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Reversible metal-dependent destabilization and stabilization of a stem-chelate-loop probe binding to an unmodified DNA target

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Abstract

Herein we report the discovery of a novel DNA probe with a stem-chelate-loop structure, wherein the stability of the probe-target duplex can be modulated lower or higher using a narrow concentration range of dilute transition metal-ions (0.1–10 μM). Oligonucleotide probes containing two terpyridine (TPY) ligands separated by 15 bases of single-stranded DNA, with or without a flanking 5 base self-complimentary DNA stem, were tested in thermal transition studies with linear target DNA and varying amounts of $ZnCl_2$. Without the stem, addition of Zn^{2+} resulted only in reversible destabilization of the probe-target duplex, consistent with assembly (up to 1 equiv. Zn^{2+}) and disassembly (excess Zn^{2+}) of the intramolecular Zn^{2+}-$(TPY)_2$ chelate. Surprisingly, probes including both the intramolecular chelate and the stem gave a probe-target duplex that was reversibly destabilized and stabilized upon addition of Zn^{2+} by ± 5–7 °C, a phenomenon consistent with assembly and then disassembly of the entire stem-Zn^{2+}-$(TPY)_2$ motif, including the DNA stem. Stem-chelate-loop probes containing dipicolylamine (DPA) ligands exhibited no metal-dependent stabilization or destabilization. The stem-Zn^{2+}-$(TPY)_2$ motif is readily introduced with automated synthesis, and may have broad utility in applications where it is desirable to have both upward and downward, reversible metal-dependent control over probe-target stability involving an unmodified DNA target.

Novel nucleic acid chemistries have potential use across many new and existing applications in nanotechnology, [1–3] genomics,[4, 5] medicine[6–8] and chemistry.[9, 10] Probe association/dissociation with a nucleic acid target according to Watson-Crick base-pairing rules is the pivotal property exploited in these applications, and is characterized by the mid-transition, or melting temperature (T_m).[11] It would be desirable to be able to tailor the thermodynamics of probe association and dissociation to a native DNA target in a facile manner for a particular application. Adding salt (*e.g.* NaCl, $MgCl_2$) is well-known to increase DNA duplex stability, but is a non-specific intervention that results in large changes in ionic strength that can have unintended effects in a given application (*e.g.* if enzymes are present that are sensitive to changes in ionic strength).[12] Previous attempts to orthogonally increase duplex stability required installations of metal-chelate moieties in both probe and target that preclude modulating binding stability to native DNA.[13, 14] Herein, we report a new approach for modulating T_m lower or higher as a function of dilute transition metal-ion concentrations using a novel DNA probe with a stem-chelate-loop structure (Figure 1).

Our findings address the challenge of orthogonally controlling probe-target duplex stability without modifying the target nucleic acid or significantly altering solution ionic strength,

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Supporting Information. Experimental procedures and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

capabilities that together will find broad utility in enzymatic reactions and other applications of probe association/dissociation with native DNA.

Terpyridyl and dipicolyl phosphoramidites, **1** and **2**, were synthesized as in Scheme 1. The secondary amines, DPA or piperidine linked TPY,[15, 16] were added to (S)-dimethoxytritylglycidol,[17–19] and the resulting secondary alcohol was phosphitylated (experimental details are provided as Supporting Information). The cyanoethyl phosphoramidites **1** and **2** were used together with the four canonical base phosphoramidities, to synthesize 6-carboxyfluorescein (FAM), Black-Hole Quencher-1®[20] (BHQ1) and chelate ligand (TPY and DPA) modified oligonucleotides **3–10**, by automated DNA synthesis (Table 1). The fluorescent target oligonucleotide **10** was complementary to each probe's 15-base non-stem DNA sequence. A bi-molecular fluorescence-quenching assay was selected so probe-target hybridization could be monitored independent of intramolecular events such as TPY intercalation, stem-formation, or fluorophore quenching by TPY (Figure 1).

