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Metal–DNA Structures

Templated Ligand Environments for the Selective Incorporation of Different Metals into DNA**

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DNA has emerged as a unique template for the construction and organization of nanostructures and arrays with precisely controlled features.[1] The incorporation of transition metals into DNA has enabled the transfer of functionality, in the form of enhanced stability, redox activity, photoactivity, and magnetic and catalytic properties, to this otherwise passive biomolecular template.[2–5] A particularly attractive goal would be the selective incorporation of different transition metals into DNA. This allows the use of the programmable character of DNA to organize transition metals into arbitrarily designed symmetric or asymmetric structures, resulting in a number of applications in artificial photosynthesis, multimetallic catalysis, nanooptics, nanoelectronics, and data storage.[3] It would also result in the expansion of the DNA "alphabet" to new metal "letters" that would increase the information content of this biomolecule and reduce errors in its assembly into nanostructures.

For this goal to reached, different DNA–ligand environments must be designed in a manner that maximizes metal-binding selectivity, promotes close interaction between the metal complex and the DNA duplex, and offers coordination programmability. The incorporation of metals into DNA constructs has been demonstrated through replacement of the hydrogen-bonded DNA base pairs with metal complexes within the interior of the DNA duplex.[2,3] The extension of this strategy to different ligand environments is, however, limited by the steric and spatial requirements of the DNA duplex, and has been successful for planar metal centers that fit in the DNA interior. Metal complexes have been appended as nucleobase and (deoxy)ribose modifications; however, this method is generally limited to a small subset of kinetically inert and unreactive metal complexes that resist the harsh conditions of automated DNA synthesis.[4]

A third approach developed by our research group is to insert ligands into the phosphodiester backbone, such that the hybridization of DNA with its complementary strand templates the assembly of a metal-coordination environment in close contact with the DNA base stack.[5] We report herein the site-specific incorporation of terpyridine (tpy) and diphenylphenanthroline (dpp) ligands into DNA strands.[6] The DNA-templated creation of three ligand environments resulted: **tpy$_2$:DNA**, **tpy:dpp:DNA**, and **dpp$_2$:DNA**. These ligand environments are highly selective for six-, five-, and four-coordinate metal ions, respectively (Figure 1a). Thermal denaturation, UV/Vis and circular dichroism spectroscopy, and gel electrophoresis revealed a strong preference of the octahedral metal ions Fe^{II} and Co^{II} for the **tpy$_2$:DNA** environment, with the highest reported thermal denaturation increase of any DNA structure upon Fe^{II} coordination. The **dpp$_2$:DNA** environment is highly selective for the tetrahedral Cu^{I} ion, whereas **tpy:dpp:DNA** exhibits preference for the five-coordinate Cu^{II} ion. Moreover, "error correction" was observed if a metal ion was placed in the incorrect environment. Thus, Cu^{I} spontaneously oxidized to Cu^{II} if added to **tpy:dpp:DNA**, and Cu^{II} underwent spontaneous reduction to Cu^{I} if placed in **dpp$_2$:DNA**. The four-coordinate Ag^{I} ion was displaced by Cu^{II} when placed in the **tpy:dpp:DNA** structure. Finally, the addition of Fe^{II} to the **tpy:dpp:DNA** structure resulted in reorganization of the ligand environment, such that two of these constructs were brought together with Fe^{II} binding to their terpyridine units. In a similar manner to the ligand pockets of metalloenzymes,[7] this new class of DNA-templated coordination environments defines a toolbox for the selective positioning of different transition metals at exact locations within DNA nanostructures.

