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High Specificity and High Spatial Restriction of Self-Biotinylation by DNA and RNA G-Quadruplexes Complexed with Heme.

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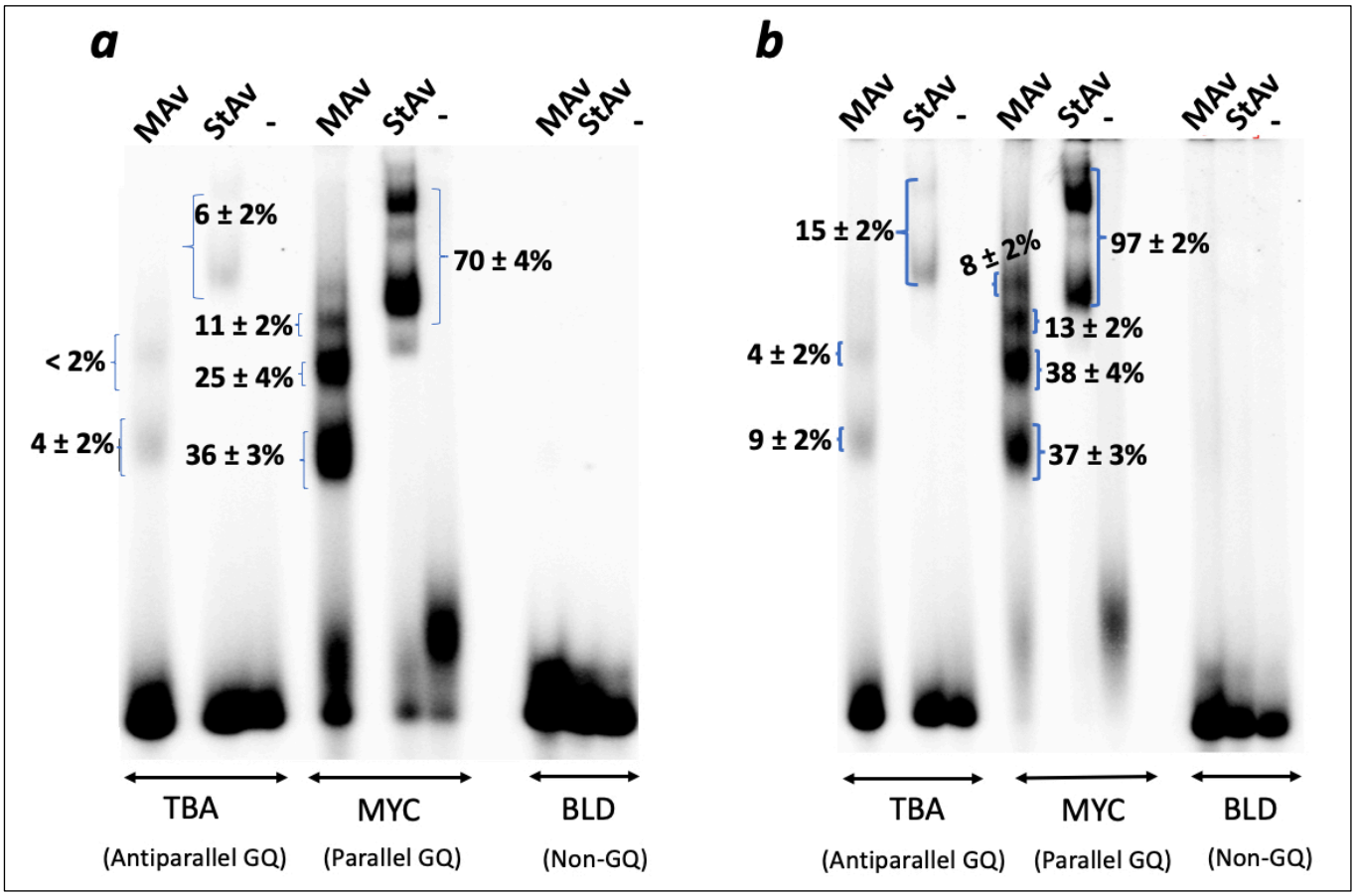
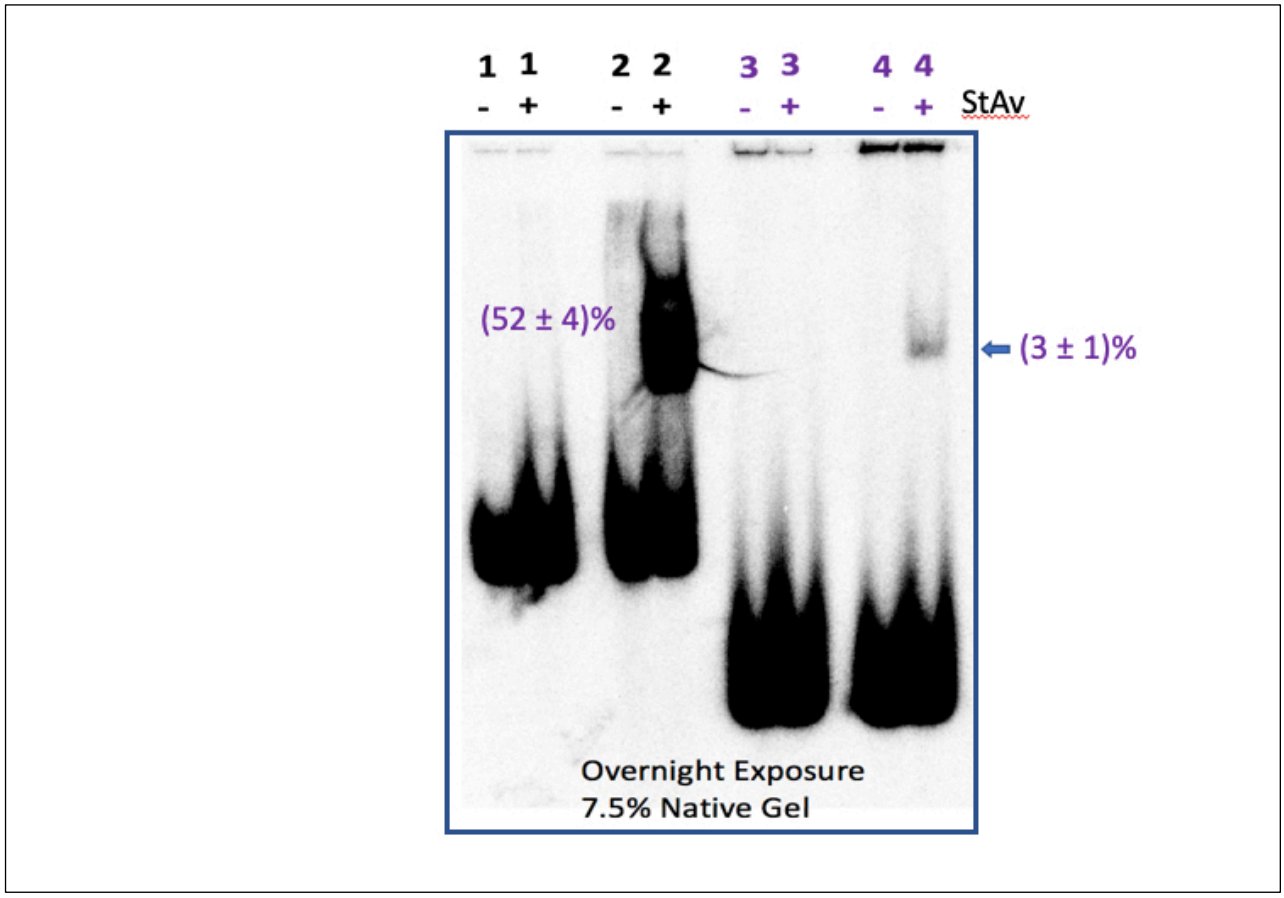
