Supplementary Information for:

Counting the Number of Magnesium Ions Bound to The Surface-immobilized Thymine Oligonucleotides That Comprise Spherical Nucleic Acids
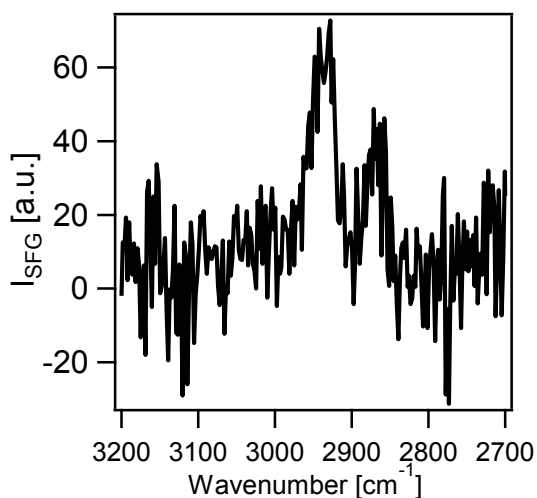
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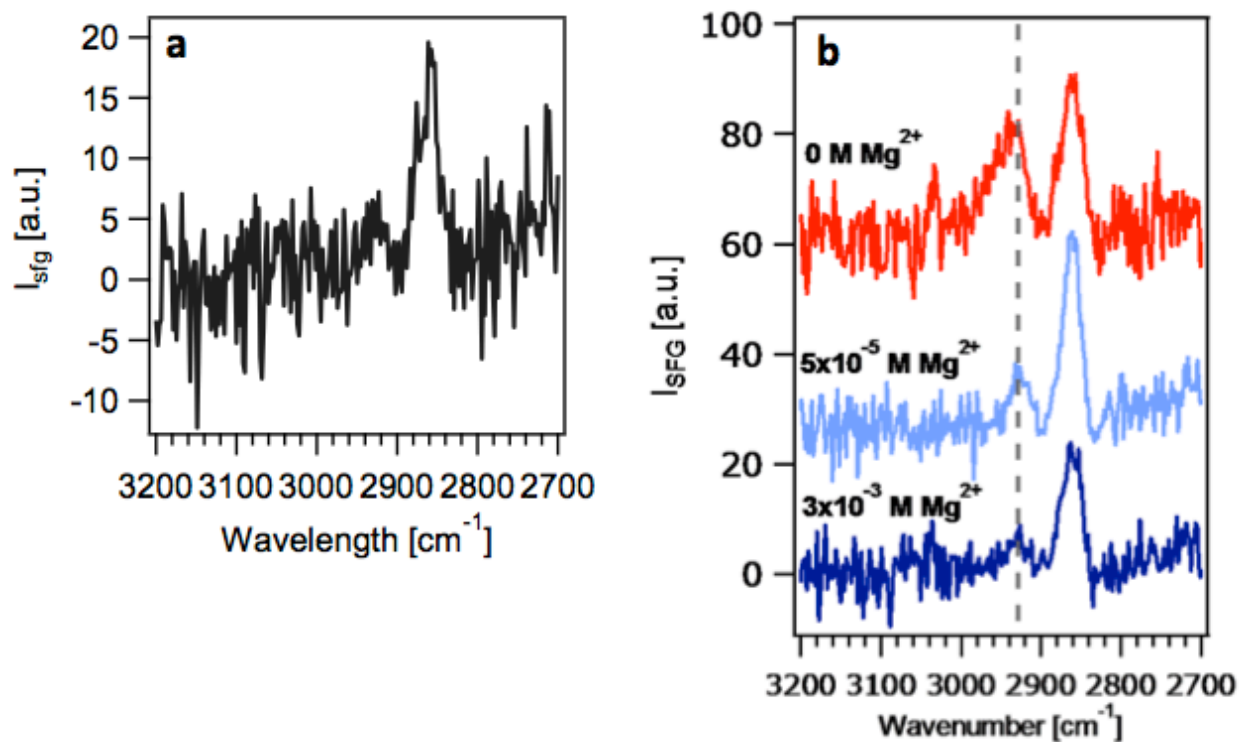[1]Department of Chemistry, Northwestern University, Evanston, IL, 60208

