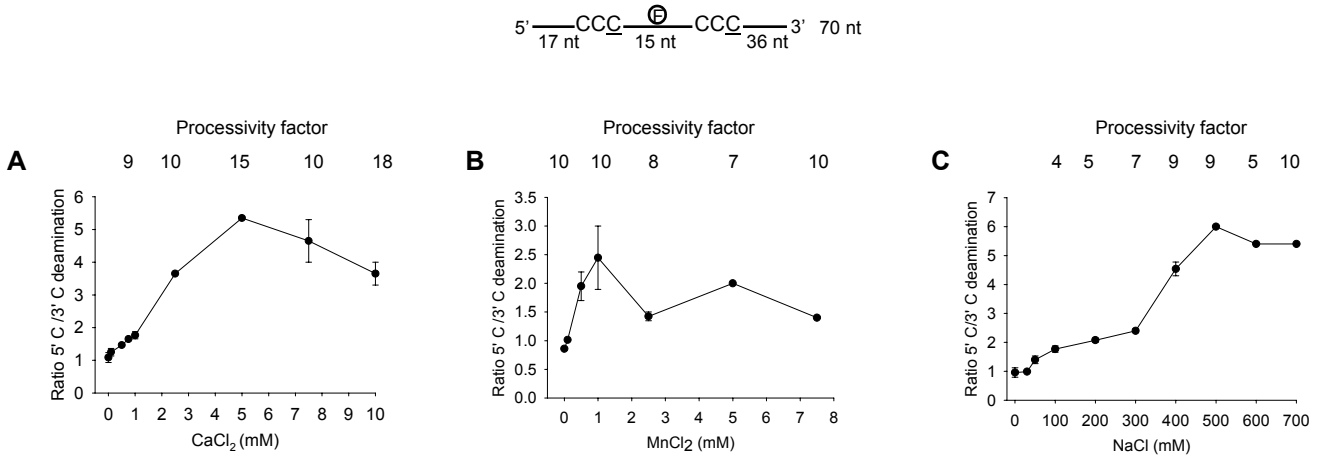
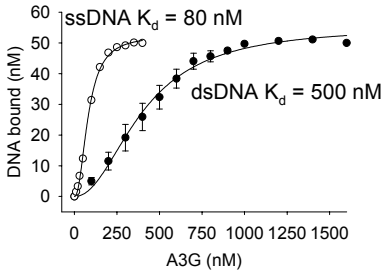


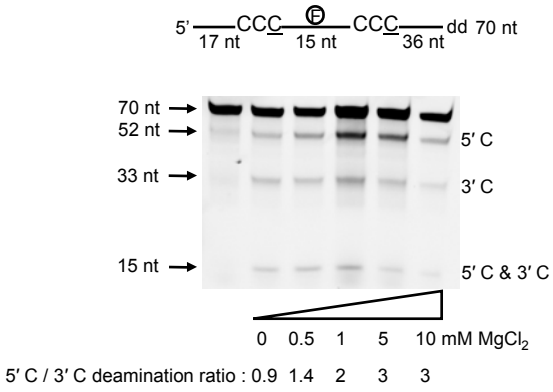
SUPPLEMENTAL FIGURE S1. **A3G deamination polarity is a general electrostatic effect.** A3G deamination of a 70 nt internally F-labeled ssDNA substrate with two CCC motifs 15 nt apart using an enzyme to DNA ratio of 1:20. The ratios of 5'C/3'C deamination, quantified from gel band intensities (not shown), are plotted as a function of, *A*, $CaCl_2$, *B*, $MnCl_2$, or, *C*, NaCl concentration. Processivity factors are shown above the graphs for select salt concentrations. Error bars show ± 1 SE from three independent experiments.

Supplemental Figure S1 - Goodman



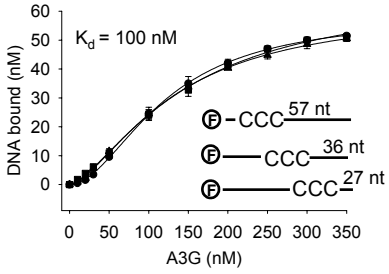
SUPPLEMENTAL FIGURE S2. **A3G binds dsDNA with much less affinity than ssDNA.** Binding of A3G to 50 nM of a 30 nt ssDNA (○) and dsDNA (●). A3G binds with an apparent K_d of 80 nM to ssDNA and 500 nM to dsDNA.