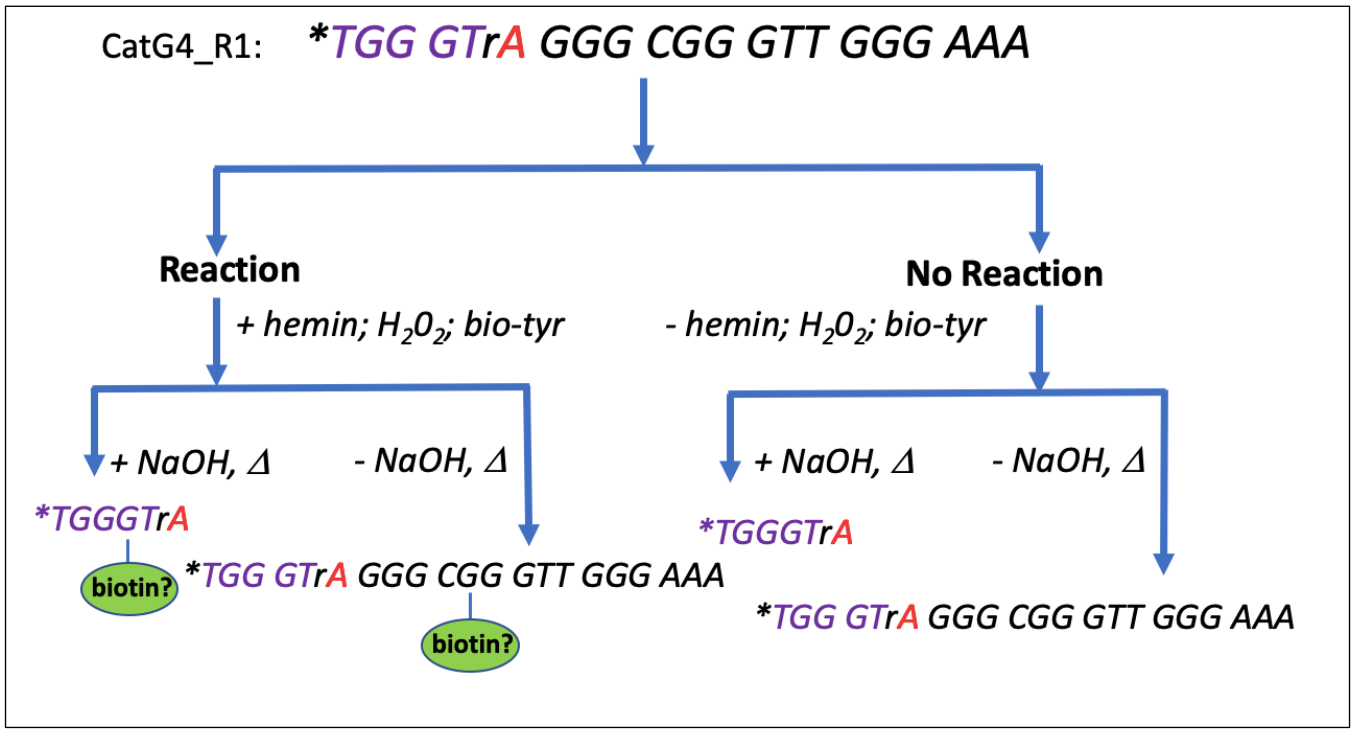
SUPPORTING INFORMATION



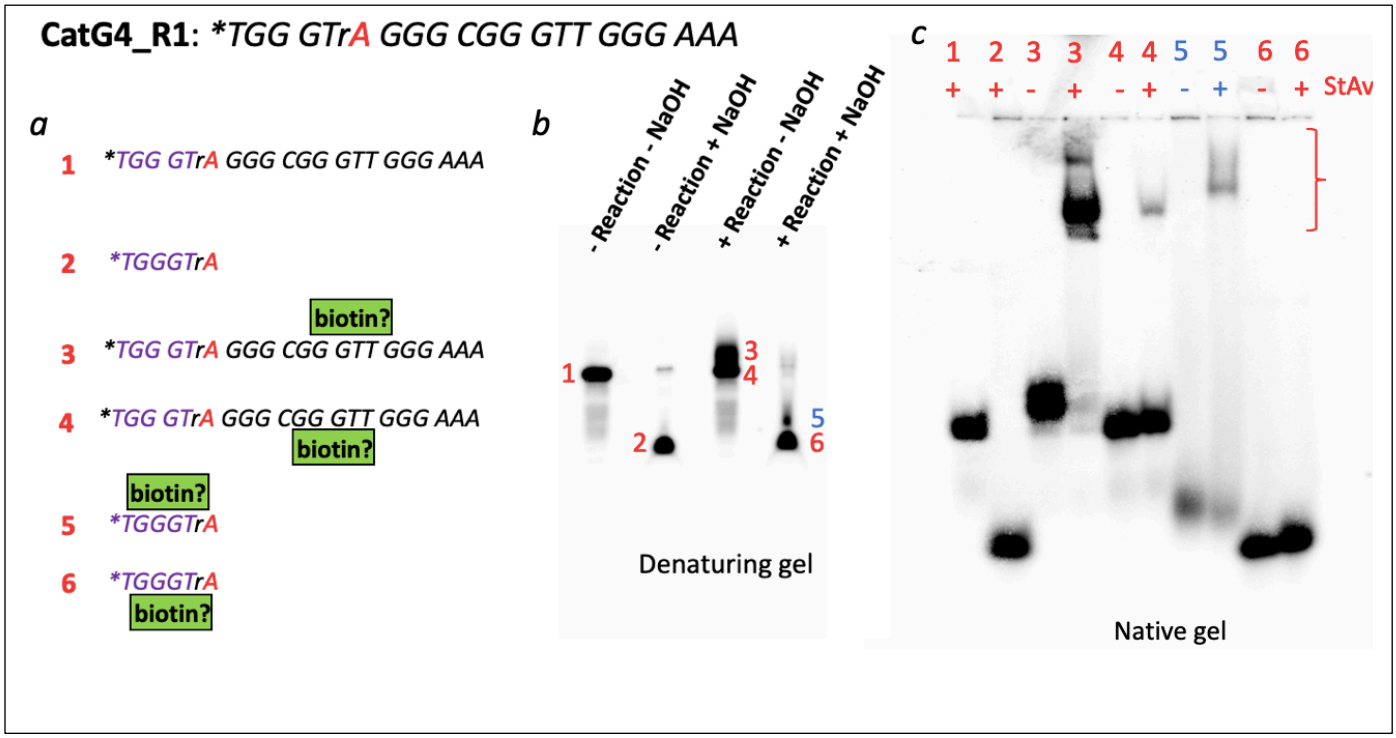
Supporting Figure S1: The consistency of GQ biotinylation levels in two independent experiments using the standard 5 μM heme (panel ***a***) and of biotinylation with a higher (50 μM) heme concentration (panel ***b***). For each of the above, two independent experiments were carried out and the error bars represent deviations from the mean. "MAv" refers to monoavidin, and "StAv" streptavidin. "-" above a gel lane indiates that neither streptavidin or monoavidin had been added to that DNA sample.