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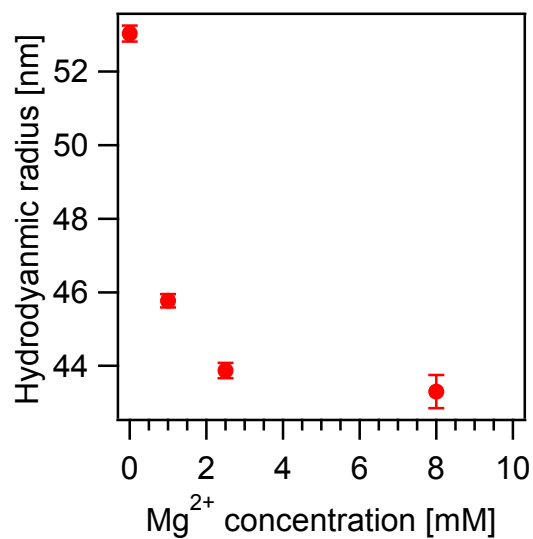
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The DNA sequences used for the nanoparticle assay include the sequence $^{3'}HS(C_3H_6)$-$T40^{5'}$ which was directly attached to the gold nanoparticle, and the self-complementary linker $^{5'}A20$-$GCGC^{3'}$.
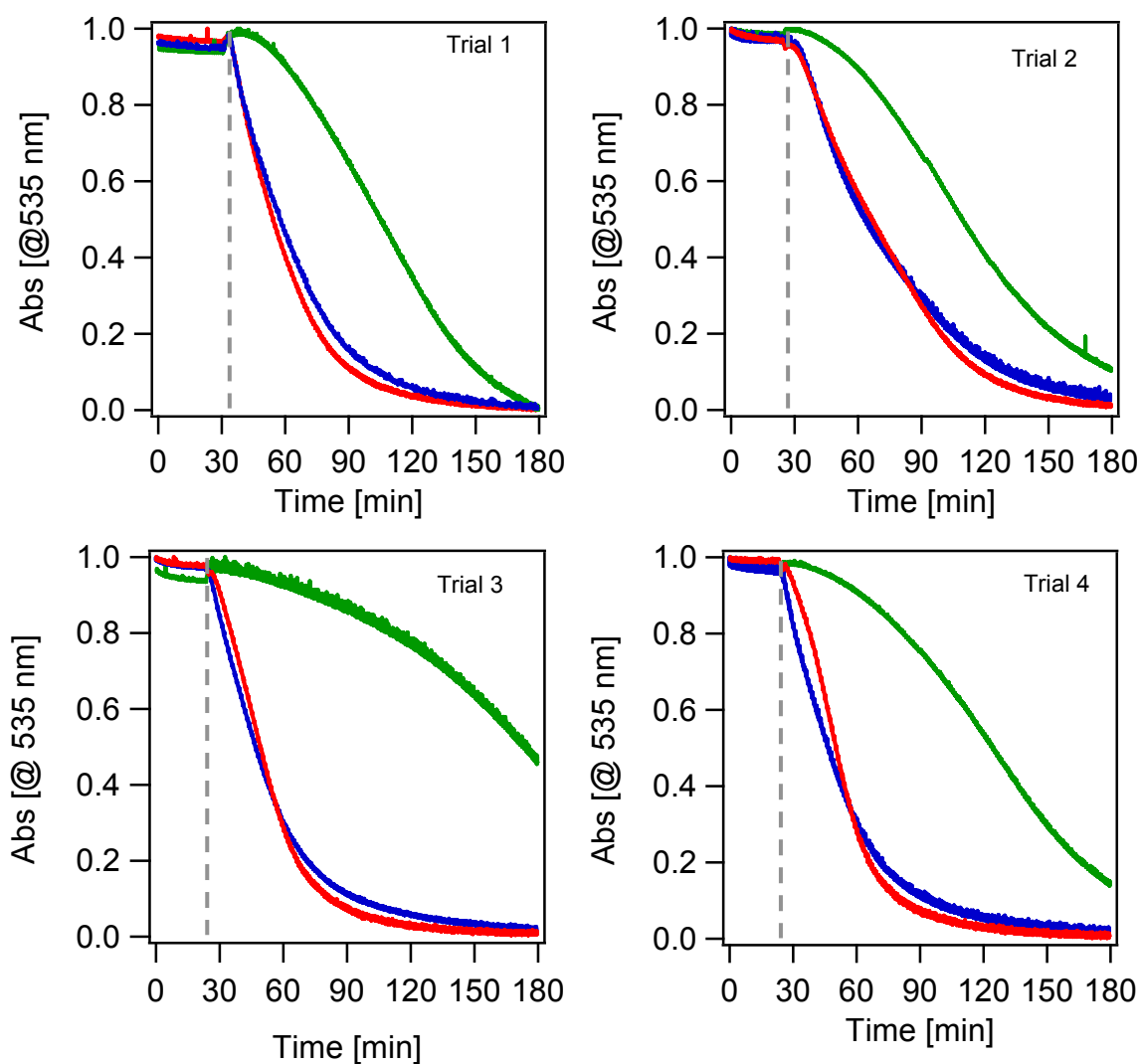

