Supporting Information for

Modulating the Bond Strength of DNA-Nanoparticle Superlattices

Soyoung E. Seo,^{‡,†,‡} Mary X. Wang,^{‡,§,‡} Chad M. Shade,[‡] Jessica L. Rouge,^{†,‡} Keith A. Brown,[‡]
Chad A. Mirkin^{*,†,‡,§}

[†]Department of Chemistry, [‡]International Institute for Nanotechnology, [§]Department of Chemical and Biological Engineering, Northwestern University, Evanston, Illinois 60208, United States

*To whom correspondence should be addressed. E-mail: chadnano@northwestern.edu

Ru^{II} Complex Binding Affinities

The apparent binding affinity (K) of each complex to an isolated binding site on free DNA was determined using a fluorescence titration assay (Table S2). These values are reported in base pair (bp) units to reflect the assumption that each DNA base pair represents a potential binding site.¹ For each assay, the fluorescence emission at 633 nm was recorded as 18 bp dsDNA was titrated into a 1 μ M solution of Ru^{II} complex in 0.05 and 0.5 M NaCl (Figure S1). By fitting this data to equations derived by Schmechel *et al.*,¹ we determined that the binding affinity of complex **1** ($2.5 \times 10^6 \text{ M}^{-1} \text{ bp}$) compares with literature reported values of the binding constant determined using equilibrium dialysis and analogous absorbance titration studies ($4.8 \times 10^6 \text{ M}^{-1} \text{ bp}$).² Additionally, we confirmed that the intercalators equilibrate into the DNA-NP superlattice at the same affinity as with free DNA at 0.5 M NaCl using binding assays (Figure S2), providing evidence that the environment of the superlattice does not prevent diffusion of the complexes. The binding affinity values of these intercalator complexes to free dsDNA were then compared against ΔT_m for the superlattice and each intercalator complex (Figure 2b). At 12.1 μ M of Ru^{II} complexes in 0.5 M aqueous NaCl, the data suggest that a good correlation can be made between the T_m and K . In principle, increasing Ru^{II} complex concentration and reducing NaCl concentrations could expand the range of K values distinguishable by ΔT_m . It is evident that individual complex binding affinities can be distinguished by simply comparing the ΔT_m of superlattice, where the high extinction coefficient of the AuNPs ($\sim 10^8 \text{ cm}^{-1} \text{ M}^{-1}$ for 15-nm AuNPs) and dramatic red shift observed upon disassembly acts as an amplifier for T_m perturbation.

By comparing the binding affinities of members of this family of Ru^{II} intercalators (Table S2) in a systematic fashion, the relative contributions of the ancillary ligand were studied. While the binding modes of different intercalating ligands are well characterized in the literature, the effect of these extended ancillary ligands on the intercalation behavior of Ru^{II} complexes into the DNA duplex has yet to be explored extensively.³⁻⁵ From the data, it is clear that the identity of the ancillary ligand has a dramatic effect on binding affinity to the DNA duplex. For this family of Ru^{II} intercalators, simply changing the

number and the nature of the pendant group affects K by two orders of magnitude in low salt conditions. To determine the effect of the steric profile of the ancillary ligand on the binding affinity, we compared complexes that possess the same overall charge but that differ in the number of bulky pendant groups. When comparing complexes **1**, **2**, and **4**, which have an overall +2 charge, significant differences are observed and can be understood based upon structural differences. For example, complex **1** has no bulky pendant groups and exhibits a binding affinity ($2.5 \times 10^6 \text{ M}^{-1} \text{ bp}$) that is greater than that of either complex **2** or complex **4** by a factor of two (1.9×10^6 and $1.3 \times 10^6 \text{ M}^{-1} \text{ bp}$, respectively). The same trend is observed for complexes **3** and **5**, which have a +1 overall charge. Indeed, complex **3** possesses one bulky pendant group ($1.5 \times 10^6 \text{ M}^{-1} \text{ bp}$) and has a binding constant an order of magnitude higher than that of complex **5** ($1.3 \times 10^5 \text{ M}^{-1} \text{ bp}$), which has two bulky pendant groups. This observation clearly illustrates a trend of decreasing binding affinity as the complex is modified with bulkier and more sterically hindering ancillary ligands.

To study the effect of that local charge of the pendant groups had on binding affinity, we compared complexes that have the same number and type of pendant groups but that differ in the charge of the ligand. Here, the observed difference is more modest. Complex **2** possesses only a slightly higher binding affinity than complex **3** at 0.05 M NaCl ($1.9 \times 10^6 \text{ M}^{-1} \text{ bp}$ vs $1.5 \times 10^6 \text{ M}^{-1} \text{ bp}$). For complexes with two pendant groups, we observe that while the presence of one negatively charged pendant group did not have a profound effect on binding affinity, the presence of two negatively charged groups had a much more pronounced effect. Complexes **4** and **5** have similar binding affinities that are an order of magnitude higher than complex **6** ($1.3 \times 10^5 \text{ M}^{-1} \text{ bp}$ for **4** and **5** vs $2.6 \times 10^4 \text{ M}^{-1} \text{ bp}$ for **6**). This effect is possibly due to the repulsion between the pendant groups and the anionic DNA phosphodiester backbone.

