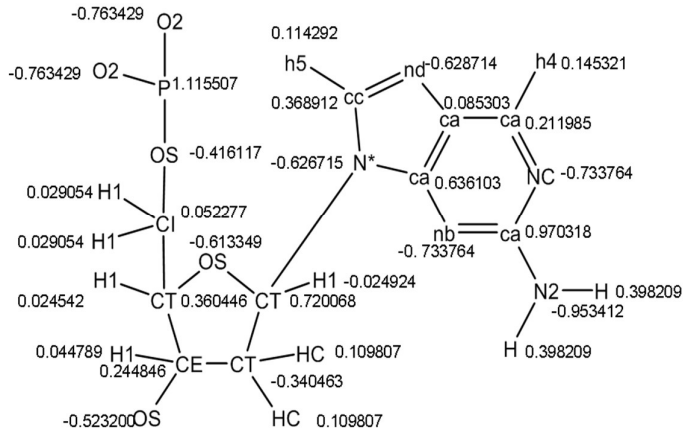

Figure S3: Atom types and partial charges for P base.

Table S1 – New atom types and non-bonded parameters.

Atom Type	Mass	σ (Å)	ε (kcal mol-1)
nR 1	14.01	1.900	0.2627
oR 1	16.00	1.670	0.2445
nK 1	14.01	1.900	0.2627
oK 1	16.00	1.670	0.2445
hB 1	1.008	1.430	0.0157
cJ 1	12.01	1.908	0.1094
cK 1	12.01	1.908	0.1094
cR 2	12.01	1.908	0.0860
cC 3	12.01	1.908	0.0860
du	1.008	0.000	0.000

1 - Taken from literature [40,41]

2 - Equivalent to GAFF c2

3 - Equivalent to GAFF ca

Table S2 – Nitroxide ring bond stretching parameters.

Bond	k_r (kcal mol^{-1} Å^{-2})	r_{eq} (Å)
cR-cR	112.20	1.368
cR-cJ	146.50	1.522
cJ-nR	131.50	1.448
cJ-c3	134.00	1.524
nR-oR	317.00	1.268
c3-hB	189.35	1.092
cC-cK [1]	328.30	1.508
cC-cC [2]	589.70	1.324
cK-c3 [3]	310.00	1.526
cK-nK [3]	370.00	1.477
nK-oK [3]	360.00	1.258

1 - GAFF c2-c3

2 - GAFF c2-c2

3 – Taken from literature [40,41]

Table S3 – Nitroxide ring angle bending parameters.

Angle	k_θ (kcal mol^{-1} rad^{-2})	r_θ (deg)
cR-cR-cJ	137.500	112.20
cR-cJ-nR	149.100	100.00
cJ-nR-cJ	130.500	115.70
cJ-nR-oR	64.000	122.20
cJ -c3-hB [1]	50.000	109.70
c3-cJ-nR [1]	60.000	109.20
c3-cJ-c3	54.300	111.10
hB-c3-hB	74.833	117.46
cC-cK-nK	66.59	97.15
cC-cC-cK [2]	64.33	123.42
cK -c3-hB [1]	50.000	109.70
cK-nK-cK	64.01	112.31
cK-nK-oK [1]	82.00	117.50
c3-cK-c3	40.00	109.50
c3-cK-nK	60.00	107.50

1 - Taken from literature [40,41]

2 – GAFF c3-c2-c2

Table S4 – Nitroxide ring dihedral/improper dihedral parameters.

Dihedral	k (kcal mol^{-1})	δ (deg)	n
oR-nR-cJ-c3 [1]	0.260	0.00	3
c3-cJ-c3-hB [1]	0.160	0.00	3
cJ-cJ-nR-oR [1]	10.10	180.00	2
cC-cK-c3-hB	0.156	0.00	3
cC-cK-nK-cK	0.300	0.00	3
cC-cK-nK-oK	0.300	0.00	3
cK-nK-cK-c3 [1]	0.204	0.00	3
c3-cK-c3-hB	0.160	0.00	3
c3-cK-nK-oK [1]	0.260	0.00	3
hB-c3-cK-nK [1]	1.400	0.00	3
cK-cK-nK-oK [1]	10.10	180.00	2

1 - Taken from literature [40,41]

Table S5 - Parameters used to describe triple bond torsional potential in C* spin label.