Thermal transitions were measured by fluorescence spectroscopy for the complementary probe-target duplexes **3:10**, **4:10**, **5:10, 6:10** and **7:10** with varying concentrations of $ZnCl_2$ from sub-stoichiometric (1/16 equiv.) to excess (32 equiv.) (see Supporting Information). The T_m of a probe:target duplex is the sum of contributions from chelate (c), non-chelate probe elements (nc), and non-specific metal-ion effects on duplex stability that vary with each metal-ion concentration (ns). The normalized melting temperature ('T_m) for each probe-target duplex was computed at each Zn^{2+} concentration by subtracting from the duplex T_m (c+nc+ns), the T_m of the corresponding no-chelate control duplex (nc+ns). The 'T_m is therefore a measure of changes in probe-target stability contributed solely by the chelate (see Supporting Information for raw data).

In control transitions absent free metal-ions (*i.e.* excess EDTA), TPY-containing probe-target duplexes **4:10** and **6:10** were both stabilized by ~2 °C relative to their TPY-free counterparts (Figure 2, gray segment). TPY-base-pair stacking interactions provide a duplex stabilizing force,[21] particularly with 3′ adjacent purine nucleobases.[13]

In the presence of Zn^{2+}, the 'T_m of stemless probe-target duplex **4:10** decreased as a function of Zn^{2+} (i.e. the probe-target duplex was destabilized), reaching a minimum at 1 equivalent (600 nM) of metal ion (Figure 2, yellow segment). Excess $ZnCl_2$ reversed the probe-target destabilization, returning the 'T_m to the value of the metal- free control (Figure 2, orange segment). Maximum destabilization of **4:10** at 1 equivalent of Zn^{2+} is consistent with assembly of all Zn^{2+}-$(TPY)_2$ chelates into **4**(Zn^{2+}) complexes (Figure 3B). Energy provided by the Zn^{2+}-$(TPY)_2$ chelate in **4**(Zn^{2+}) must be overcome in order for the probe to hybridize to the target, resulting in destabilization of the probe-target duplex at equilibrium.[22, 23] Excess Zn^{2+} (>1 equivalent) populates both TPY ligands of **4**, resulting in disassembly of the Zn^{2+}-$(TPY)_2$ chelate and formation of unconstrained probe **4**$(Zn^{2+})_2$ (Figure 3C) having probe-target duplex stability equal to that of unconstrained probe **4** in the metal-free control (Figure 3A).

Similar to the duplex **4:10**, the 'T_m of the duplex **6:10** also decreased as a function of Zn^{2+}, reaching a minimum at 1 equivalent of metal ion (Figure 2, yellow segment). Surprisingly however, excess $ZnCl_2$ beyond 1 equivalent not only reversed the destabilization of the probe-target duplex, but resulted in a net 5 °C stabilization of the probe-target duplex relative to the metal-free control; a significant increase in 'T_m of 12 °C overall (Figure 2, orange segment). Maximum destabilization of the duplex **6:10** at 1 equivalent of Zn^{2+} is consistent with assembly of all stem-Zn^{2+}-$(TPY)_2$ motif chelates into **6**(Zn^{2+}) complexes analogous to **4**(Zn^{2+}) complexes (Figure 4B). In distinction to **4**(Zn^{2+}), the energy provided

by both the Zn^{2+}-$(TPY)_2$ chelate and the duplex stem must be overcome in order for **6**(Zn^{2+}) to hybridize to target. The stem alone contributes a 5 °C reduction in probe-target T_m compared to the stemless analog (see Supporting Information). If excess Zn^{2+} (>1 equivalent) populated the two TPY ligands of **6** and resulted in only the disassembly of the Zn^{2+}-$(TPY)_2$ chelate, the probe-target duplex stability would be predicted to equal that of **6** in the metal-free control in analogous fashion to the stemless probe **4**. However, relative to the metal-free control, excess Zn^{2+} resulted in stabilization of the **6:10** probe-target duplex by an amount equal to that provided by the duplex stem (i.e. ~5 °C), a phenomenon consistent with the disassembly of the entire stem-Zn^{2+}-$(TPY)_2$ motif, including the DNA stem.