Supplemental Figure S2 - Goodman



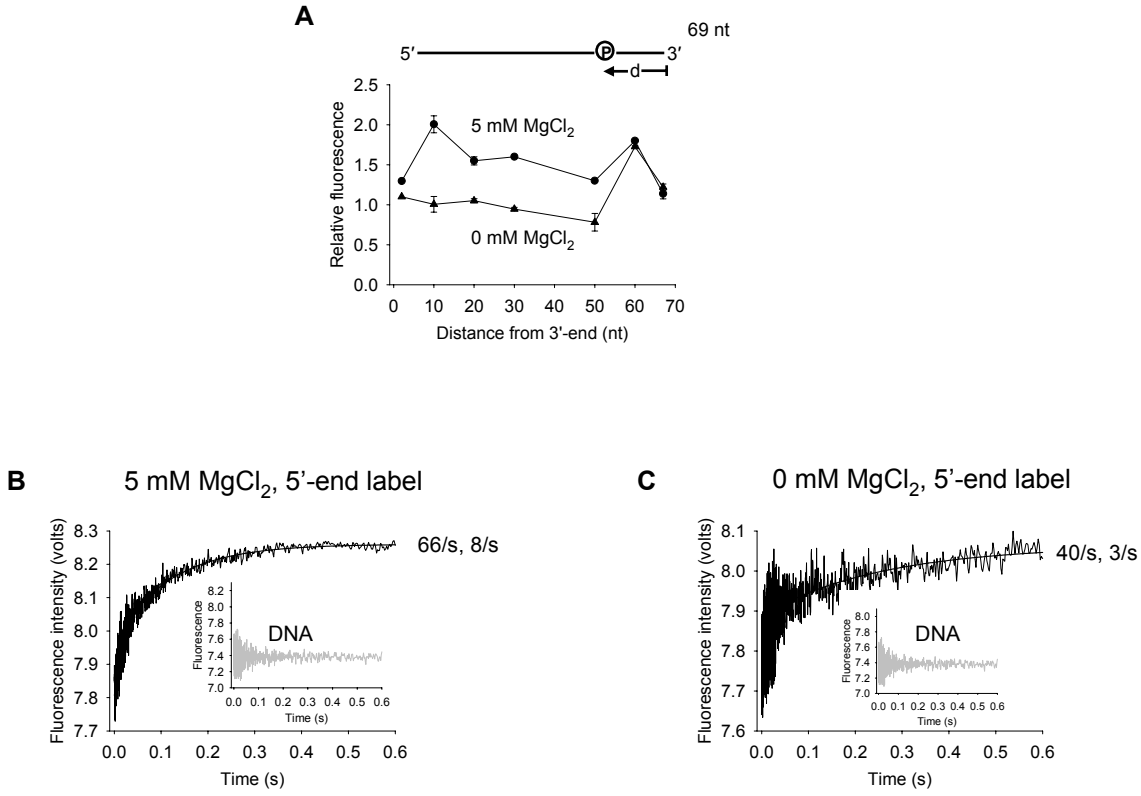
SUPPLEMENTAL FIGURE S3. **The deamination polarity of A3G is not affected by the 3'hydroxyl.** A3G directional bias was characterized on an F-labeled 3'-dideoxy (dd) ssDNA substrate (70 nt) containing two CCC motifs 15 nt apart using an enzyme to DNA ratio of 1:20. Directional bias is measured by the 5'C/3'C deamination ratio (below gel).

Supplemental Figure S3 - Goodman



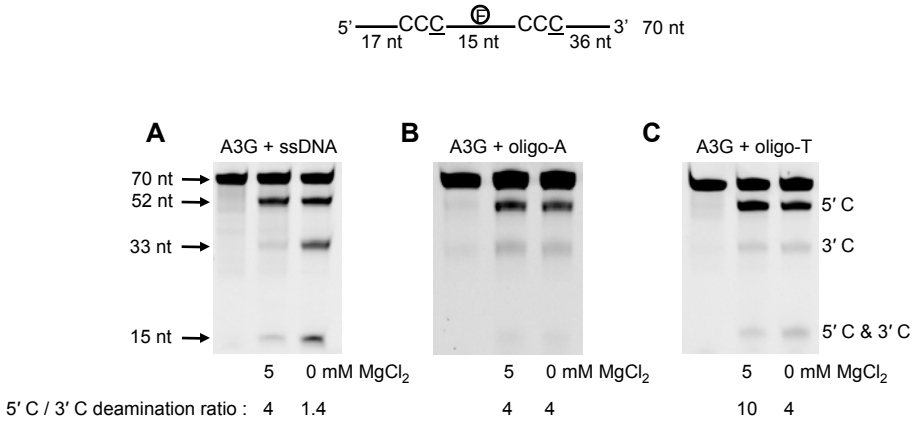
SUPPLEMENTAL FIGURE S4. **A3G binds ssDNA with the same affinity regardless of the CCC motif location.** Binding of A3G to 50 nM of a 69 nt ssDNA with a CCC motif 57- (●), 36- (■), or 27- (▲) nt from the 3'-end has an apparent K_d of 100 nM.