Parameter	Force Constant	Equilibrium Value	Source
ch-cC	625.0	1.307	GAFF c1-c2
cg-du	375.9	1.066	GAFF c1-ha
ch-du	375.9	1.066	GAFF c1-ha
cg-cg-du [1]	45.0	90.0	n/a
ch-cg-du [1]	45.0	90.0	n/a
cc-cg-du	0	90.00	n/a
cC-ch-du	0	90.00	n/a
ch-cC-cC	78.5	126.94	DFT
X-cc-cg-du [2]	14.5	180.00 (n = 2)	GAFF X-ca-ca-X
du-cg-ch-du [2]	14.5	180.00 (n = 2)	GAFF X-ca-ca-X
cC-cC-ch-du [3]	0.25	180.00 (n = 2)	DFT
cJ-cC-ch-du [3]	0.25	180.00 (n = 2)	DFT

1 - Term to hold dummy atoms perpendicular to C≡C.

2 - Term to keep dummy atoms in plane with base ring .

3 - Term used to introduce additional torsional restraint to triple bond.

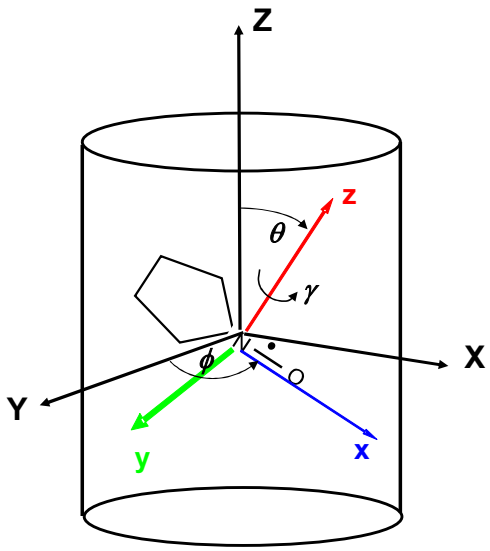

Figure S4: Relative orientation between the DNA fixed frame (black axes) and the directions of the principle components of the partially averaged magnetic tensor (coloured axes) defined by rotational angles θ, ϕ and γ.

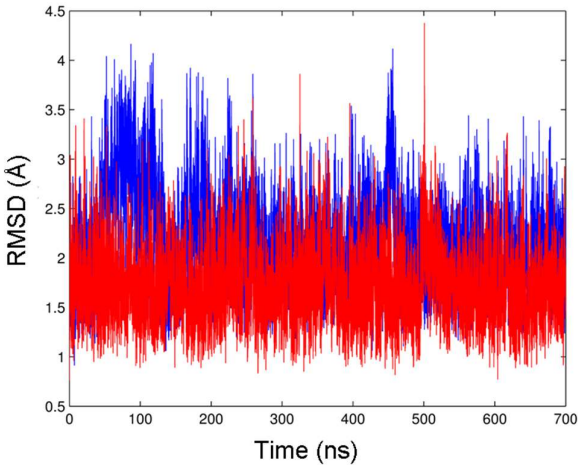

Figure S5: RMSD with terminal base pairs removed for Q (blue) and C* (red) labelled duplex DNA at 293K.

Table S6: Sequence average conformational parameters obtained from full trajectories for the unlabelled C and C* labelled base/pair in duplex DNA at 293K. Angles are reported in degrees and distances are reported in Angstroms.

Parameter	Unlabelled	C* Labelled
Shear	-0.11	0.46
Stretch	-0.15	-0.56
Stagger	0.06	-0.33
Buckle	2.56	6.94
Propeller	-5.88	-1.33
Opening	-0.29	0.10
Shift	0.60	1.25
Slide	0.00	0.42
Rise	3.17	3.27
Tilt	3.59	6.54
Roll	3.29	9.36
Twist	31.74	31.68
Phase	143.7	144.3
Amp	41.0	43.2
α	-70.1	-72.9
β	168.2	169.4
γ	55.4	53.5
δ	133.7	137.1
ε	-140.2	-127.8
ζ	-143.8	-173.4
χ	-104.0	-97.0