To characterize the mechanism underlying this stem disassembly further, two control probes (**7** and **8**) were made with a sequence identical to **6**, except bearing only a single TPY each (Table 1). The duplex **7:10** was increasingly stabilized as a function of metal (Figure 5). The duplex **8:10** exhibited no 2 °C TPY-duplex stabilization or change in 'T_m as a function of metal, possibly because the TPY is neighbored with a 3′ purine and thus is alternatively engaged in more favorable stem base-stacking interactions. Therefore, the stem disruptor function is embodied in the 5′ TPY lacking an adjacent purine, as in **7:10**. Neither duplex **7:10** nor **8:10** exhibited a duplex destabilizing effect at 1 equivalent of Zn^{2+}, indicating both TPY motifs are required for stemstabilization. The structural basis for the metal-mediated disruption of the DNA stem by the 5′ TPY is unknown, but is believed to involve the formation of a putative TPY-Zn^{2+}-nucleobase complex that precludes Watson-Crick base-pairing in the stem (Figure 4C). The complex returns the probe not to a bi-metal stem-constrained probe, but instead to the unconstrained configuration **6**$(Zn^{2+})_2$, which exhibits a net 5 °C stabilization of the probe-target duplex relative to the stem-constrained metal-free control **6** (Figure 4A).

Except for replacement of the two TPY ligands with DPA ligands, the DPA -containing probe-target duplex **9:10** is identical to duplex **6:10** (Table 1). DPA and TPY have equivalent ability to chelate Zn^{2+}, and yet the duplex **9:10** exhibited no metal-dependent change in 'T_m and no metal-free stabilization of its T_m relative to the DPA -free counterpart **5:10** (Supporting Information). The basis for these striking differences in metal-dependent and metal-independent behavior between duplexes **9:10** and **6:10** are unclear. However, the lack of metal-free stabilization in **9:10** suggests that compared to TPY, DPA has limited ability to stack with adjacent base-pairs in the probe-target duplex. Additionally, terpyridyl and dipicolyl phosphoramidites **1** and **2** install TPY and DPA ligands with slightly different inter-stem spacing, respectively (Scheme 1). The metal-dependent and metal-independent phenomena seen with TPY -containing probes **4** and **6**, may therefore mechanistically depend on base-stacking and/or precise placement of the TPY ligands.

Other metallo-chelate-nucleobase polymer assemblies have been described in the literature. We previously reported the development of PNA-based probes that exhibited enhanced binding specificity.[23] In an analogous arrangement, Krämer[24] and Moreau[21] introduced oligonucleotides with antipodal TPY groups that underwent macrocyclization in the presence of a coordinating transition metal. Chelate-modified oligonucleotides consisting of unnatural metallo-base pairs (*e.g.* T-Hg^{+}-T) are known that exhibit exceptional duplex stability. [25, 26–34] However, to our knowledge this is the first report of a novel stem-chelate-loop nucleobase polymer assembly that permits metal-dependent stabilization and destabilization of probe-target binding to unmodified DNA. We demonstrated here that a DNA probe with the stem-chelate-loop structure consisting of two TPY ligands is maximally stabilized with 1 equivalent of Zn^{2+} via an intramolecular chelate (i.e. TPY-Zn^{2+}-TPY). However, with increasing amounts of supra-stoichiometric Zn^{2+}, the probe-target duplex exhibits increased stability consistent with disassembly of both the

intramolecular chelate and adjacent Watson-Crick base-paired stem. The ‘ T_m of the probe-target duplex was modulated by a total of 12 °C over a narrow concentration range of Zn^{2+} ions (0.1–10 μM). This concentration range is 4 and 5 orders of magnitude more dilute than the range of $MgCl_2$ and NaCl that non-specifically affects a similar change in duplex T_m, respectively.[12]