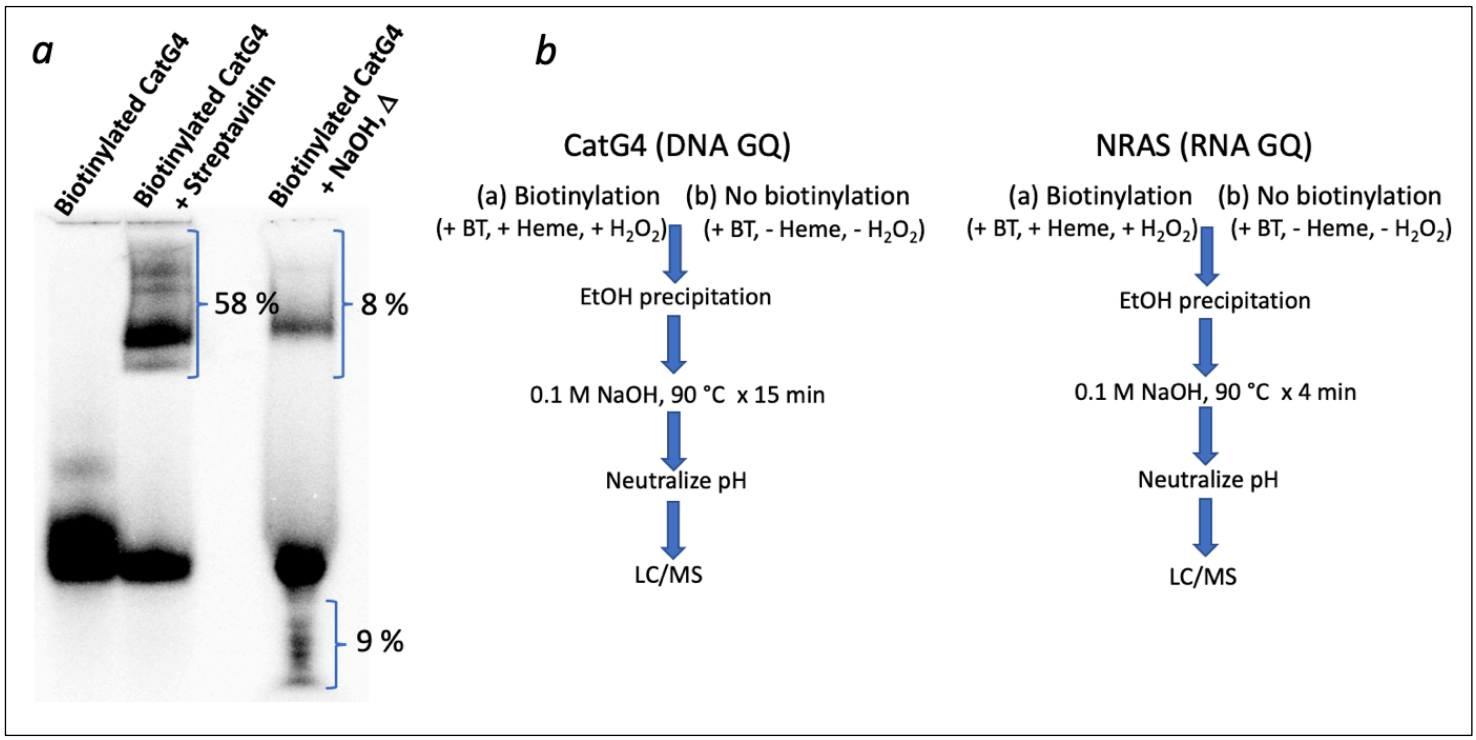
Supporting Figure S2: A longer exposure of the gel shown in **Figure 5** ***b***.