Details of the synthesis, characterization, and solid-phase incorporation of the phosphoramidite derivatives of the dpp and tpy ligands into the DNA (Figure 1c) can be found in the Supporting information.[8] The tpy and dpp derivatives were both designed to have flexible diethylene glycol spacers that would enable placement of their aromatic moieties in close proximity to the DNA base stack to promote strong interaction and chirality transfer from the B-DNA duplex upon metal binding (Figure 1b). The selective insertion of **tpy** and **dpp** at the 5′ and 3′ termini of complementary DNA strands **a** and **b**, followed by hybridization, resulted in the formation of three DNA-templated coordination spheres: **tpy$_2$:ab**, **tpy:dpp:ab**, and **dpp$_2$:ab** (Figure 1a). In contrast to metal-driven supramolecular coordination,[9,10] the programmed self-assembly of duplex DNA can template the formation of any combination of ligands, whether homoleptic or mixed, in close proximity.

We studied the thermal stability of DNA duplexes **tpy$_2$:ab** and their metal complexes (Figure 2a). For these duplexes, even without the addition of a metal, the melting temperature increased from 43 °C for the unmodified DNA to 66 °C for **tpy$_2$:ab**. This increase is possibly a result of π stacking of the tpy ligands on the DNA base stack, or interactions of tpy ligands with Na^+ or H^+ ions in the buffer solution.

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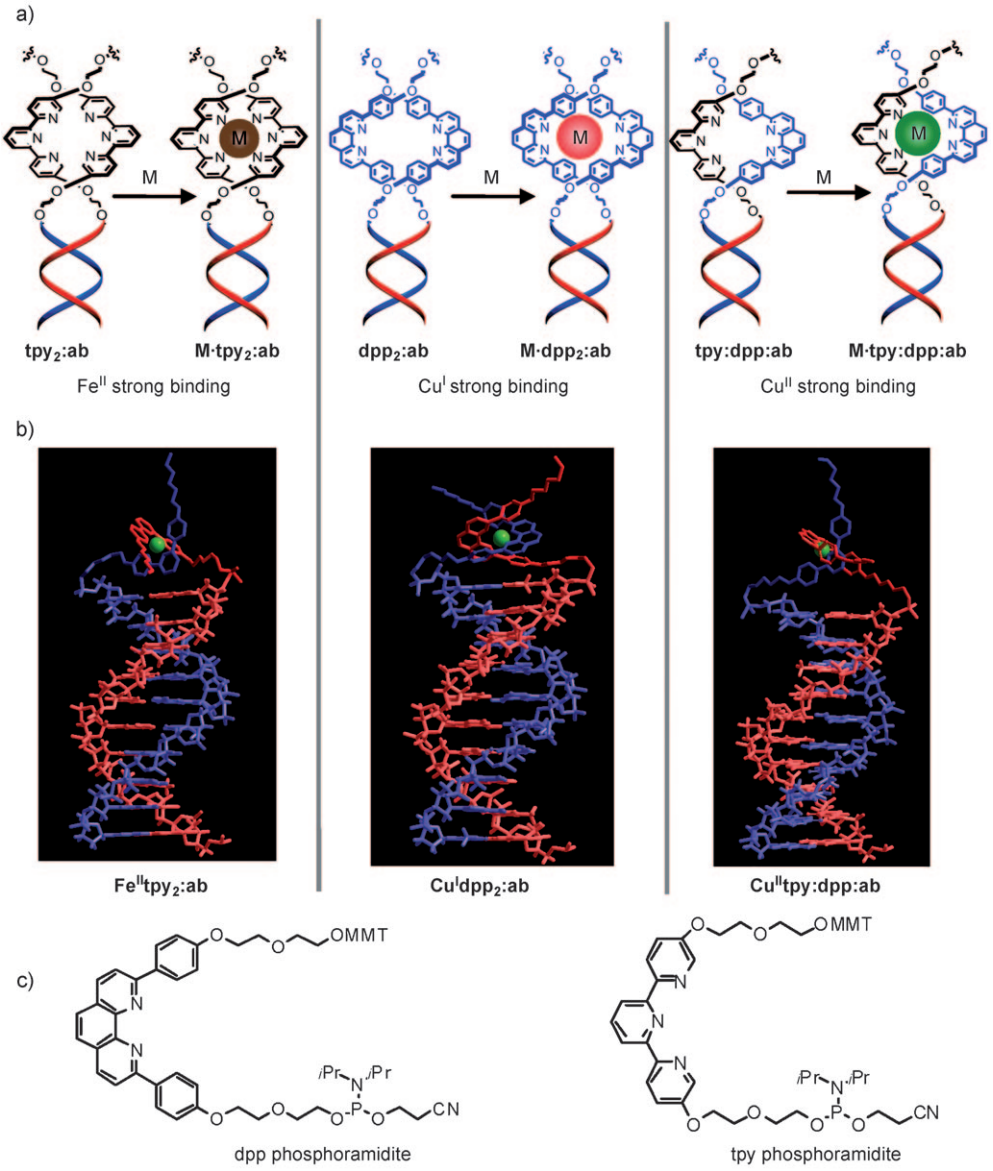

