Supporting Information

for

Direct observation of the hole protonation state and hole localization site in DNA-oligomers

by

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Supporting Information:

Analyses of the ESR spectra of ds DNA-oliomers shown in Figure S5 using guanine neutral radical (G(–H)•) from dGuo and guanine neutral radical (G*(–H)•) from G* [G* = 8-D-dGuo, 96% deuterated]

Figure S1: Analyses for deuteration levels in the G* labeled ds DNA-oligomers

We have analyzed the ds DNA-oligomer d[G*CG*CG*CG*C]$_2$ for the fraction of deuteration with the help of benchmark spectra of deprotonated guanine cation radical G(–H)•, and G*(–H)• , obtained from dGuo and G* [G* = 8-D-dGuo] via one-electron oxidation by Cl_2•$^-$ at 155 K in the dark at pD *ca.* 9. We find that the method of preparation had an affect on the deuteration level resulting. For non HPLC purified samples, we have found that the one-electron oxidized d[G*CG*CG*CG*C]$_2$ is *ca.* 80 % deuterated; whereas, the extent of deuteration (i.e., incorporation of G*) in HPLC purified oligo was found to be *ca.* 50% (see Figure S1 below). We found that the extent of G* incorporation in each of the DNA-oligomers which were not HPLC-purified, *e.g.*, TG*T, d[G*CG*CG*CG*C]$_2$, d[G*GGCCC]$_2$, d[GG*GCCC]$_2$, and d[GGG*CCC]$_2$ – were also found to be *ca.* 80%.

