Supplemental Material for:

Thermodynamics of Ligand Binding to a Heterogeneous RNA Population in the Malachite Green Aptamer

Joshua E. Sokoloski[1], Sarah E. Dombrowski[1,2], and Philip C. Bevilacqua[1]*

[1]Department of Chemistry, The Pennsylvania State University, University Park, PA 16802.

[2]Present Address: School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15213.

*Author to whom correspondence should be addressed: Phone: (814) 863-3812; Fax: (814) 865-2927; e-mail: pcb5@psu.edu.

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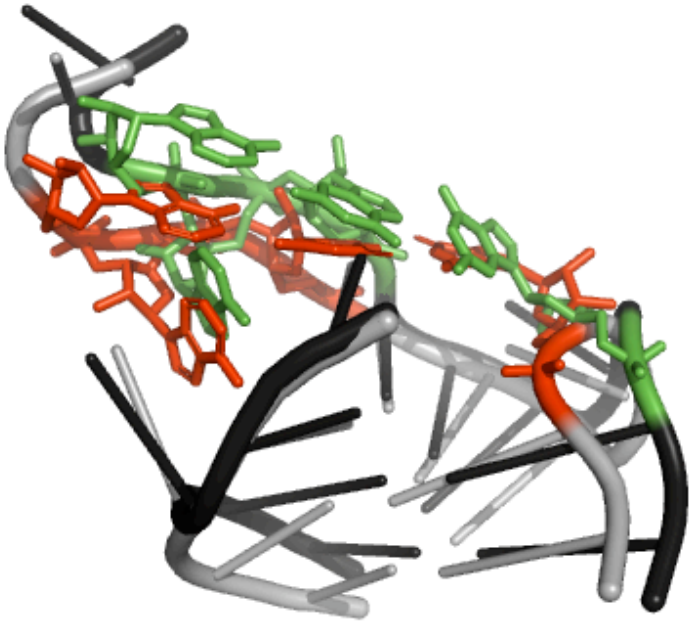

Figure S1: Overlay of the binding pocket of the MG-bound[1] (green) and TMR-bound[2] (red) structures, with the ligand deleted showing differences in base orientations among the *syn* bases. Bases that are *syn* in the MG complex are G24, G29, and A31, and bases that are *syn* in the TMR-bound complex are G24 and A30. Bases 24, 29, 30, and 31 are colored for each ligand.

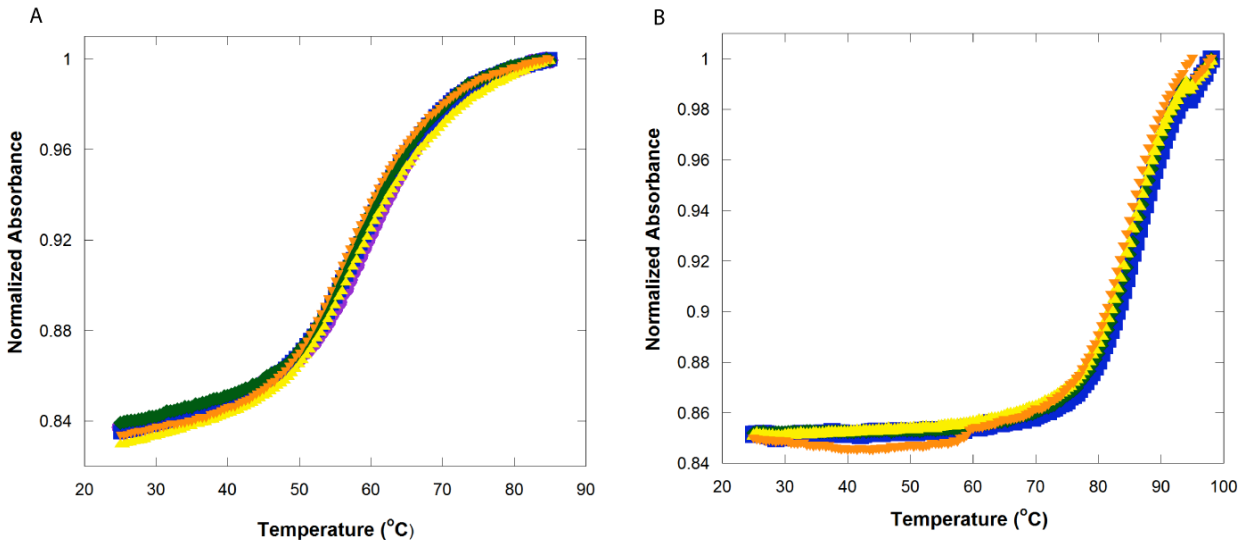

Figure S2: Concentration-dependence of thermal denaturation of MG aptamer monitored by UV absorbance. Thermal denaturation of MG aptamer at 260 nm in **A.)** The absence of divalent metal ions and **B.)** The presence of 10 mM Mg^{2+}, with 1.3 to 20 μM RNA aptamer. Buffer conditions for both panels were 10 mM sodium cacodylate (pH 5.8), 10 mM KCl. Curves are colored as follows, with concentrations of MG aptamer provided: Purple (1.3 μM), Blue (2.5 μM), Green (5 μM), Yellow (10 μM), Orange (20 μM).