Figure 1. a) Metal coordination to three different DNA–ligand environments. b) Minimized structures (AMBER force field) of **Fe^{II}tpy$_2$:ab**, **Cu^{I}dpp$_2$:ab**, and **Cu^{II}tpy:dpp:ab**. c) Structures of the **dpp** phosphoramidite and **tpy** phosphoramidite. MMT = monomethoxytrityl.

Zn^{II}, Ag^{I}, and Cu^{II} are relatively labile metals in terms of their binding with the **tpy$_2$:ab** junction. The melting temperatures of the resulting complexes are close to that of unmetalated **tpy$_2$:ab**. The binding of these ions to tpy:tpy DNA was evidenced by circular dichroism (CD). In most previous examples in which metal complexes are appended to DNA, only minimal changes are observed in the CD spectrum upon metal binding.[12] In our case, however, the close contact of the ligands with the DNA duplex causes chirality transfer from the right-handed B-DNA to the metal–ligand coordination environment. This interaction results in a *P* (plus) form helical structure, which displays a large induced CD signal with a positive Cotton effect in the region of the ligand absorbance (300–400 nm),[13] and is a sensitive probe for metal binding. The CD spectra demonstrated that Fe^{II}, Co^{II}, Zn^{II}, Ag^{I}, and Cu^{II} all bind with **tpy$_2$:ab** (Figure 2c,d). A metal–DNA binding ratio of 1:1 was confirmed by the CD-monitored titration of **tpy$_2$:ab** with Zn^{II} and Fe^{II}, as representatives of metals that show labile and strong binding, respectively.[14] Cu^{I} showed very poor binding with **tpy$_2$:DNA**. When Cu^{I} was added to **tpy$_2$:ab**, no change in the T_m value or in the ligand absorption region of the CD spectrum was observed (Figure 2e). Thus, the **tpy$_2$:DNA** environment is most stabilized by Fe^{II} and Co^{II} ions.

Fe^{II} and Co^{II} greatly increase the thermal stability of the six-coordinate **tpy$_2$:ab** structure, a result consistent with their strong binding affinity for terpyridine. The melting temperature of **Fe^{II}tpy$_2$:ab** is 83 °C. To the best of our knowledge, this melting-temperature increase (from 43 °C for unmodified DNA) is the highest reported for the modification of a DNA strand with a metal complex, and is higher than that observed upon the inclusion of any single synthetic DNA modification (including modification with locked nucleic acid (LNA)[11]). The melting-temperature curve is biphasic with a broad transition around 55 °C (Figure 2b), which indicates partial denaturation without the dissociation of the metal complex. The complexation of Co^{II} led to the next highest T_m increase to 74 °C. Thus, the stability of metal–DNA duplexes can be tuned by the use of metals with different binding affinity.