The stem-Zn^{2+}-$(TPY)_2$ motif is introduced readily with automated oligonucleotide synthesis, and the range of ‘ T_m values accessible could be expanded at the synthesis stage by adjusting stem stability by altering the stem length or G/C content, or by employing alternate stem types.[22, 25, 35–39] The stem-chelate-loop structure may thus have broad utility in life science applications, as well as in the development of DNA-based nano-devices and electronic components, all applications where it may be useful to have reversible upward and downward, metal-dependent control over duplex stability.[1, 40]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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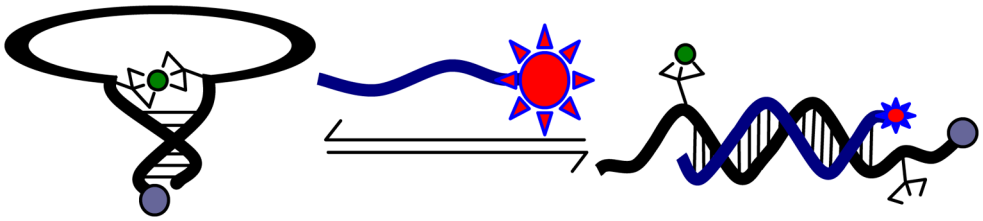


Figure 1.
Representative bi-molecular fluorescence-quenching assay design for detecting probe hybridization with target DNA. Target hybridization results in stem and chelate dissociation, probe-target duplex formation and fluorescence quenching. Key: Thick black line, probe DNA; blue line, target DNA; green circle, metal–ion; gray circle quencher; large red star, un-quenched fluorophore; and small red star, quenched fluorophore.

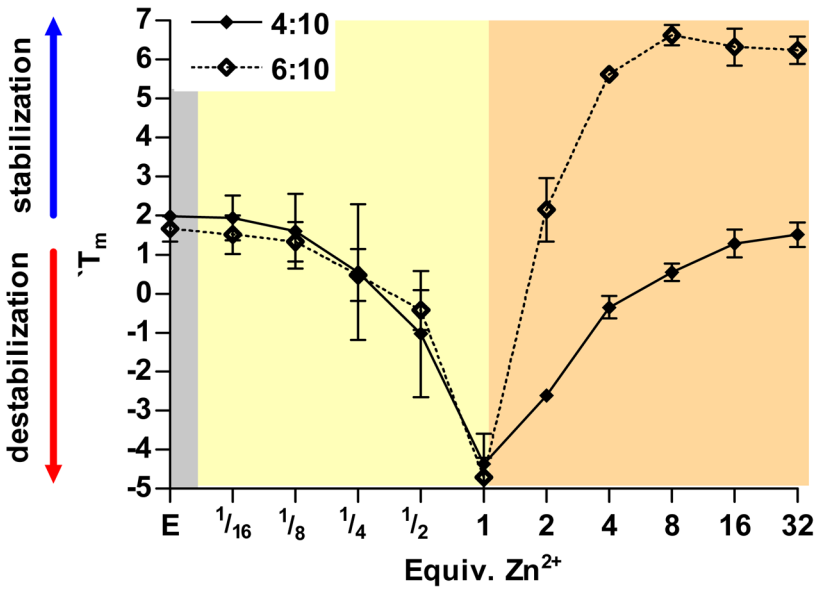


Figure 2.

Probe-target stability (‘Tm) of stemless (**4:10**) and stemmed (**6:10**) TPY-containing probe-target duplexes in the absence of free transition metal-ions, i.e. 5 μM EDTA (E), or in the presence of the indicated equivalents of $ZnCl_2$; [probe] = 600 nM, [target **10**] = 100 nM, 10 mM MOPS, 1 mM $MgCl_2$, pH = 7.5. Normalized melting temperature (‘Tm) at each E or Zn^{2+}: **4:10** ‘T_m = **4:10** T_m − **3:10** T_m and **6:10** ‘T_m = **6:10** T_m − **5:10** Tm. Error bars are the S.D. for three repetitions (Supporting Information).