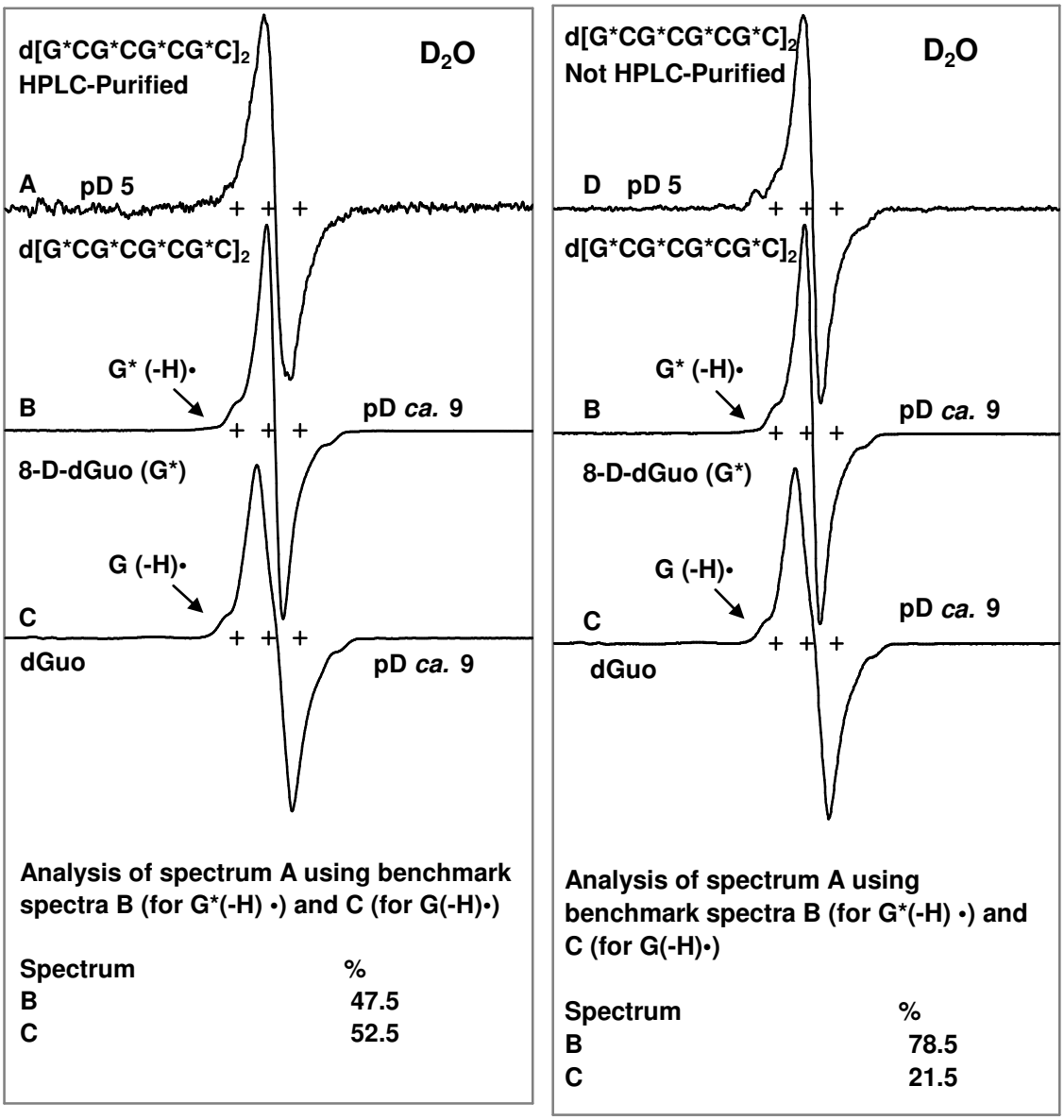

Figure S1. Spectral analyses of one-electron oxidized guanine obtained from the glassy sample (7.5 M LiCl/D_2O) of the HPLC-purified (spectrum A) and not purified by HPLC (spectrum D) of the ds DNA-oligomer d[G*CG*CG*CG*C]$_2$ at the native pD *ca.* 5 of 7.5 M LiCl in D_2O using benchmark spectra of G*(-H)• from G* (spectrum B) and G(-H)• from dGuo (spectrum C) have been presented. Spectra A and D were recorded at 77 K. These analyses show that in one-electron oxidized guanine formed in glassy sample of HPLC-purified d[G*CG*CG*CG*C]$_2$, both G*(-H)• and G(-H)• could be present almost in equal amount i.e., the extent of G* incorporation in the HPLC-purified ds DNA-oligomer d[G*CG*CG*CG*C]$_2$ is *ca.* 50 %. On the other hand, in the one-electron oxidized guanine formed from d[G*CG*CG*CG*C]$_2$ which was not purified by HPLC, the dominance (*ca.* 80%) of the G*(-H)• clearly indicates that this oligomer was *ca.* 80% deuterated.