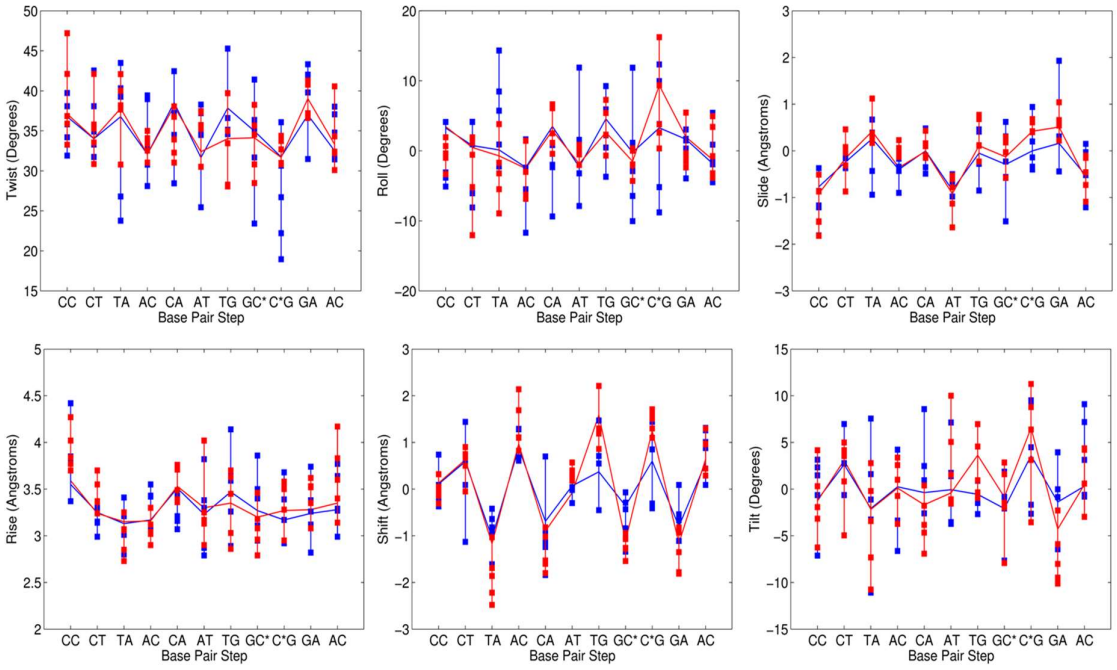

Figure S6: Average base pair step helical parameters as defined in {Dickerson, 1989 #58} for standard (blue) and spin labelled (red) [5'-d(GCC-TAC-ATG-**C**(/**C***)GA-CG)-5'-d(CG-TCG-CAT-GTA-GGC)] duplex DNA at 293K. Error bars determined from the structure of five randomly selected frames. Terminal base pairs were excluded from the analysis.

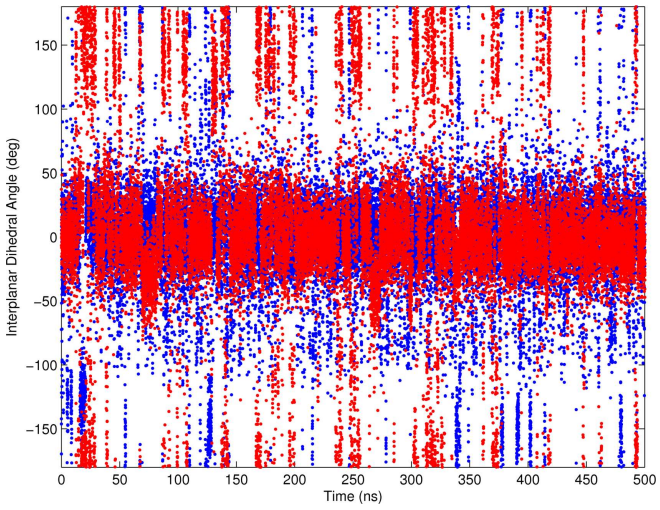

Figure S7: Inter-planar angle between C base and nitroxide bearing ring in C* probe in duplex (blue) and single strand (red) DNA at 293K.

dQ ([5'-d(GCC-TAC-ATG-QGA-CG)-5'-d(CGT-CPC-ATG-TAG-GC)])

Local Motion

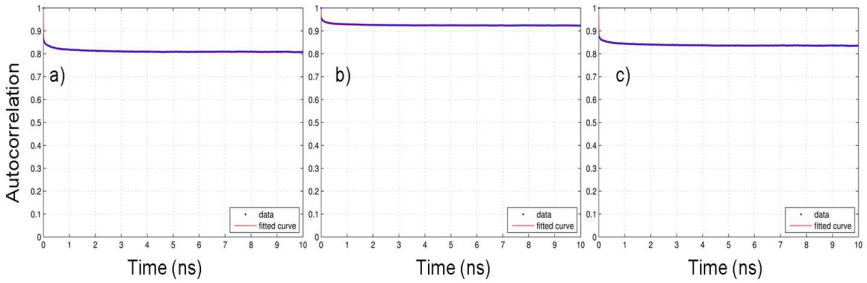

Figure S8: Fitted autocorrelation functions of Q-labelled duplex DNA magnetic z (a), y (b) and x (c) axes at 293K using tri-exponential expression with global motion excluded.

Table S7: Fitted parameters Q-labelled duplex DNA magnetic axes using tri-exponential expression with global motion excluded.