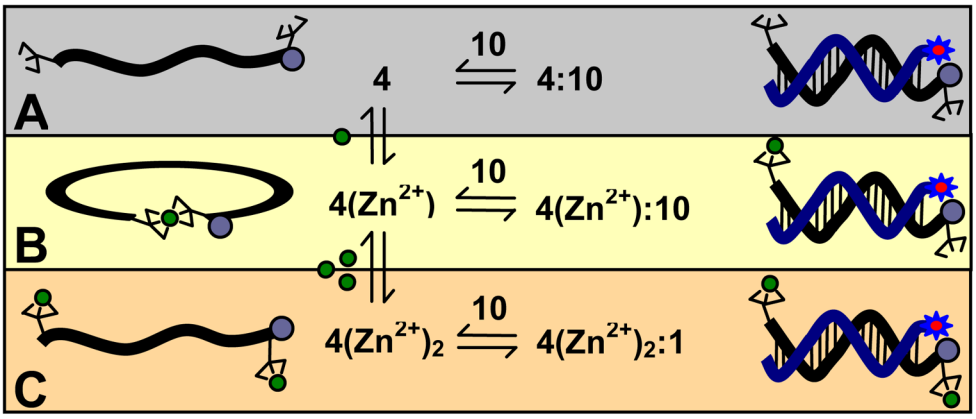


Figure 3.
Model of probe **4** and target **10** hybridization with (A) EDTA, (B) 1 equivalent of Zn^{2+} or (C) excess Zn^{2+}. The color coding in each panel designates predominant probe and target species in the corresponding colored segments in Figure 2.

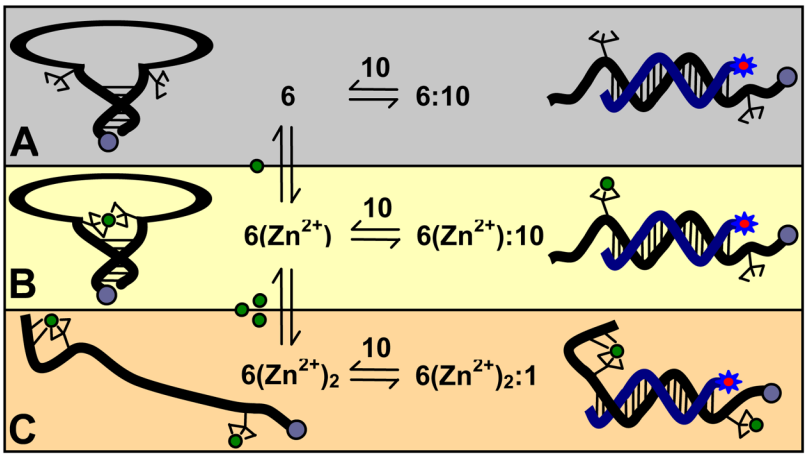


Figure 4.
Model of stemmed probe **6** and target **10** hybridization with (A) EDTA, (B) 1 equivalent of Zn^{2+} or (C) excess Zn^{2+}. The color coding in each panel designates predominant probe and target species in the corresponding colored segments in Figure 2.