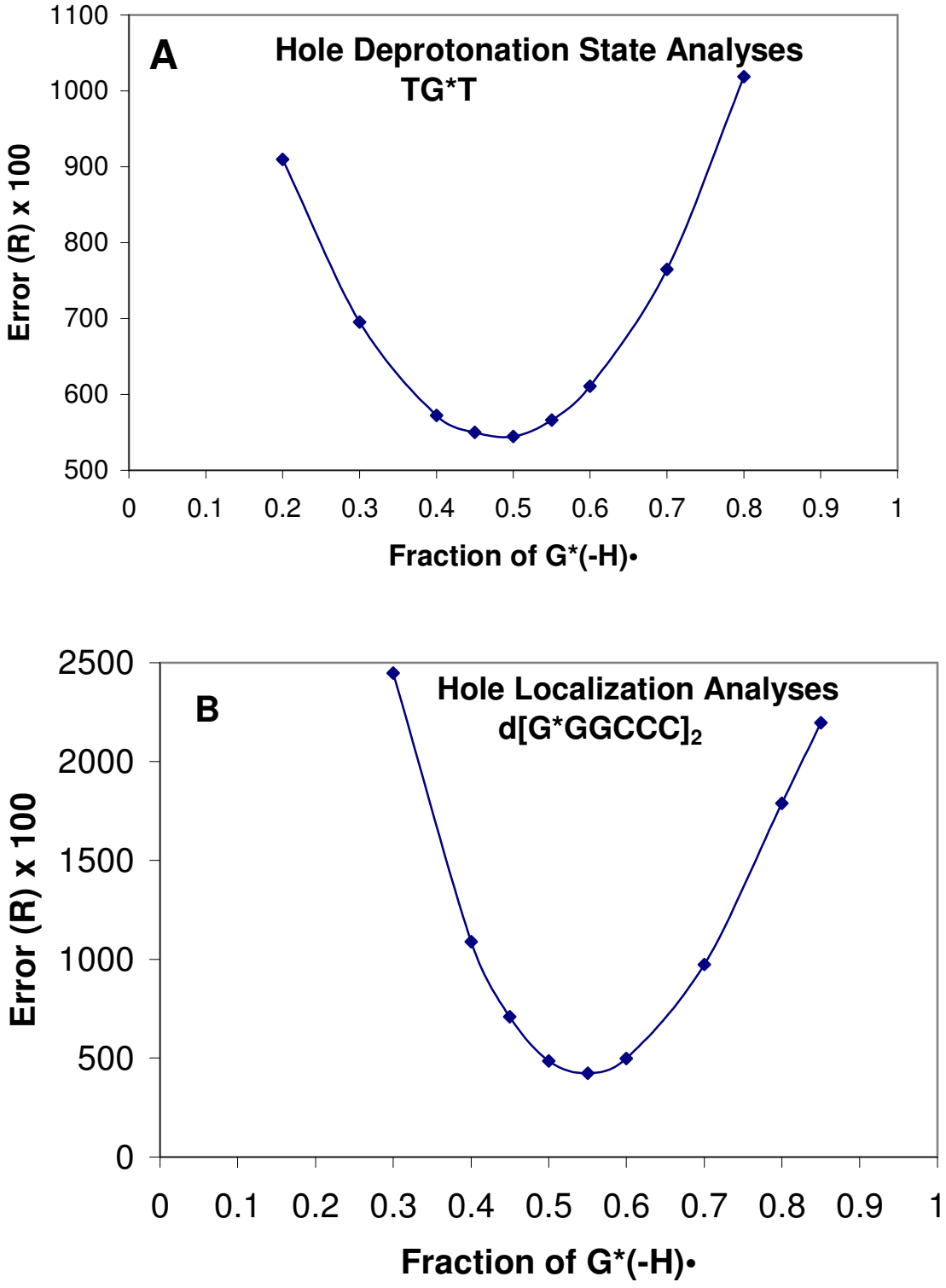

Figure S2. ESR spectra of (A) one-electron oxidized ss DNA-oligomer TG*T (pD *ca.* 5) and (B) one-electron oxidized ds DNA-oligomer d[G*GGCCC]$_2$ are employed as

examples analyses. Figure A is an example of the fit for the protonation state and Figure B is an example for fit for hole localization. We estimate the error by statistical comparison (R) of the experimentally observed spectrum using with varying amounts of two benchmark spectra. For (A) the spectrum of one-electron oxidized ss DNA-oligomer TG*T, fits were obtained by employing the ESR spectra of G*•$^{+}$ and G*(-H)• found in 8-D-dGuo (G*) as benchmarks (Figure 2A in the manuscript). For (B) the spectrum of one-electron oxidized ds DNA-oligomer d[G*GGCCC]$_2$, fits were obtained by employing the ESR spectra of G(-H)• and G*(-H)• found in d[GCGCGCGC]$_2$ and d[G*CG*CG*CG*C]$_2$ as benchmarks (Figures 3A and 3B in the manuscript). The error was a minimum at the composition reported in the manuscript (46% for A and 58% for B) found via a global least squares minimization routine. From this figure we estimate error limits of our fits of about ±10% in A and somewhat less for B.