Supplemental Figure S4 - Goodman



SUPPLEMENTAL FIGURE S5. **Fluorescence change of pyrrolo-C labeled ssDNA incubated with A3G.** *A*, steady state fluorescence change caused by A3G binding pyrrolo-C labeled ssDNA (69 nt). Fluorescence emissions of 100 nM pyrrolo-C labeled DNA were monitored after the addition of 1 μM A3G. Emissions are plotted as fluorescence value relative to DNA alone against distance of pyrrolo-C label from the 3'-end. Emissions were determined in the presence of 5 mM $MgCl_2$ (●) and its absence (▲). *B-C*, presteady state changes in fluorescence of 100 nM pyrrolo-C labeled ssDNA (*inset graph*) upon the addition of 1 μM A3G (*black line*) were monitored as a function of time. The pyrrolo-C label was placed 10 nt from the 5'-end. The fluorescence change was fit to a double exponential equation. *B*, with 5 mM $MgCl_2$ the fluorescence change of pyrrolo-C ssDNA began with a rapid rate of 66±6/s (38% amplitude) and was followed by a slower rate of 8±0.2/s (62% amplitude). *C*, in the absence of $MgCl_2$ the fluorescence change of pyrrolo-C ssDNA began with a rapid rate of 40±7/s (44% amplitude) and was followed by a slower rate of 3±0.6 /s (56% amplitude).

Supplemental Figure S5 - Goodman



SUPPLEMENTAL FIGURE S6. **A3G deamination of ssDNA, oligo-A, and oligo-T substrates at an enzyme to DNA ratio of 1:2.** A3G was incubated with a 70 nt internally F-labelled ssDNA substrate with two CCC motifs 15 nt apart and at an enzyme to DNA ratio which corresponds with the conditions for AFM (Figure 6, enzyme to DNA ratio of 1:2). Directional bias as measured by the 5'C/3'C deamination ratio (below gel) was calculated in the presence and absence of 5 mM $MgCl_2$ for A3G deamination on, *A*, ssDNA, *B*, oligo-A, and *C*, oligo-T.

Supplemental Figure S6 - Goodman

SUPPLEMENTAL TABLE S1. **DNA substrates.**
FdT = Fluorescein dT; PdC = Pyrrolo-dC

NAME	SEQUENCE
3P3	GAA GAG TAA TAT AAG AAA GAG AAA TGT GAT GAA AGT TGA TGA GAT TAA TGT GTG TAA ATA AGA CCC AAA
3P9	GAA GAG GAA TGT AAG AAA GAG AAA TGT GAG GAA AGT TGA TGA TGT TAA TGT GTG AGA CCC AAA TAA ATA
3P21	GAA GAG GAA TGT AAG AAA GAG AAA TGT GAG GAA AGT TGA TGA AGA CCC AAA TGT TAA TGT GTG TAA ATA
3P27	GAA GAG TAA TAT AAG AAA GAG AAA TGT GAT GAA AGT AGA CCC AAA TGA GTA TGT TAA TGT GTG TAA ATA
3P29	GAA GAG GAA GGA AGA AAG AGA AAG TGA AGA AAG GAG ACC CAA AGT TGA GGA TGT TAA TAT GTG TAA ATA
3P31	GAA GAG GAA GTG AAG AAA GAG AAA GTG GAG AAA GAC CCA AAA GTG TGA GGA TGT TAA TAT GTG TAA ATA
3P33	GAA GAG GAA TGT AAG AAA GAG AAA TGT GAG AGA CCC AAA GAA AGA TGA TGA TGT TAA TGT GTG TAA ATA
3P36	GCT AGT TTA GTC GTT TGT ATA GAA TTA ATA CCC AAA GAA GTG TAT GTA ATT GTT ATG ATA AGA TTG AAA
3P48	GAA GAG TAA TAT AAG AGA CCC AAA AAA GAG AAA TGT GAG GAA AGT TGA GGA GGT TAA TGT GTG TAA ATA
3P57	GAA GAG AGA CCC AAA GAA TAT AAG AAA GAG AAA TGT GAG GAA AGG TGA GGA GGT TAA TGT GTG TAA ATA
3P63	AGA CCC AAA GAA GAG TAA TAT AAG AAA GAG AAA TGT GAG GAA AGT TGA GGA GGT TAA TGT GTG TAA ATA
20 nt dsDNA	CTT TCA TAC ACA TAT ATC AC
L15 (AFM & deamination)	AAA GAG AAA GTG AGA CCC AAA GAA (FdT)GA AGA CCC AAA TGT TAG AAT TGT TAA TGT GTG TGA TGA TGT TGA
L15 OLIGO-A (AFM & deamination)	AAA AAA AAA AAA AAA CCC AAA AAA A(FdT)A AAA ACC CAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA A
L15 OLIGO-T (AFM & deamination)	TTT TTT TTT TTT TTT CCC TTT TTT T(FdT)T TTT TCC CTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT T
L15 OLIGO-A (AID)	AAA AAA AAA AAA AAA AGC AAA AAA A(FdT)A AAA AAG CAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA A
L30	AAA GAG AAA GTG ATA CCC AAA GAG TAA AGT (FdT)AG ATA GAG AGT GAT ACC CAA AGA GTA AAG TTA GTA AGA TGT GTA AGT ATG TTA A
L60	GAA TAT ATG TTG AGA CCC AAA GTA ATG AGA GAT TGA (FdT)TA GAT GAG TGT AAT GTG ATA TAT GTG TAT GAA AGA TAT AAG ACC CAA AGA GTA AAG TTG TTA ATG TGT GTA GAT ATG TTA A
30 nt (binding)	TTA GAT GAG TGT AA(FdT) GTG ATA TAT GTG TAT