Figure S1. Representative SSP-polarized SFG spectrum at the silica/D_2O interface of NHS-linker as used for spectra presented in main text.
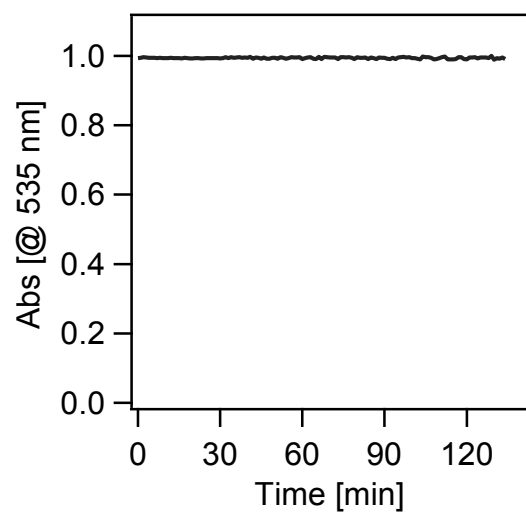
Figure S2. **a)** SSP-polarized SFG spectrum of NHS-linker with little contribution in the asymmetric region. **b)** T_{40} ssDNA (deposited on NHS-linker from same batch as Fig. S3a) with increasing concentrations of Mg^{2+}.
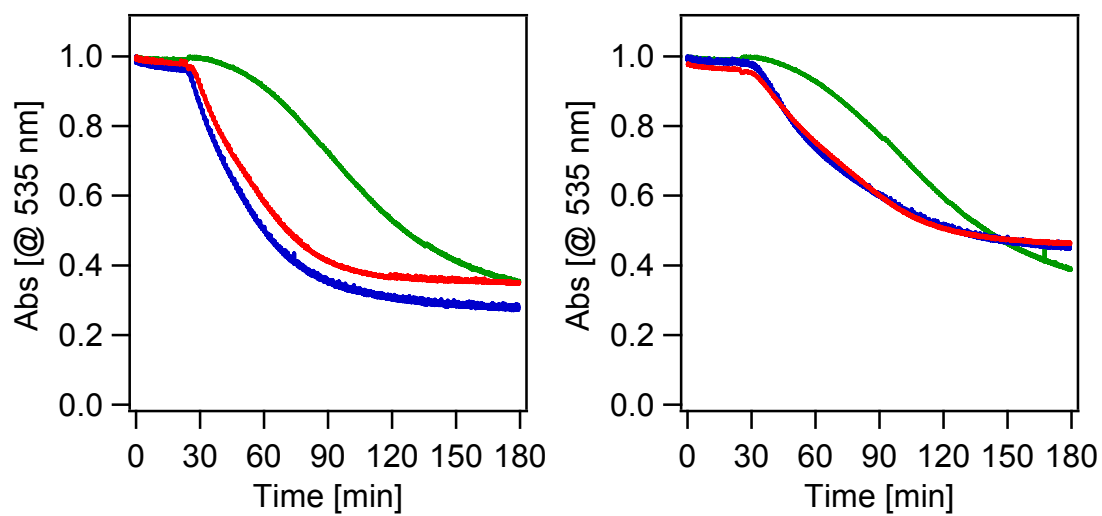
Figure S3. Hydrodynamic radius of T_{40}-functionalized 30 nm gold nanoparticles in the presence of 0, 1, 2.5, and 8 mM Mg^{2+} as measured by dynamic light scattering (DLS).