T (K)	Axis	w_1		τ_1 (ns)		w_2		τ_2 (ns)		w_3		τ_3 (ns)		S^2
		Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	
293	X	0.800	0.794/ 0.800	0.007	0.007/ 0.007	0.121	0.121/ 0.127	0.275	0.268/ 0.284	0.079	0.079/ 0.079	2.22	2.19/ 2.25	0.835
	Y	0.649	0.636/ 0.662	0.009	0.008/ 0.010	0.234	0.221/ 0.247	0.201	0.188/ 0.216	0.117	0.104/ 0.117	1.94	1.87/ 2.02	0.923
	Z	0.755	0.750/ 0.766	0.007	0.006/ 0.007	0.141	0.135/ 0.146	0.197	0.182/ 0.215	0.104	0.099/ 0.104	1.49	1.44/ 1.55	0.808

Global Motion

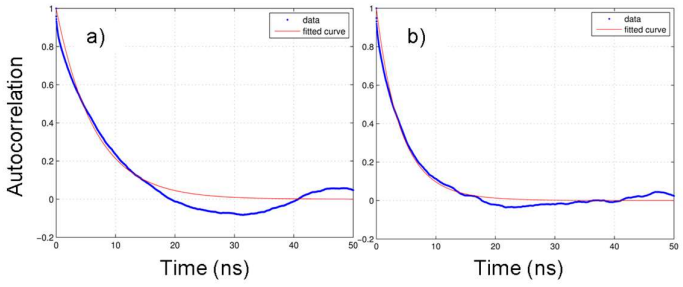

Figure S9: Fitted autocorrelation functions of Q-labelled duplex DNA global Z (a) and X/Y (b) axes at 293K.

Table S8: Fitted parameters for Q-labelled duplex DNA principal axes at 293K.

T (K)	$\tau_{\perp}$ (ns)		$\tau_{\parallel}$ (ns)	
	Fit	Min/Max	Fit	Min/Max
293	6.21	6.12/6.30	2.99	2.93/3.06

dC ([5'-d(GCC-TAC-ATG-C*GA-CG)-5'-d(CG-TCG-CAT-GTA-GGC)])

Local Motion

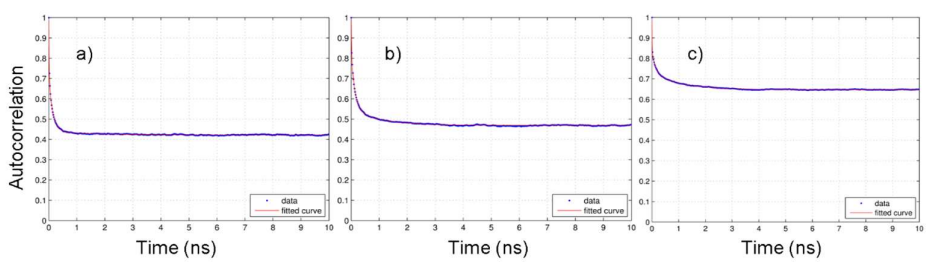

Figure S10: Fitted autocorrelation functions of C*-labelled duplex DNA magnetic z (a), y (b) and x (c) axes at 273K using tri-exponential expression with global motion excluded.

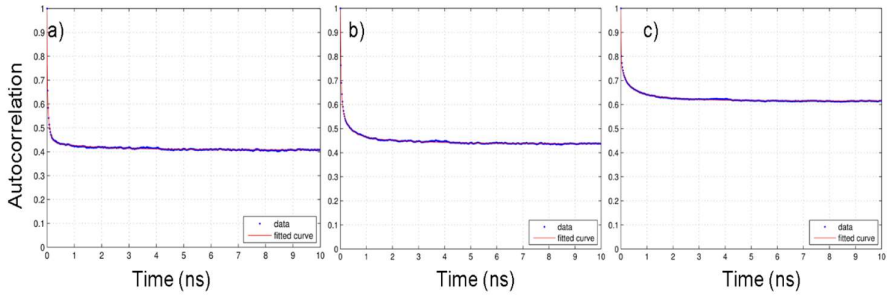

Figure S11: Fitted autocorrelation functions of C*-labelled duplex DNA magnetic z (a), y (b) and x (c) axes at 293K using tri-exponential expression with global motion excluded.

Table S9: Fitted parameters C*-labelled duplex DNA magnetic z (a), y (b) and x (c) axes using tri-exponential expression with global motion excluded.