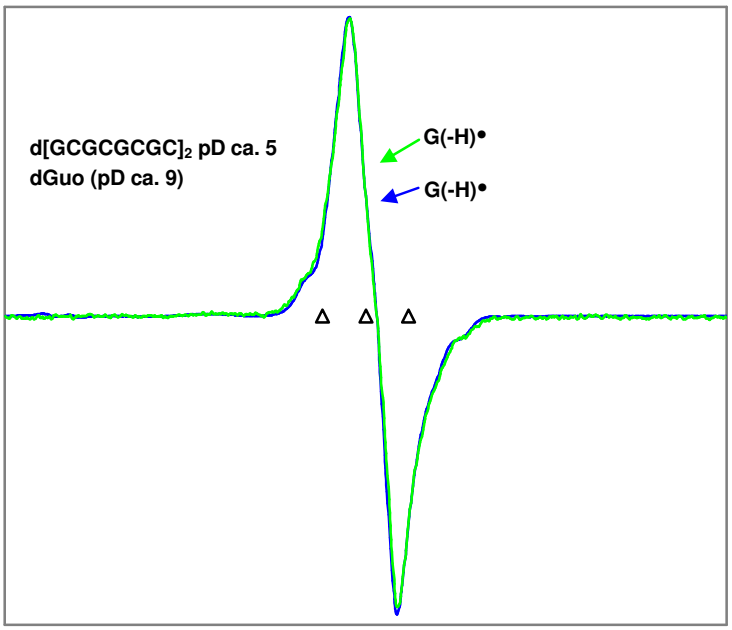

Figure S3. ESR spectrum (blue color) of the deprotonated guanine neutral radical (G(–H)•) formed by $Cl_2^{\bullet -}$ oxidation of dGuo (3 mg/mL) in a homogeneous glassy (7.5 M LiCl in D_2O) solution at pD *ca.* 9 via thermal annealing at 150 – 155 K in the dark. ESR spectrum (green) of the one-electron oxidized ds DNA oligomer $d[GCGCGCGC]_2$ produced by $Cl_2^{\bullet -}$ oxidation of $d[GCGCGCGC]_2$ (2 mg/mL) at the native pD *ca.* 5 in a homogeneous glassy (7.5 M LiCl in D_2O) solution via thermal annealing at 150 – 155 K in the dark. Both spectra were recorded at 77 K. The very close similarities in overall hyperfine splitting, anisotropic nitrogen hyperfine couplings, lineshape, and the center of these two spectra show they are for the same radical and establish that the one-electron oxidized ds DNA oligomer $d[GCGCGCGC]_2$ is from G(–H)•.