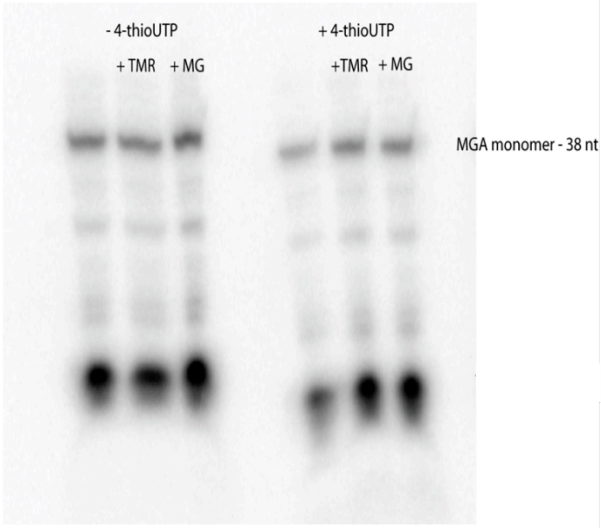

Figure S3: 4-thioUTP UV crosslinking experiment. The group of three lanes on the left is the wild-type MG aptamer under ITC conditions. The group of three lanes on the right consists of MG aptamer ('MGA') that had 4-thioUTP incorporated during transcription. Both sets of samples were exposed to 312 nm light for 30 min. TMR and MG ligands were added to the labeled lanes to test if the ligand influences oligomerization. No evidence of dimers or higher–order species was observed for any conditions in the 10% denaturing polyacrylamide gel. The bands on the bottom of the lanes are unincorporated α-labeled GTP.

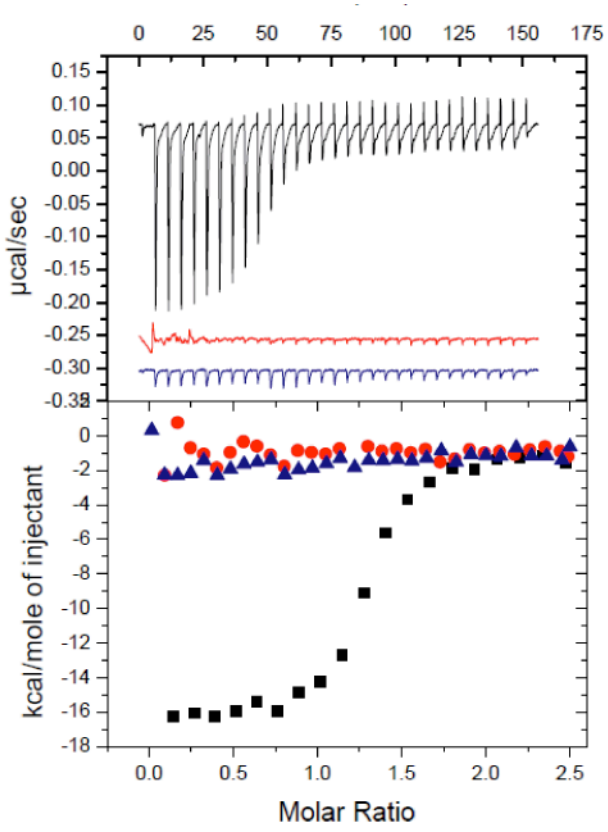

Figure S4: MG aptamer vs tRNA control data for TMR binding. The top panel shows raw data for TMR titrated into the MG aptamer (black line), TMR titrated into tRNA (blue line), and TMR titrated into buffer (red line). The bottom panel shows the integrated enthalpy data for TMR titrated into the MG aptamer (black squares), TMR titrated into tRNA (blue triangles), and TMR titrated into buffer (red circles).

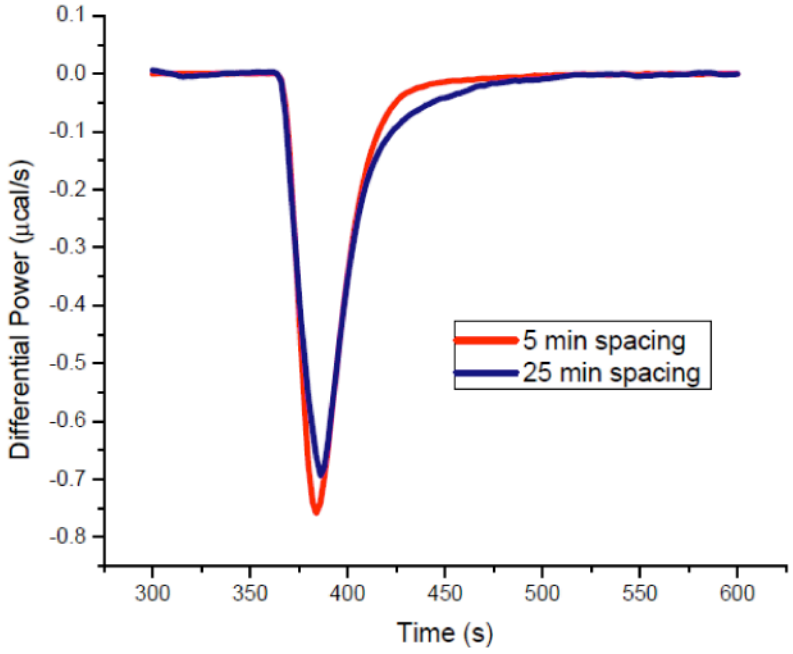

Figure S5. Overlay of two typical injections for MG titration into MG aptamer showing that the injection peak returns to baseline in less than 2 min for both the 5 min (red line) and 25 min (blue line) spacings. No change in signal was detected for the additional ~20 min in the 25 min spacing (not shown). These data were collected at 25 ^{o}C.

References

(1) Flinders, J., DeFina, S. C., Brackett, D. M., Baugh, C., Wilson, C., and Dieckmann, T. (2004) Recognition of planar and nonplanar ligands in the malachite green-RNA aptamer complex. *Chembiochem 5*, 62-72.

(2) Baugh, C., Grate, D., and Wilson, C. (2000) 2.8 A crystal structure of the malachite green aptamer. *J. Mol. Biol. 301*, 117-128.