T (K)	Axis	w_1		τ_1 (ns)		w_2		τ_2 (ns)		w_3		τ_3 (ns)		S^2
		Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	
273	X	0.470	0.459/ 0.482	0.008	0.007/ 0.009	0.297	0.289/ 0.306	0.138	0.131/ 0.146	0.232	0.227/ 0.238	1.08	1.06/ 1.11	0.647
	Y	0.322	0.305/ 0.337	0.013	0.012/ 0.015	0.529	0.518/ 0.542	0.132	0.127/ 0.137	0.149	0.143/ 0.154	1.06	1.02/ 1.10	0.469
	Z	0.493	0.490/ 0.497	0.011	0.011/ 0.011	0.476	0.472/ 0.477	0.136	0.135/ 0.137	0.031	0.031/ 0.033	1.47	1.43/ 1.51	0.422
293	X	0.624	0.621/ 0.629	0.013	0.012/ 0.013	0.252	0.250/ 0.257	0.266	0.260/ 0.273	0.125	0.119/ 0.127	1.44	1.42/ 1.47	0.615
	Y	0.680	0.676/ 0.685	0.028	0.028/ 0.029	0.244	0.240/ 0.247	0.255	0.249/ 0.260	0.077	0.075/ 0.078	1.75	1.72/ 1.78	0.438
	Z	0.600	0.593/ 0.608	0.011	0.011/ 0.011	0.346	0.338/ 0.353	0.083	0.081/ 0.085	0.054	0.054/ 0.061	1.78	1.75/ 1.81	0.408

Global Motion

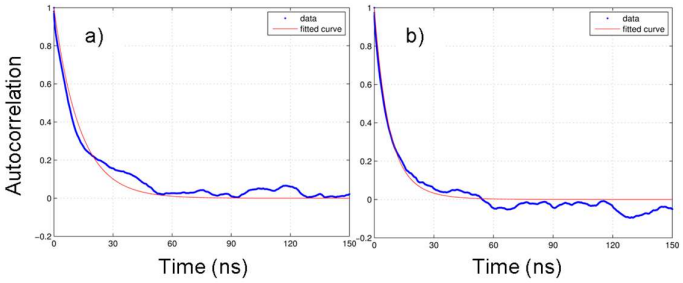

Figure S12: Fitted autocorrelation functions of C*-labelled duplex DNA global Z (a) and X/Y (b) axes at 273K.

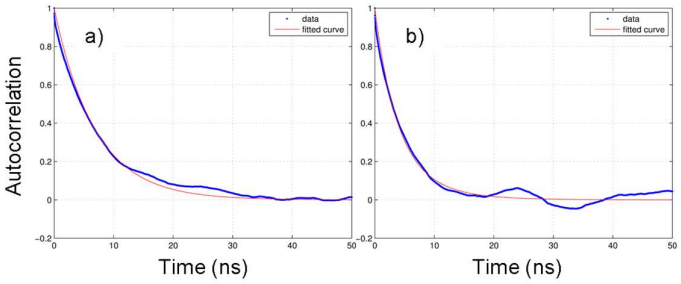

Figure S13: Fitted autocorrelation functions of C*-labelled duplex DNA global Z (a) and X/Y (b) axes at 293K.

Table S10: Fitted parameters for C*-labelled duplex DNA principal axes.

T (K)	$\tau_{\perp}$ (ns)		$\tau_{\parallel}$ (ns)	
	Fit	Min/Max	Fit	Min/Max
273	13.10	12.99/13.20	5.23	5.14/5.30
293	7.02	6.95/7.09	2.88	2.83/2.93

sQ ([5'-d(GCT-TAA-GCT-QCG-CG])

Local Motion

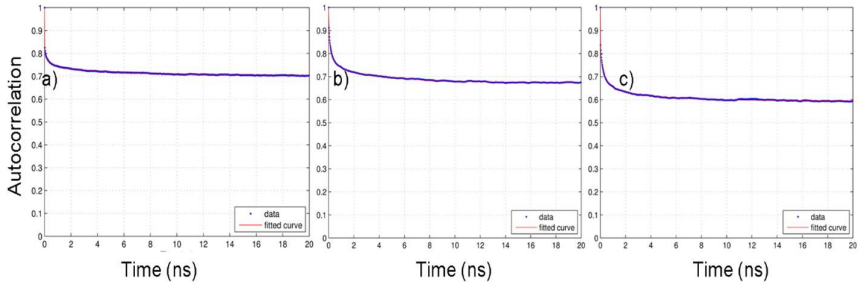

Figure S14: Fitted autocorrelation functions of Q-labelled single strand DNA magnetic z (a), y (b) and x (c) axes at 293K using tri-exponential expression with global motion excluded.