Although Cu^{I} shows no propensity to bind to **tpy$_2$:DNA**, it displays strong binding to the four-coordinate tetrahedral **dpp$_2$:DNA** environment.[6] Thus, a large induced CD signal and a T_m increase from 43 °C for unmodified DNA to 80 °C for **Cu^{I}dpp$_2$:ab** were observed (Figure 3b,c). The melting curve of this complex shows a single transition, which indicates that the thermal denaturation is more cooperative than that of **Fe^{II}tpy$_2$:ab**. Molecular modeling demonstrated that the phenyl groups on the phenanthroline ligands are better able to mediate π–π stacking interactions between the terminal

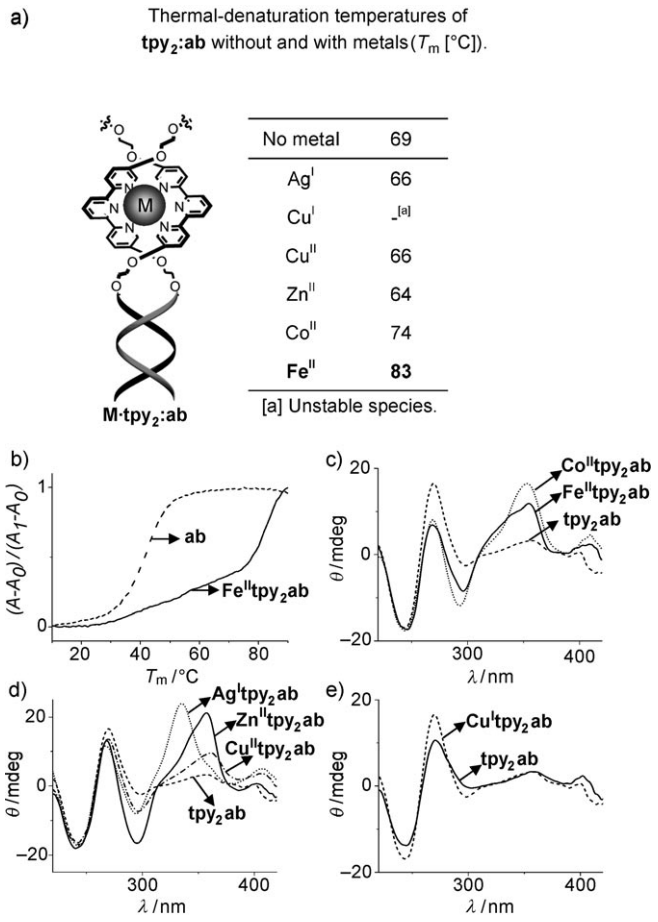

Figure 2. a) Thermal-denaturation temperatures of **tpy$_2$:ab** without and with metal ions. b) Thermal-denaturation curves for **Fe^{II}tpy$_2$:ab** and the unmodified DNA **ab**. c–e) CD spectra of **tpy$_2$:ab** with the strongly binding metal ions Co^{II} and Fe^{II} (c), with ions Ag^{I}, Cu^{II}, Zn^{II}, and Cu^{I}, which form labile complexes with **tpy$_2$:ab** (d), and with Cu^{I}, which does not bind to **tpy$_2$:ab** (e). In each case the spectrum of unbound **tpy$_2$:ab** is shown for comparison.