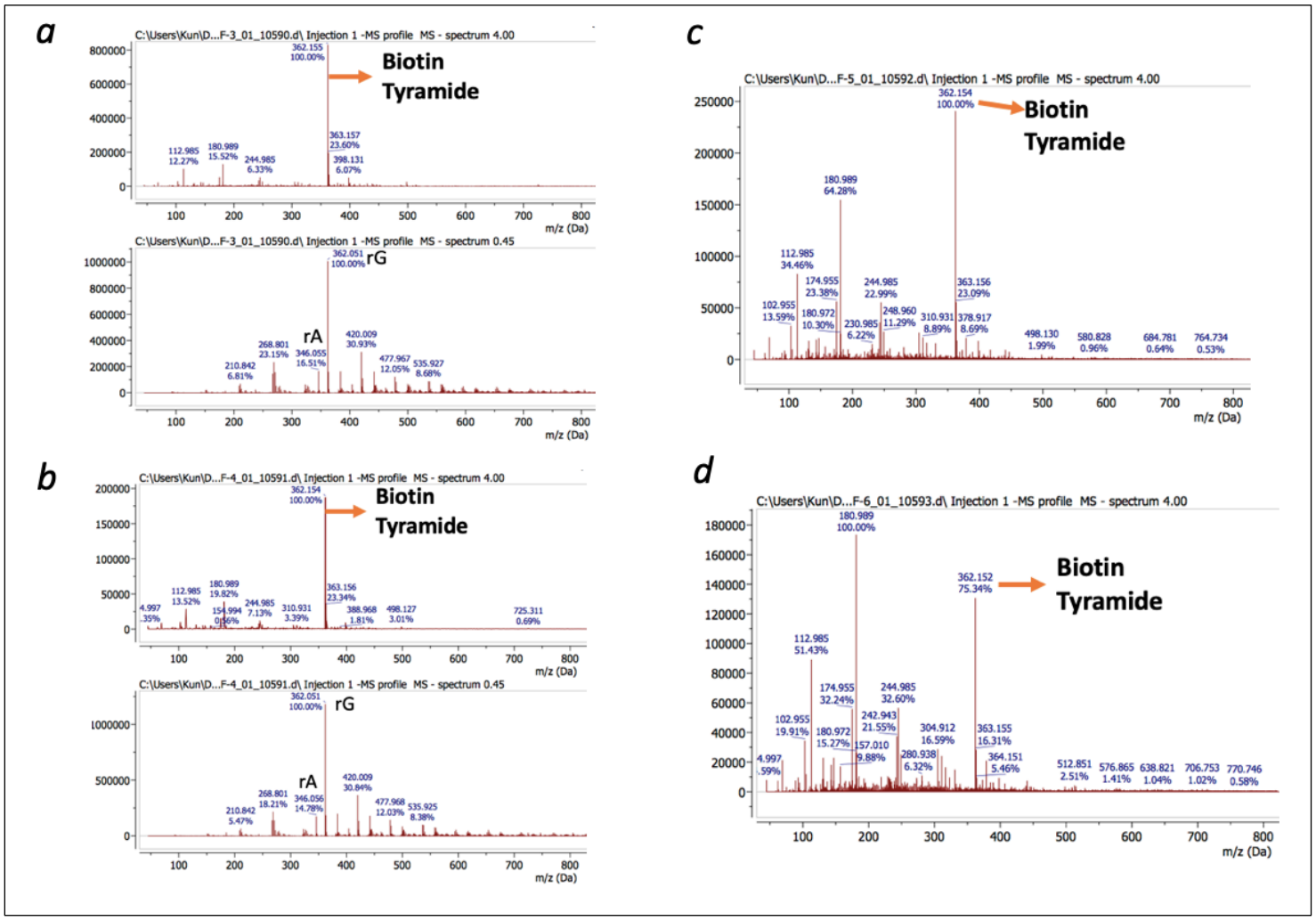
Supporting Figure S3: Schematic showing the experimental approach to determining the distribution of appended biotins in the GQ formed by "CatG4".



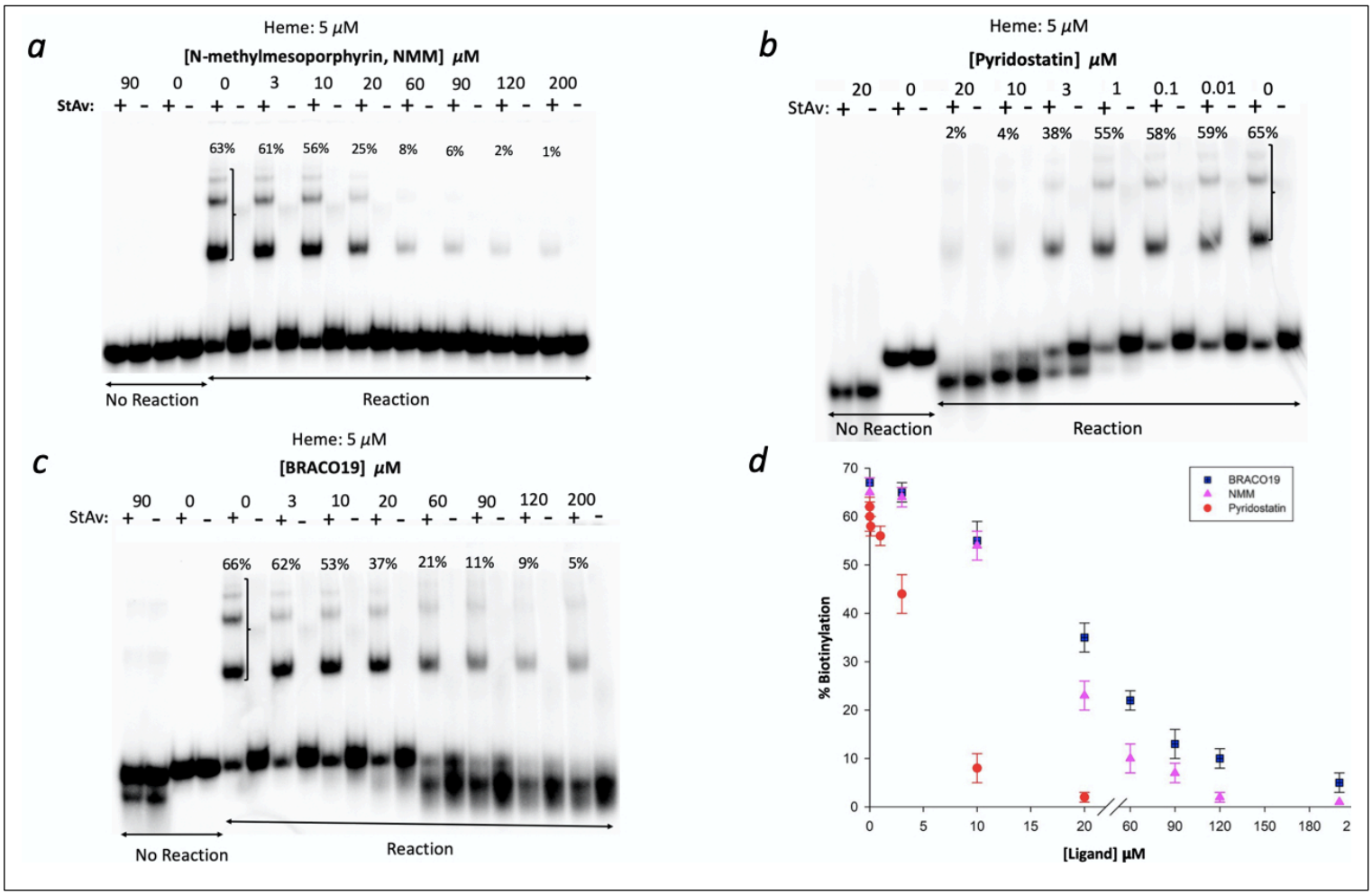
Supporting Figure S4: Determination of the extent of biotinylation within two component pieces (sequences 1 and 2 shown in ***a***) of the oligonucleotide "G4_R1" (or CatG4_R1). ***b***: A denaturing gel showing G4_R1 treated with hemin, BT, and H_2O_2 ("Reaction") or not treated, either cleaved in two by heating with NaOH (or not). The six ^{32}P-labeled fragments, 1-6, were purified, and then mixed with soluble streptavidin (StAv). These were then run on a native gel (***c***). The red bracket in ***c*** shows the streptavidin-retarded bands.