3P36 (binding)	GC(FdT) AGT TTA GTC GTT TGT ATA GAA TTA ATA CCC AAA GAA GTG TAT GTA ATT GTT ATG ATA AGA TTG AAA
3P57 (binding)	GAA GAG AGA CCC AAA GAA TAT AAG AAA GAG AAA TGT GAG GAA AGG TGA GGA GGT TAA TGT GTG TAA A(FdT)A
3P27 (binding)	GAA GAG TAA TAT AAG AAA GAG AAA TGT GAT GAA AGT AGA CCC AAA TGA GTA TGT TAA TGT GTG TAA A(FdT)A
PYRROLO-C-1	GC(PdC) AGT TTA GTC GTT TGT ATA GAA TTA ATA CCC AAA GAA GTG TAT GTA ATT GTT ATG ATA AGA TTG AAA
PYRROLO-C-2	GCT AGT TTA G(PdC)C GTT TGT ATA GAA TTA ATA CCC AAA GAA GTG TAT GTA ATT GTT ATG ATA AGA TTG AAA
PYRROLO-C-3	GCT AGT TTA GTC GTT TGT A(PdC)A GAA TTA ATA CCC AAA GAA GTG TAT GTA ATT GTT ATG ATA AGA TTG AAA
PYRROLO-C-4	GCT AGT TTA GTC GTT TGT ATA GAA TTA ATA CCC AA(PdC) GAA GTG TAT GTA ATT GTT ATG ATA AGA TTG TAA
PYRROLO-C-5	GCT AGT TTA GTC GTT TGT ATA GAA TTA ATA CCC AAA GAA GTG TAT GTA A(PdC)T GTT ATG ATA AGA TTG TAA
PYRROLO-C-6	GCT AGT TTA GTC GTT TGT ATA GAA TTA ATA CCC AAA GAA GTG TAT GTA ATT GTT ATG A(PdC)A AGA TTG tAA
PYRROLO-C-7	GCT AGT TTA GTC GTT TGT ATA GAA TTA ATA CCC AAA GAA GTG TAT GTA ATT GTT ATG ATA AGA TTG (PdC)AA

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Steady state rotational anisotropy and fluorescence assays–A3G-DNA binding were monitored by changes in steady state fluorescence depolarization (rotational anisotropy). Reaction mixtures (70 µl), containing an F-labeled DNA (50 nM) in buffer (50 mM HEPES, pH 7.3, 1 mM DTT and 5 mM $MgCl_2$) and varying concentration of 0 to 1600 nM A3G, were incubated at 37 °C. Rotational anisotropy was measured using a QuantaMaster QM-1 fluorometer (Photon Technology International) with a single emission channel. Samples were excited with vertically polarized light at 494 nm and both vertical and horizontal emission was monitored at 520 nm (4 nm band pass). The fraction of DNA bound to protein were determined as described previously [1].

Experiments using pyrrolo-C labeled DNA and A3G were performed by exciting 100 nM pyrrolo-C DNA at 345nm (6 nm band pass) in buffer (50 mM HEPES, pH 7.3, 1 mM DTT and 5 mM $MgCl_2$ where indicated) and monitoring the emissions from 410 to 510 nm with an emissions scan (0.1 s integration time). The 450 nm emissions peak was used to calculate the relative fluorescence emissions of A3G-DNA compared to DNA alone. A3G was added to a final concentration of 1 µM.

REFERENCES

1. Bertram, J.G., Bloom, L.B., O'Donnell, M., and Goodman, M.F. (2004) *J Biol. Chem.* **279**, 33047-33050.