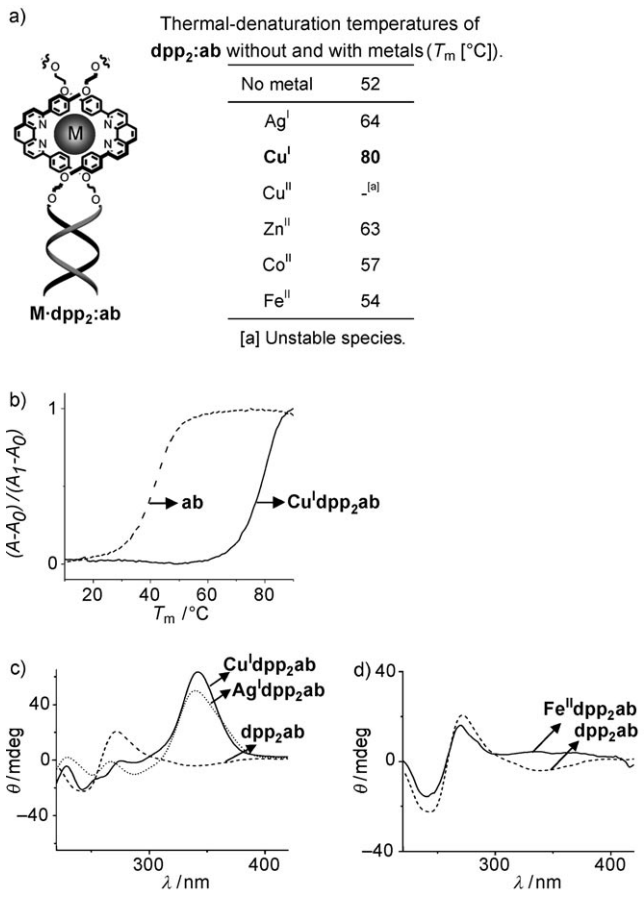

Figure 3. a) Thermal-denaturation temperatures of **dpp$_2$:ab** without and with metal ions. b) Thermal-denaturation curves of **Cu^{I}dpp$_2$:ab** and the unmodified DNA **ab**. c,d) CD spectra of **dpp$_2$:ab** with the strongly binding metal ions Cu^{I} and Ag^{I} (c) and poorly binding Fe^{II} (d). In each case the spectrum of unbound **dpp$_2$:ab** is shown for comparison.

base pair of the duplex and the ligand than the unsubstituted terpyridine ligands in the tpy:tpy environment (Figure 1b). Upon metal binding, the thermal-denaturation temperatures increased dramatically for Cu^{I}, and to a lesser extent for Ag^{I}, Zn^{II}, and Co^{II}, and the CD signatures showed chirality transfer from B-DNA to the metal environments (Figure 3c).[14] On the other hand, the addition of Fe^{II} did not result in stabilization of the duplex towards thermal denaturation, nor was a significant induced CD signal observed for this metal (Figure 3d); these results indicated little binding affinity of this octahedral metal ion for the strongly tetrahedral ligand environment.

For the **tpy:dpp:DNA** mixed-ligand environment, Cu^{II} is the preferred metal ion, the addition of which led to a significant increase in melting temperature (Figure 4). Complexes with Ag^{I}, Zn^{II}, Cu^{I}, and Co^{II} all showed an induced CD signal[14] but no significant increase in melting temperature relative to that of **tpy:dpp:DNA** without a metal. These results with Ag^{I}, Zn^{II}, Cu^{I}, and Co^{II} are consistent with binding and the formation of more labile complexes with **tpy:dpp:DNA**.

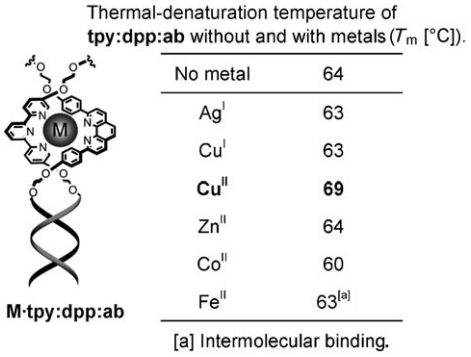

Figure 4. Thermal-denaturation temperatures of **tpy:dpp:ab** without and with metal ions.

Thus, the three ligand environments show clear selectivity for specific metal ions. The **tpy:tpy:DNA** octahedral environment most stably binds Fe^{II} and to a lesser extent Co^{II}. The **dpp$_2$:DNA** structure shows a strong preference for the tetrahedral Cu^{I} ion, and the **tpy:dpp:DNA** mixed environment binds five-coordinate Cu^{II} most stably. Ag^{I} and Zn^{II}

form more labile complexes with all three ligand environments.