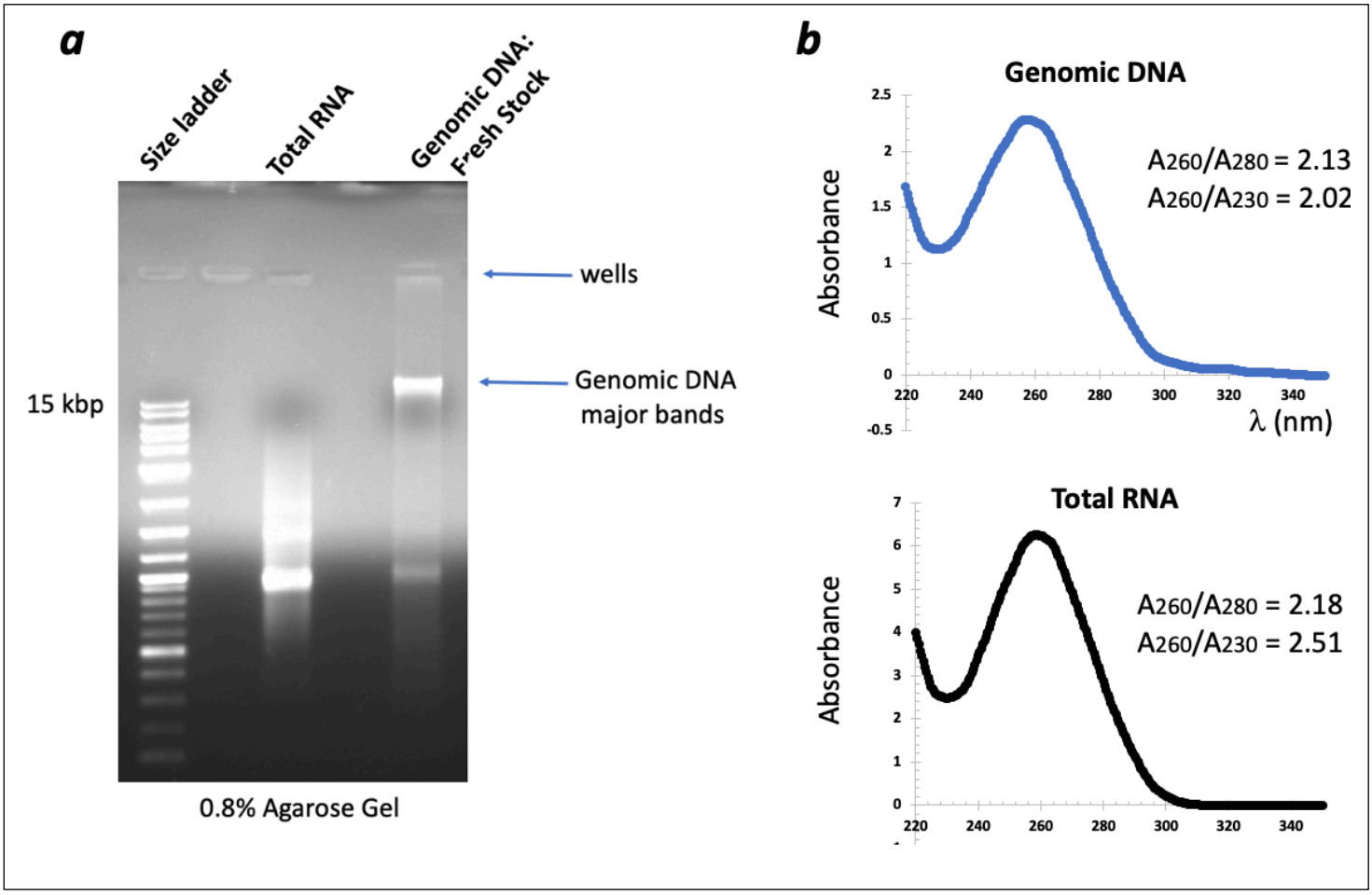
Supporting Figure S5 ***a:*** Effect of 0.1 M NaOH and heating upon the biotinylated GQ, CatG4. Shown is a 7.5% native (non-denaturing) gel, whose first two lanes on the left show, respectively, biotinylated CatG4 (generated by the standard heme/H_2O_2/biotin-tyramide reaction) without and with streptavidin added prior to loading in the gel. From the second lane, it can be seen that 58% of the DNA has been biotinylated (retarded by streptavidin binding). Treatment with 0.1 M NaOH at 90°C (lane at far right), however, shows that such a treatment leaves only 8% of the DNA biotinylated. A portion of the DNA (9%) is also shown to be broken down in size by this treatment. ***b***: A reaction scheme for an