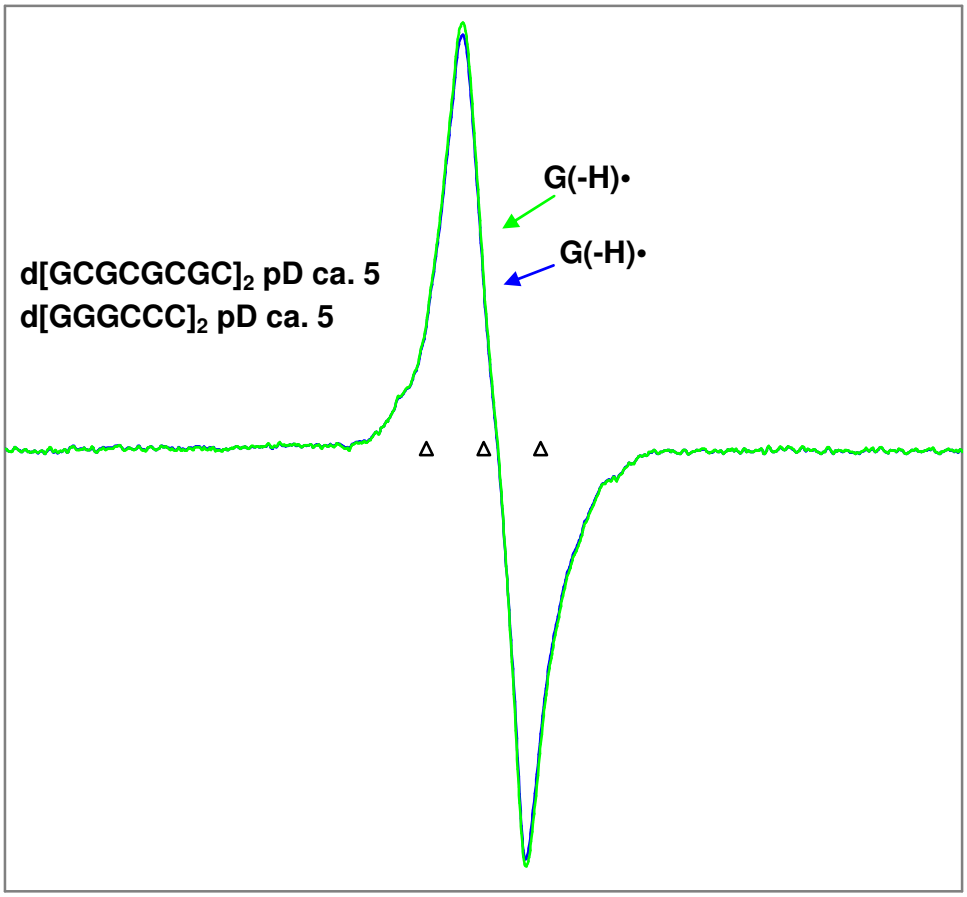

Figure S4. ESR Spectra of the one-electron oxidized dsDNA-oligomers *viz.* $d[GGGCCC]_2$ (blue) and $d[GCGCGCGC]_2$ (green). One-electron oxidation of each oligomer (2 mg/mL) was caused by $Cl_2^{\bullet-}$ at the native pD *ca.* 5 in the homogeneous glassy (7.5 M LiCl in D_2O) solution via thermal annealing at 150 – 155 K in the dark. Both spectra were recorded at 77 K. It is evident from Figure S3 that the one-electron oxidized $d[GCGCGCGC]_2$ was assigned to G(–H)•.

The very close similarities regarding the overall hyperfine splitting, anisotropic nitrogen hyperfine couplings, lineshape, and the center of these two spectra clearly establish that the one-electron oxidation in these two ds DNA oligomers leads to form the same radical species. Thus, one-electron oxidized $d[GGGCCC]_2$ was assigned to G(–H)• as well.