To further test the ability of this DNA system to discriminate between metals, we added the "incorrect" metal ion to our structures. In the first case, we added Cu^{I} to the **tpy:dpp:DNA** structure, which should show a preference for Cu^{II}. Within 30 min, the CD band at 336 nm corresponding to Cu^{I} decreased in intensity and was replaced by the CD spectrum for Cu^{II} (Figure 5a). This change is consistent with the spontaneous and rapid oxidation of Cu^{I} to Cu^{II}, which is the favored metal within this ligand environment. In contrast, the Cu^{I} complex of **dpp$_2$:DNA** is stable for long periods in this oxidation state under the same conditions.

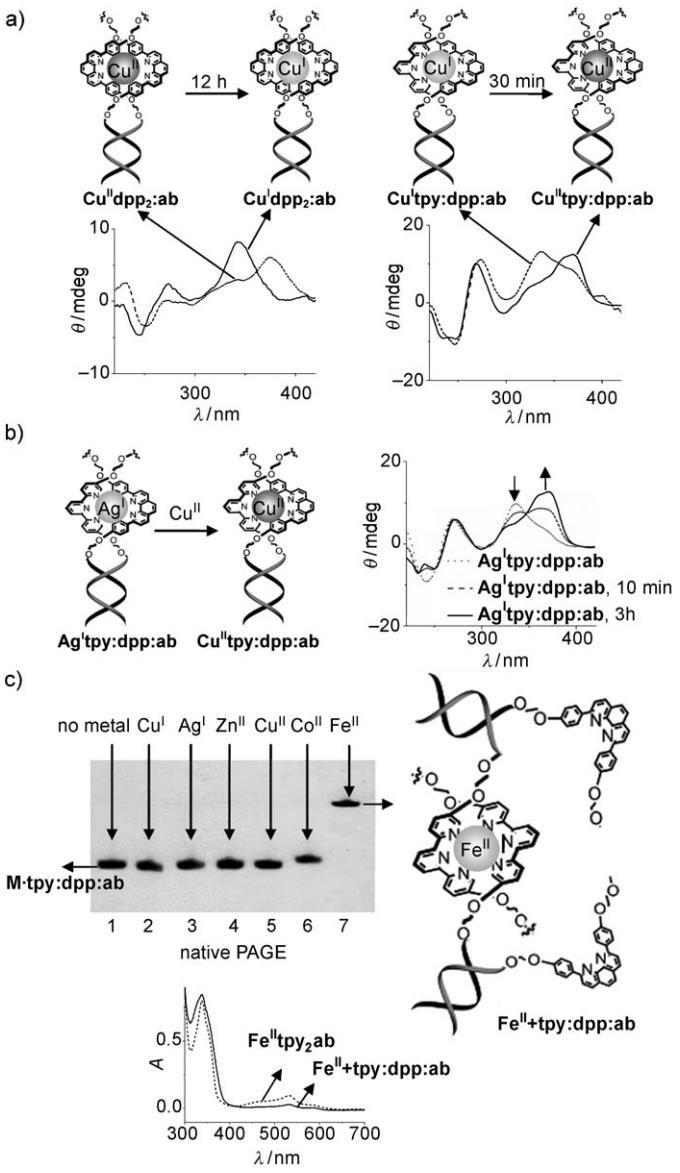

Figure 5. a) Spontaneous Cu^{II}/Cu^{I} redox reaction in the **dpp$_2$:ab** and **tpy:dpp:ab** environments, as monitored by CD. b) Replacement of Ag^{I} with Cu^{II} in the **tpy:dpp:ab** environment, as monitored by CD. c) Native polyacrylamide gel electrophoresis (PAGE) and UV/Vis spectral analysis of Fe^{II} binding with **tpy:dpp:ab**.