LC/MS-based scheme for looking at whether biotin tyramide (or a related product) is in fact released from biotinylated DNA (CatG4) and RNA (NRAS) GQs following the hot base treatment. The MS data are shown in Supporting Figure S6.



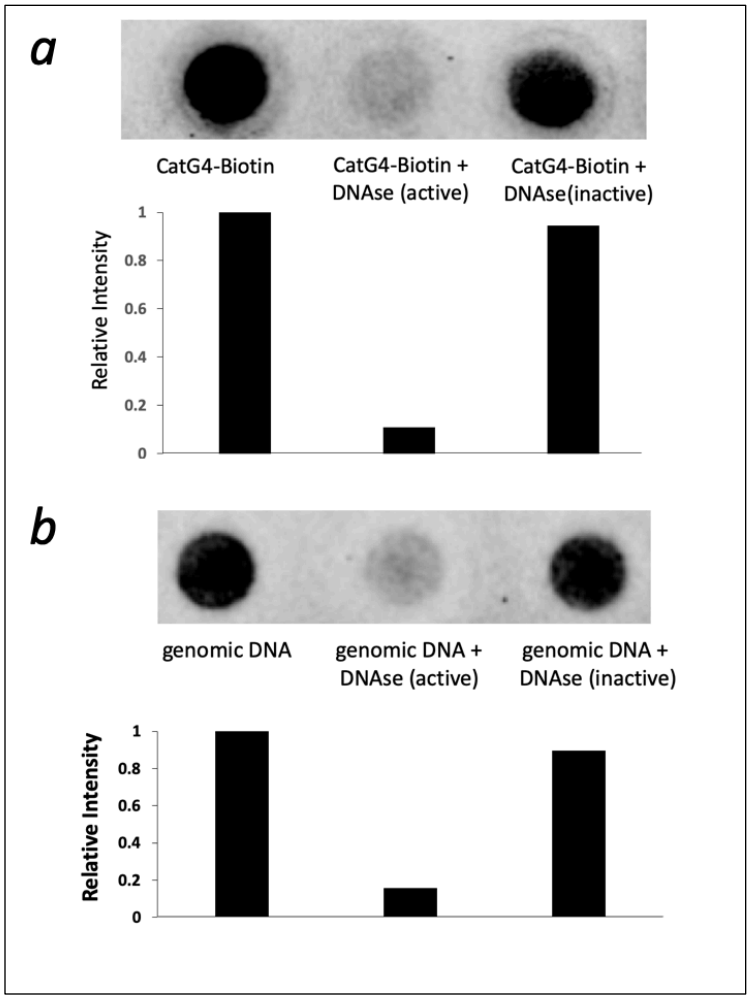
Supporting Figure S6: LC- ESI Mass spectrometry analysis of solutions (***a***) "NRAS RNA biotinylation"; (***b***) "NRAS RNA no biotinylation"; (***c***) "CatG4 DNA biotinylation"; (***d***); "CatG4 DNA no biotinylation". ***a*** and ***b*** show both biotin-tyramide mass and abundances, as well as the mass and abundances of rGMP and rAMP generated from alkaline hydrolysis of the NRAS RNA (rCMP and rUMP are very small peaks). ***c*** and ***d*** show biotin-tyramide mass and abundances.