Figure S4. Multiple nanoparticle hybridization assay trials. Hybridization initiated by addition of complementary linker (indicated by dashed gray line). Hybridization tracked in the presence of 150 mM NaCl (red trace), 3 mM Mg^{2+}/150 mM NaCl (blue trace), and 3 mM Mg^{2+} (green trace). Data was normalized to 1 and referenced to 0 for direct comparison.

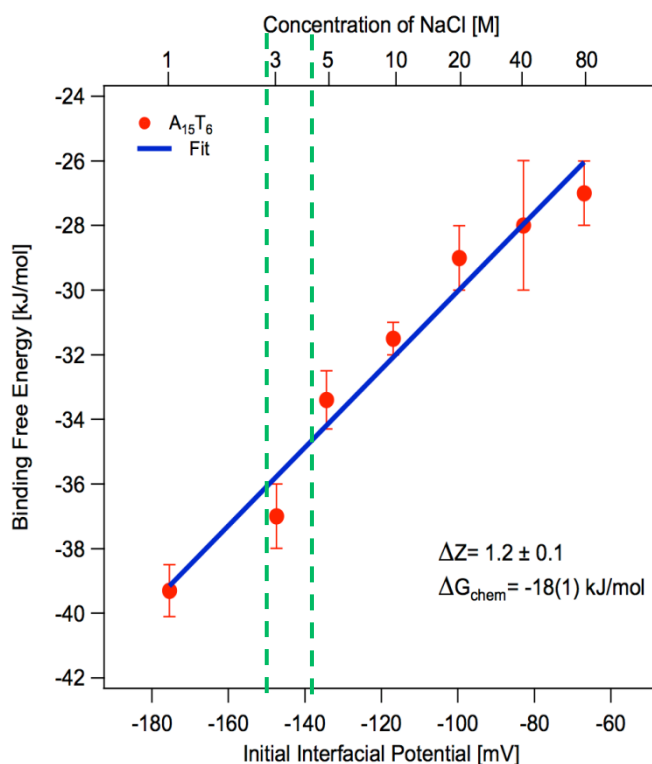**Figure S5**. Nanoparticle hybridization assay in 16 mM NaCl solution.

Figure S6. Example nanoparticle hybridization assay trials, normalized to 1 but not referenced to 0.

Table S1. SFG fit parameters for each magnesium concentration studied.

Peak		Magnesium Concentration						
		0 M	5×10^{-7} M	5×10^{-6} M	5×10^{-5} M	5×10^{-4} M	3×10^{-3} M	5×10^{-3} M
2930 cm^{-1}	amplitude	190(11)	160(12)	145(11)	135(10)	95(10)	64(11)	85(10)
	Γ_v	24(1)	21(2)	20(1)	19(1)	15(2)	15(3)	16(2)
2868 cm^{-1}	amplitude	94(9)	104(9)	105(9)	103(9)	96(8)	81(8)	92(8)
	Γ_v	14(1)	15(1)	14(1)	15(1)	14(1)	14(2)	15(1)

*The average Chi-nonresonant parameter was fit as 0.42

In general, the binding constant will change as a function of bulk Mg^{2+} concentration; however, this change is negligible in the range of concentrations that we employ. Since the concentration of NaCl is held constant at 10 mM, one can assume that slight changes in the interfacial potential do not affect the calculated binding free energies for our system (Figure S7, green dashed lines). At the highest Mg(II) concentration studied (3 mM Mg^{2+}) analysis via the Gouy-Chapman equation shows that the interfacial potential for the T_{40} surface changes from -150 mV (for 10 mM NaCl) to -138 mV (10 mM NaCl + 3 mM $MgCl_2$), a change which is marked by the green dashed lines in the figure above. From this figure (adapted from Holland, et al. *J. Phys. Chem. B* **2011**, *115*, 8338), it is apparent that the change in free energy of binding over the range of Mg^{2+} concentration used in this work is less than our experimental error.

Figure S7. Calculated free energy of binding as a function of electrolyte concentration (top axis) and initial interfacial potential (bottom axis) for Mg^{2+} at the oligonucleotide-functionalized fused silica/water interface, at pH 7 and with NaCl. The solid line is the linear least-squares fit to the data. Green dashed lines indicate the range of interfacial potential studied in the current work on T_{40} sequences.