Interestingly, when Cu^{II} was placed in the **dpp$_2$:DNA** environment, which shows a strong preference for Cu^{I}, spontaneous reduction of this metal ion occurred over 12 h, as evidenced by the disappearance of its CD trace and the appearance of the CD band corresponding to Cu^{I} (Figure 5a). These examples show the strong selectivity of the DNA-templated systems, which force the metal ions to undergo a redox process to the correct oxidation state for their environment. Whereas trace oxygen in the reaction medium might cause the oxidation of Cu^{I} to Cu^{II} in the first case, the reduction of Cu^{II} to Cu^{I} occurs in phosphate buffer, and the source of electrons in this case is unclear. The reducing reagent could be a trace amount of an impurity or, more interestingly, bases (such as guanine) in the adjacent DNA strand in the buffer solution.[15]

In a third experiment, the addition of Ag^{I} to **tpy:dpp:ab** resulted in the formation of **Ag^{I}tpy:dpp:ab**, as confirmed by its CD spectrum (Figure 5b). We then added Cu^{II}, which is the preferred metal for this environment. Over the course of 3 h, the CD band corresponding to the Ag^{I} complex disappeared and was replaced by the CD trace of **Cu^{II}tpy:dpp:ab**. This result is consistent with the displacement of Ag^{I} by Cu^{II}, and once again shows the ability of these ligand environments to select for the correct metal ion.

In the final experiment, we added Fe^{II} to the **tpy:dpp:DNA** structure. As described above, Fe^{II} has a very strong preference for the tpy ligand over the dpp ligand and showed essentially no binding to **dpp$_2$:DNA**. When added to **tpy:dpp:ab**, Fe^{II} did not bind in a 1:1 fashion. Instead, gel electrophoresis showed a single band of reduced mobility with respect to that of the other metalated **tpy:dpp:DNA** structures (Figure 5c). UV/Vis spectroscopy showed a band at 530 nm, which is characteristic of an $[Fe(tpy)_2]^{2+}$ complex.[16] On the basis of the strong preference of Fe^{II} for **tpy**, its weak binding to **dpp**, and the spectroscopic and electrophoretic evidence, we propose that Fe^{II} coordinates to two **tpy:dpp:DNA** structures, whereby it binds selectively in an intermolecular fashion to the terpyridine ligands and not to either of the phenanthroline moieties. This behavior could be of interest in terms of the construction of higher-order assemblies through the use of the Fe(**tpy**)$_2^{2+}$ complex to link together two DNA strands. These results demonstrate that ligand-modified DNA structures can bind one metal selectively over another, and that error checking occurs in response to less favored binding.

In summary, we have synthesized two ligands that can be attached site specifically to DNA strands by using conventional methods of automated synthesis. These structures can be assembled in a programmable manner through DNA hybridization to template the formation of three unique ligand environments: **tpy**:**tpy**, **dpp**:**dpp**, and **tpy**:**dpp**, which are in close proximity to the DNA base stack. We have found that specific metal ions display a strong preference for a single coordination environment: Fe^{II} and Co^{II} show a clear preference for binding with **tpy$_2$**, Cu^{I} prefers **dpp$_2$**, and Cu^{II} prefers the **tpy:dpp** mixed-ligand environment. These tightly bound metals confer a dramatic enhancement in duplex stability on these DNA structures, especially in the case of the **Fe^{II}tpy$_2$:DNA** complex, the formation of which led to the

highest melting-temperature increase reported for a single modification with a metal complex. We were also able to demonstrate the first "error-checking" metal system placed within DNA, whereby a metal spontaneously adjusts its redox state when placed in the incorrect environment, displaces a relatively labile metal to form a more stable complex, or causes reorganization of the coordination site to create the favored ligand complex. The site-specific incorporation of ligands and their subsequent DNA-templated assembly by simple duplex formation represents a facile method to prepare well-defined metal-coordination geometries within a DNA framework. This method may lead to an expanded DNA "alphabet" with an enriched information content that could be used for the site-specific incorporation of various transition metals within higher-order DNA-based nanostructures.

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