Table S1. Oligonucleotide sequences. “Sp18” denotes a commercially available hexaethyleneglycol phosphosphate spacer.

Thiolated Strands for AuNP	
Name	Sequence (5'-3')
A-SH	HS (Sp18) ₅ CAT CCA TCC TTA TCA ACT
B-SH	HS (Sp18) ₅ AAC GAC TCA TAC TCA CCT
DBCO Strands for QdNP	
A-DBCO	DBCO TEG (Sp18) ₅ CAT CCA TCC TTA TCA ACT
B-DBCO	DBCO TEG (Sp18) ₅ AAC GAC TCA TAC TCA CCT
Linker Strands	
Name	Sequence (5'-3')
A Linker	TT CC TTT Sp18 AGT TGA TAA GGA TGG ATG
B Linker	AAA GG AA Sp18 AGG TGA GTA TGA GTC GTT
dsDNA for Fluorescence Binding Assay	
Name	Sequence (5'-3')
C	TCA ACT ATT CCT ACC TAC
C Linker	GTA GGT AGG AAT AGT TGA

Table S2. Ru^{II} complex binding affinities for the DNA duplex.

	0.05 M NaCl	0.5 M NaCl
Complex 1	2.5 x 10 ⁶	1.0 x 10 ⁵
Complex 2	1.9 x 10 ⁶	8.6 x 10 ⁴
Complex 3	1.5 x 10 ⁶	4.0 x 10 ⁴
Complex 4	1.3 x 10 ⁵	1.1 x 10 ⁴
Complex 5	1.3 x 10 ⁵	1.2 x 10 ⁴
Complex 6	2.6 x 10 ⁴	1.0 x 10 ⁴

K (/M bp) determined by fluorescence titration assay.

Table S3. Binding affinities (K in M⁻¹ bp) of complex 1 and 6 to spherical nucleic acids and DNA-programmable superlattices and DNA-programmable superlattices in 0.5 M NaCl.

	Complex 1	Complex 6
Spherical Nucleic Acid	2.0 x 10 ⁵	7.8 x 10 ⁴
Superlattice	2.1 x 10 ⁵	8.4 x 10 ⁴

Figure S1. Free dsDNA binding isotherms. Fluorescence assays wherein dsDNA is titrated into 1 μM of each complex at varying salt concentrations. (green --) 0.05 M NaCl, (blue --) 0.25 M NaCl, (red --) 0.5 M NaCl.

Figure S2. Superlattice and spherical nucleic acid binding isotherms. Binding assay of Ru^{II} complex **1** and **6** into SNA (a) and DNA-programmable superlattice (b).

Figure S3. SAXS scattering patterns of DNA-programmable superlattices. Example scattering patterns and structure factor $S(q)$ of (a) slow cooled single crystal DNA-programmable superlattices. (b) slow cooled crystals intercalated with complex **1**. (c) Slow cooled crystals intercalated with complex **6**. All samples exhibit bcc symmetry.

Figure S4. Intercalated superlattice maintains structure at elevated temperatures. SAXS patterns of control and DNA-NP bcc superlattice (50 nM nanoparticles) each incubated with 12.1 μM complexes **1-6** at increasing temperature (ramp 1 $^{\circ}\text{C}/\text{min}$).

Figure S5. Peak fitting for 1D SAXS scattering patterns. Representative SAXS patterns fit to bcc crystal lattice symmetry (spacegroup Im-3m). (a) Slow cooled crystals intercalated with 0.7 μM of complex **1** and (b) Non-intercalated slow cooled crystals.

Figure S6. SAXS lineshape analysis. An example of the peak fitting and Williamson-Hall analysis performed on (a) unintercalated single crystals and (b) after addition of 2 μM complex **6**.

Figure S7. Intercalator is stable in superlattice. Interparticle distance of complex **1** intercalated superlattice (as calculated from the q_0 peak) does not change after 2 days at room temperature (b) and after the slow cool procedure (c), demonstrating that complex **1** remains within the superlattice without diffusing away during that time. (a) SAXS pattern of control bcc superlattice.

Figure S8. TEM images of Au and Qd single crystals. (a) Slow cooled Au single crystals and (b) slow cooled Qd single crystals. Colloidal density of gold and quantum dot affects the contrast of these crystals under TEM.

Figure S9. SAXS analysis and fast Fourier transformation images showing that core-shell superlattice crystal is ordered. (a) SAXS analysis reveals a single set of bcc diffraction peaks with no peak broadening and (b) FFT on the selected TEM image reveal that both the shell (top) and core (bottom) portions of the structure are ordered.

Additional References

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