Table S11: Fitted parameters Q-labelled single-strand DNA magnetic z (a), y (b) and x (c) axes using tri-exponential expression with global motion excluded.

T (K)	Axis	w_1		τ_1 (ns)		w_2		τ_2 (ns)		w_3		τ_3 (ns)		S^2
		Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	
293	X	0.495	0.490/ 0.502	0.014	0.013/ 0.014	0.340	0.335/ 0.342	0.345	0.339/ 0.351	0.165	0.163/ 0.165	3.61	3.58/ 3.65	0.595
	Y	0.405	0.402/ 0.408	0.036	0.036/ 0.037	0.368	0.362/ 0.368	0.325	0.323/ 0.328	0.227	0.227/ 0.230	4.05	4.04/ 4.06	0.674
	Z	0.668	0.664/ 0.668	0.010	0.010/ 0.010	0.191	0.191/ 0.195	0.393	0.389/ 0.397	0.141	0.141/ 0.144	5.59	5.57/ 5.61	0.702

Global Motion

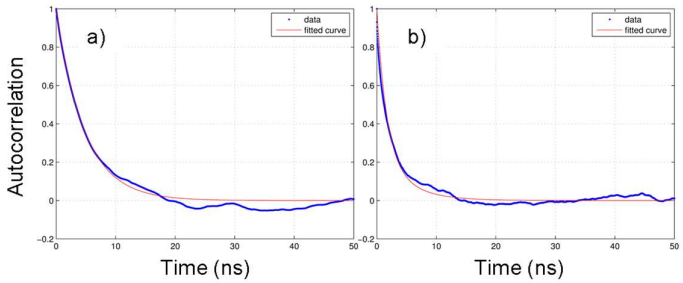

Figure S15: Fitted autocorrelation functions of Q-labelled single-strand DNA global Z (a) and X/Y (b) axes at 293K.

Table S12: Fitted parameters for Q-labelled single-strand DNA principal axes.

T (K)	$\tau_{\perp}$ (ns)		$\tau_{\parallel}$ (ns)	
	Fit	**Min/Max**	**Fit**	**Min/Max**
293	4.76	4.73/4.80	1.23	1.21/1.25

sC ([5'-d(GCC-TAC-ATG-C*GA-CG])

Local Motion

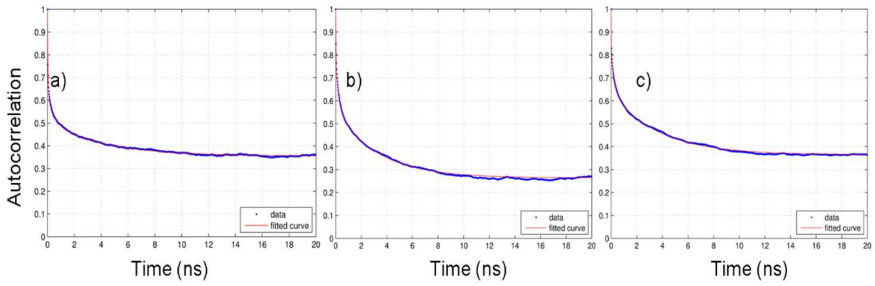

Figure S16: Fitted autocorrelation functions of C*-labelled single-strand DNA magnetic z (a), y (b) and x (c) axes at 273K using tri-exponential expression with global motion excluded.

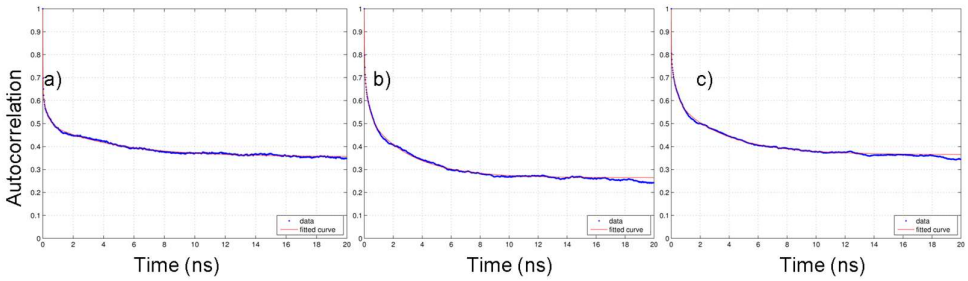

Figure S17: Fitted autocorrelation functions of C*-labelled single strand DNA magnetic z (a), y (b) and x (c) axes at 293K using tri-exponential expression with global motion excluded.

Table S13: Fitted parameters C*-labelled single-strand DNA magnetic z (a), y (b) and x (c) axes using tri-exponential expression with global motion excluded.