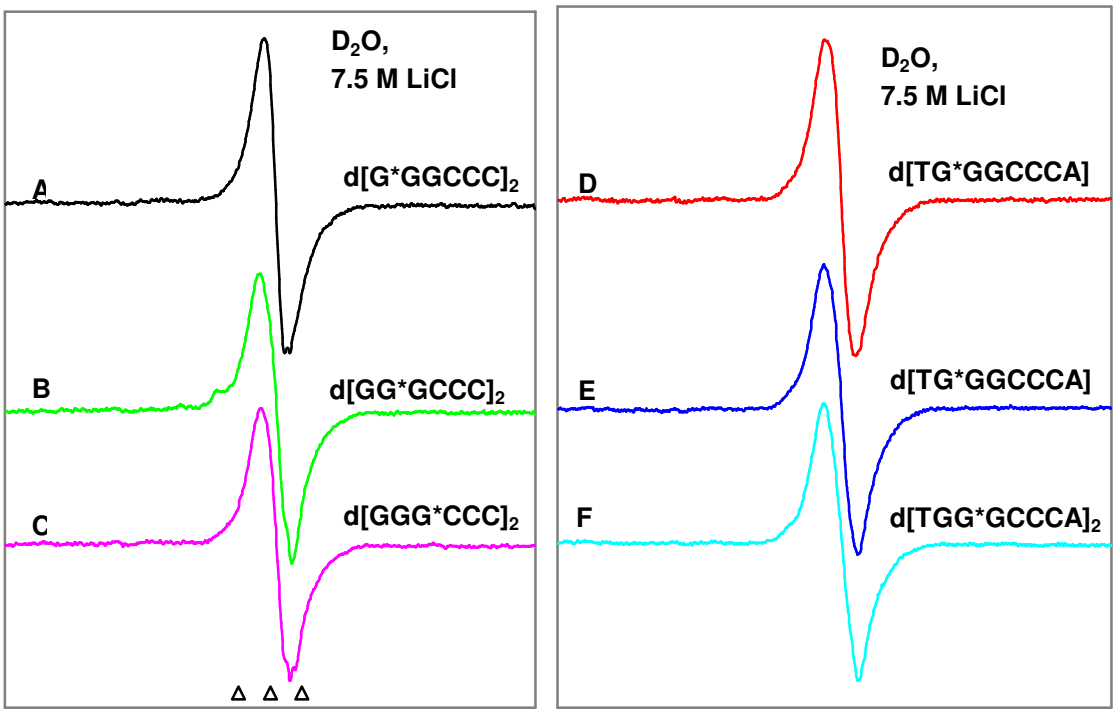

Figure S5. ESR Spectra of the one-electron oxidized ds DNA-oligomers with 5'-OH and 3'-OH [(A) d[G*GGCCC]$_2$ (black), (B) d[GG*GCCC]$_2$ (green) and (C) d[GGG*CCC]$_2$ (pink)], and with 5'-T and 3'-T having G* [G* = 8-D-dGuo] at 5'-end, in the middle, and at the 3'-end [(D) d[TG*GGCCCA]$_2$ (red), (E) d[TGG*GCCCA]$_2$ (blue), and (F) d[TGGG*CCCA]$_2$ (turquoise)]. Levels of deuteration were 80% in A, B, C and 50% in D, E, F.

One-electron oxidation of each oligomer (1.5 – 2.5 mg/mL) was caused by $Cl_2^{\bullet -}$ at the native pD *ca.* 5 in the homogeneous glassy (7.5 M LiCl in D_2O) solution via thermal annealing at 150 – 155 K in the dark. All spectra were recorded at 77 K.

Analyses of the ESR spectra of ds DNA-oliomers shown in Figure S5 using guanine neutral radical (G(–H)•) from dGuo and guanine neutral radical (G*(–H)•) from G* [G* = 8-D-dGuo, 96% deuterated]

As a second test of our analyses, we also employed the G*(-H)• spectrum from G* and G(-H)• spectrum from dGuo (96% deuterated) as shown in Figure 2A in the manuscript, to analyse the ESR spectra of one-electron oxidized HPLC-purified ds DNA-oligomers, viz. d[TG*GGCCCA]$_2$, d[TGG*GCCCA]$_2$, and d[TGGG*CCCA]$_2$. The results of these analyzes are presented in Table T1 as raw data.

Table T1. Fractional composition of G(-H)• (from dGuo) and G*(-H)• (from G*, G* = 8-D-dGuo) in one-electron oxidized d[TGGGCCCA]$_2$ by selective incorporation of G* (ca. 10% relative error)

ds DNA oligomer		Corrected[a] (Raw)Radical percentages	
Nature	Name	G*(–H)•	G(–H)•
5'-T and 3'-A	D[TG*GGCCCA]$_2$	**48** (31)	52 (69)
	D[TGG*GCCCA]$_2$	**32** (21)	68 (79)
	D[TGGG*CCCA]$_2$	**21** (14)	79 (86)

[a]These DNA-oligos have G* incorporation as *ca.* 50% (see supporting information Figure S1); thus the raw values are corrected appropriately.

Assuming the sum total of the fractional composition of G*(-H)• in these ds DNA-oligomers as 100 %, we find that the relative percentages G*(–H)• in one-electron oxidized HPLC-purified d[TG*GGCCCA]$_2$, d[TGG*GCCCA]$_2$, and d[TG*GGCCCA]$_2$ as ca. 48%, 32%, and 21%. These values of fractional compositions of G*(-H)• and G(-H)• spectra (from monomers, G* and dGuo) in the ESR spectra observed in these one-electron oxidized HPLC-purified ds DNA-oligomers are found to be agree nicely to the corresponding values (55%, 30%, and 20%) obtained using G*(-H)• spectrum from HPLC-purified d[G*CG*CG*CG*C]$_2$ when corrected for the level of deuteration and G(-H)• spectrum from d[GCGCGCGC]$_2$ (see Table 1 in the manuscript). Thus, these

analyzes clearly show that that the 5’-G is the preferential site of hole localization in a GGG sequence even with phosphorylation at the 5’-end.