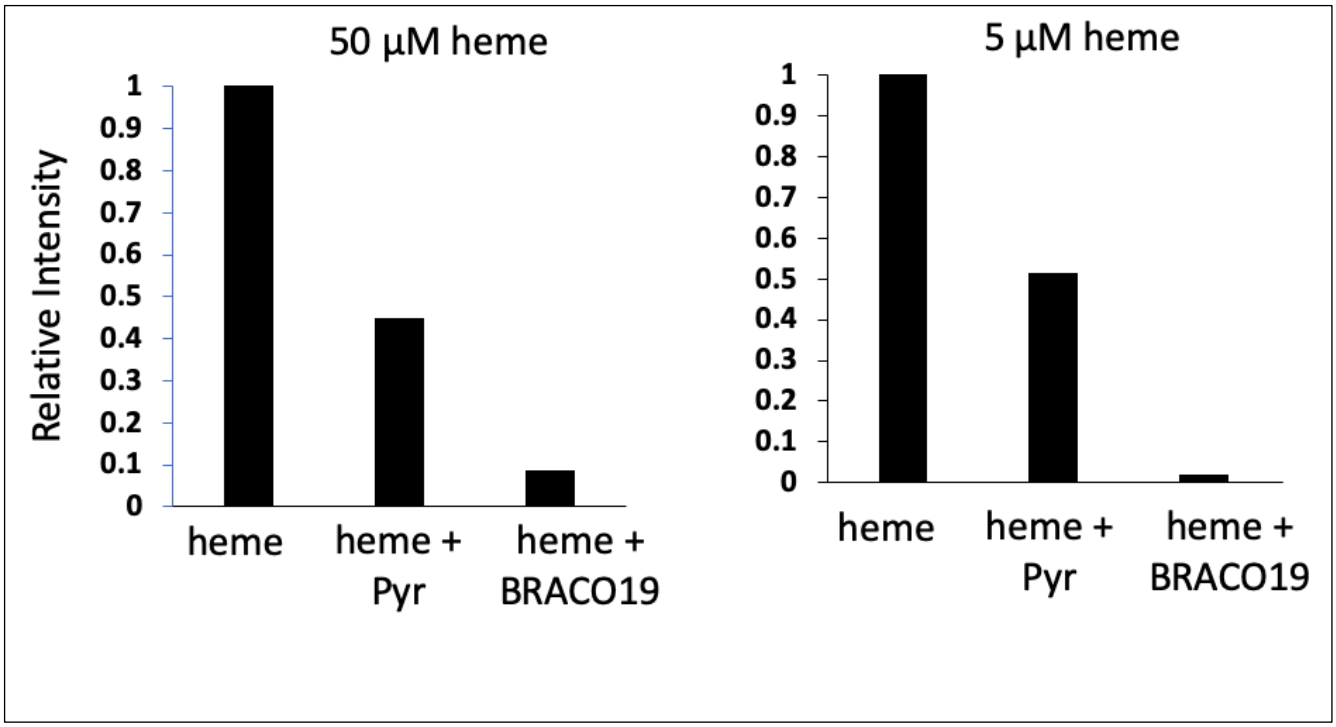
Supporting Figure S7: Impact of the titration of three non-heme GQ-binding ligands upon the GQ self-biotinylation reaction. Titration of 0-200 μM N-methylmesoporphyrin (NMM) (***a***); 0-20 μM Pyridostatin (***b***); and 0-200 μM BRACO19 (***c***) to 5 μM heme•"CatG4-ext" complex results in gradual abolition of GQ self-biotinylation. In all three panels, the bracket indicates streptavidin (StAv)-retarded DNA species. Panel ***d*** plots percent GQ biotinylation measured in response to concentration of added ligand (here the data represent quantitation of three independent experiments carried out with each of the GQ-binding ligands, NMM, Pyridostatin, and BRACO19. The error bars indicated represent one standard deviation from the mean.



Supporting Figure S8: Examination of the purity of biotinylated DNA and RNA extracted using commercial kits from treated Drosophila embryo salivary glands. Panel ***a*****:** A 0.8% agarose gel showing (left) total RNA and (right) genomic DNA purified from the glands. Panel ***b***: UV-Vis spectra of the purified total RNA and genomic DNA. The A_{260}/A_{280} ratios, which are > 1.8, indicate high purities (free of proteins) for both DNA and RNA preparations.



Supporting Figure S9: Evidence that the biotin detected, using streptavidin-HRP-generated chemiluminescence output signal, is from biotinylated DNA (and not biotinylated proteins) extracted from treated *Drosophila* embryonic salivary glands. Panel ***a***: A chemically biotinylated oligonucleotide (3'-biotinyl CatG4) was used as a positive control, spotted by itself (left); treated with DNAase I (centre); and with heat-deactivated DNAase I (right). Panel ***b***: the same analysis as in panel ***a***, except on total genomic DNA extracted from *Drosophila* 3rd instar larval salivary glands.



Supporting Figure S10: Quantiation of the chemiluminescence output data from the biotinylated total RNA blots shown in **Figure 9d**.