T (K)	Axis	w_1		τ_1 (ns)		w_2		τ_2 (ns)		w_3		τ_3 (ns)		S^2
		Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	Fit	Min/ Max	
273	X	0.306	0.290/ 0.323	0.012	0.010/ 0.014	0.265	0.254/ 0.274	0.289	0.273/ 0.308	0.430	0.427/ 0.435	3.67	3.64/ 3.70	0.365
	Y	0.287	0.263/ 0.313	0.027	0.023/ 0.032	0.307	0.288/ 0.328	0.262	0.241/ 0.286	0.405	0.401/ 0.411	3.27	3.23/ 3.31	0.264
	Z	0.526	0.522/ 0.529	0.024	0.024/ 0.025	0.230	0.228/ 0.233	0.361	0.357/ 0.365	0.245	0.243/ 0.245	4.01	4.00/ 4.02	0.355
293	X	0.335	0.331/ 0.342	0.010	0.010/ 0.011	0.274	0.271/ 0.277	0.324	0.318/ 0.330	0.391	0.389/ 0.392	3.432	3.420/ 3.445	0.365
	Y	0.345	0.319/ 0.371	0.016	0.013/ 0.019	0.273	0.257/ 0.289	0.276	0.251/ 0.306	0.382	0.375/ 0.389	3.002	3.950/ 3.056	0.264
	Z	0.567	0.551/ 0.582	0.012	0.011/ 0.013	0.200	0.190/ 0.207	0.297	0.277/ 0.319	0.234	0.231/ 0.235	4.502	4.443/ 4.563	0.355

Global Motion

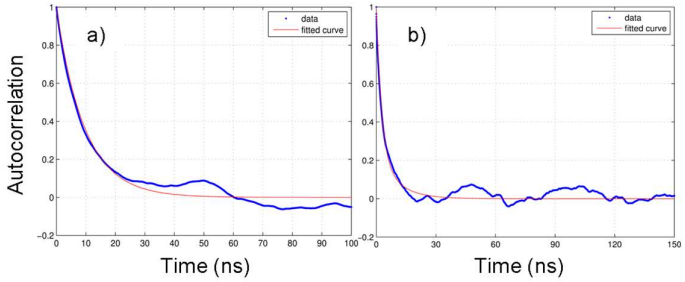

Figure S18: Fitted autocorrelation functions of C*-labelled single-strand DNA global Z (a) and X/Y (b) axes at 273K.

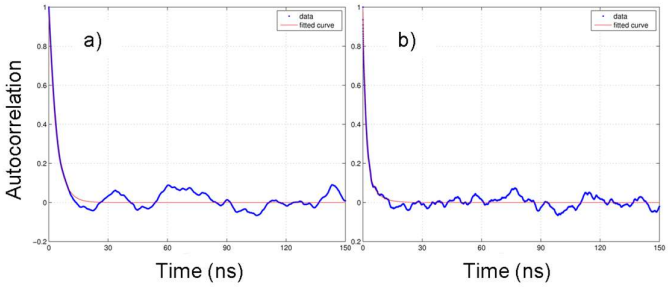

Figure S19: Fitted autocorrelation functions of C*-labelled single strand DNA global Z (a) and X/Y (b) axes at 293K.

Table S14: Fitted parameters for C*-labelled single-strand DNA principal axes.

T (K)	$\tau_{\perp}$ (ns)		$\tau_{\parallel}$ (ns)	
	Fit	**Min/Max**	**Fit**	**Min/Max**
273	9.65	9.57/9.74	1.91	1.88/1.93
293	3.753	3.711/3.796	0.968	0.948/0.987

Example with double helix DNA labelled with Q spin probe demonstrating the sensitivity of EPR spectrum predicted from MD to the choice of force fields for DNA modelling.

Table S15: Motional and order parameters of duplex DNA labelled with Q probe obtained from the fitting of relevant autocorrelation functions generated from MD simulation with the parm99/TIP3P force field.

Label	Temp (K)	$\tau_{\perp}$ (ns)	$\tau_{\parallel}$ (ns)	$\overline{\tau}_x$ (ns)	$\overline{\tau}_y$ (ns)	$\overline{\tau}_z$ (ns)	S_x	S_y	S_z
Q	293	3.13	1.84	0.93	0.20	0.29	0.92	0.96	0.91

Table S16: Magnetic parameters of Q spin label in duplex DNA generated from MD simulation with the parm99/TIP3P force field and partially averaged by the local motion.

Label	T (K)	g_{xx}	g_{yy}	g_{zz}	A_{xx}	A_{yy}	A_{zz}	φ	ϑ	γ
Q	293	2.0085	2.0060	2.0023	6.60	7.26	32.21	49.37	41.71	35.44

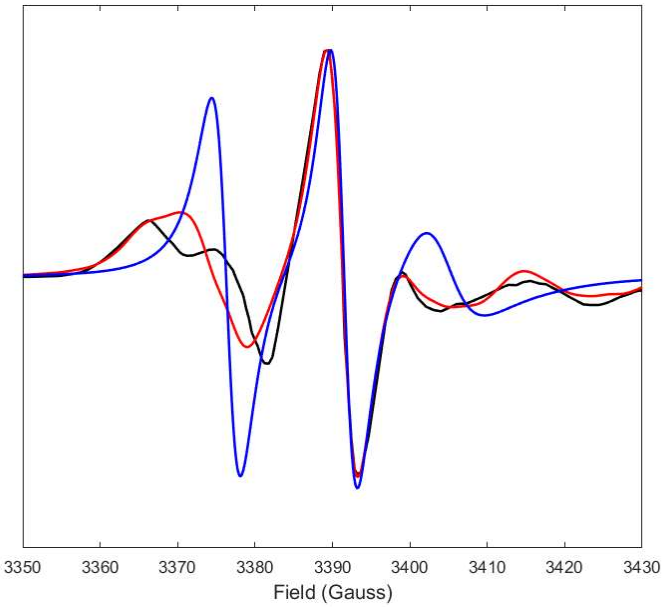

Figure S20: Comparison between experimental EPR spectrum (black) and those predicted from MD using different force field parameters of Q-labelled duplex DNA at 293K. The red and bluer lines represent EPR spectra generated from MD simulations employing parmbsc1/SPC/E and parm99/TIP3P force fields, respectively. Experimental EPR spectrum is reproduced from [10] with permission from the American Chemical Society.

Example with single strand DNA labelled with C spin probe demonstrating the sensitivity of EPR spectrum predicted from MD to the choice of force fields for DNA modelling.*

Table S17: Motional and order parameters of single-strand DNA labelled with C*Q probe obtained from the fitting of relevant autocorrelation functions generated from MD simulation with the parm99/SPC/E force field.

Label	Temp (K)	$\tau_{\perp}$ (ns)	$\tau_{\parallel}$ (ns)	$\overline{\tau}_x$ (ns)	$\overline{\tau}_y$ (ns)	$\overline{\tau}_z$ (ns)	S_x	S_y	S_z
C*	273	5.24	2.90	4.20	3.86	5.03	0.45	0.41	0.49

Table S18: Magnetic parameters of C*spin label in single-strand DNA generated from MD simulation with the parm99/SPC/E force field and partially averaged by the local motion.

Label	T (K)	g_{xx}	g_{yy}	g_{zz}	A_{xx}	A_{yy}	A_{zz}	φ	ϑ	γ
C*	273	2.0067	2.0063	2.0038	9.64	11.63	24.93	28.82	-88.38	-29.82

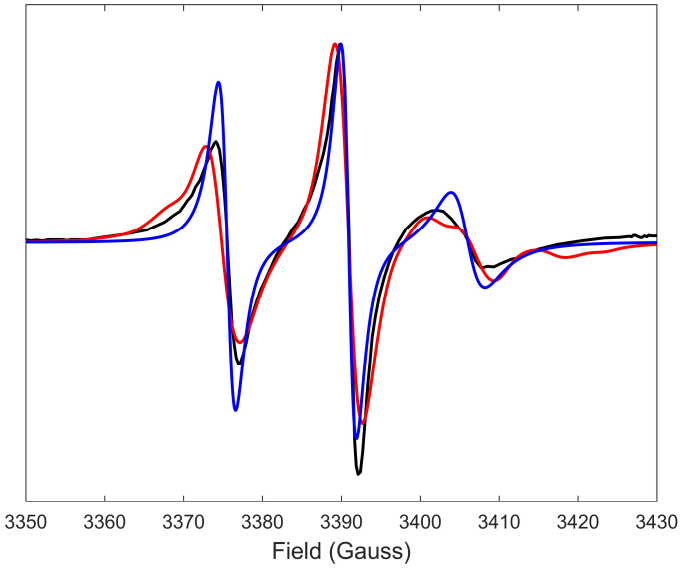

Figure S21: Comparison between experimental EPR spectrum (black) and those predicted from MD using different force field parameters of C*-labelled single strand DNA at 273K. The red and bluer lines represent EPR spectra generated from MD simulations employing parmbsc1/SPC/E and parm99/SPC/E force fields, respectively. Experimental EPR spectrum is reproduced from [20] with permission from Taylor and Francis.