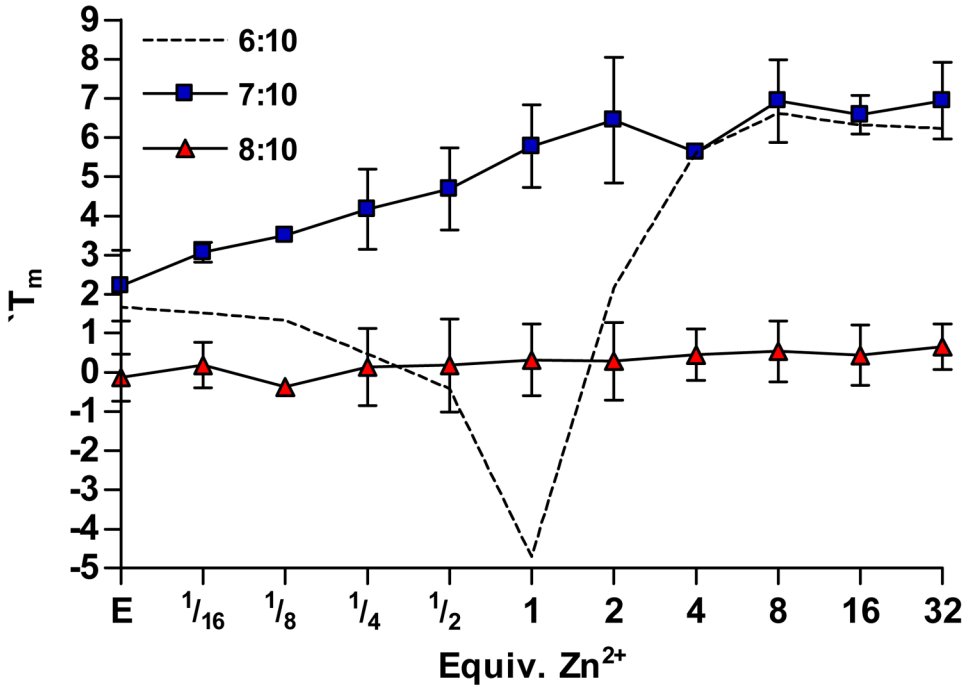
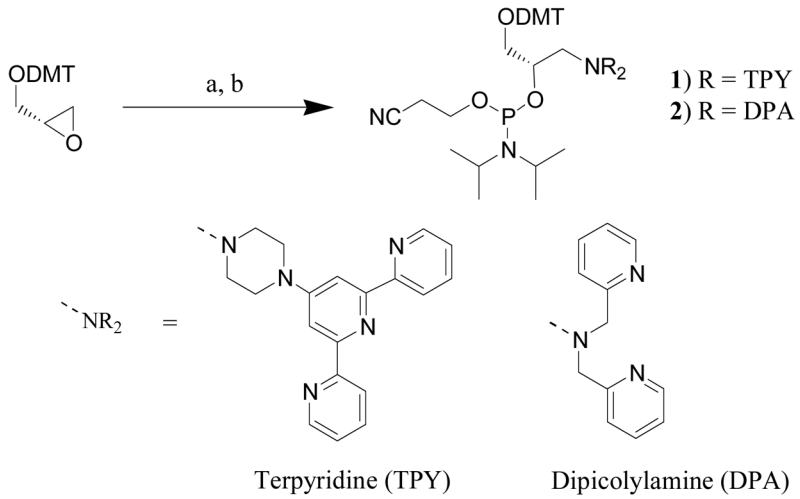


Figure 5.

Probe-target duplex stability (‘Tm) of mono-terpyridine controls (**7:10**) and (**8:10**) in the absence of free transition metal-ions, i.e. 5 μM EDTA (E), or in the presence of the indicated equivalents of $ZnCl_2$.



Scheme 1.

Synthesis of TPY and DPA cyanoethyl phosphoramidites

a) HNR_2, K_2CO_3, CH_3CN, 80 °C; b) 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphorodiamidite, 1H-tetrazole, CH_3CN.

Table 1

Oligonucleotides: no-chelate control (**3**, **5**), chelate-ligand modified (**4**, **6**, **7**, **8**, **9**) and target (**10**)

#	modified oligonucleotide[a]
3	CCA-$(AAA)_3$-ACC-BHQ1
4	TPY- CCA-$(AAA)_3$-ACC-TPY-BHQ1
5	CGCTC-CCA-$(AAA)_3$-ACC-GAGCG-BHQ1
6	CGCTC-TPY-CCA-$(AAA)_3$-ACC-TPY-GAGCG -BHQ1
7	CGCTC-TPY-CCA-$(AAA)_3$-ACC-GAGCG -BHQ1
8	CGCTC-CCA-$(AAA)_3$-ACC-TPY-GAGCG -BHQ1
9	CGCTC-DPA-CCA-$(AAA)_3$-ACC-DPA-GAGCG -BHQ1
10	FAM-GGT-$(TTT)_3$-TGG

[a]Underlined base sequences indicate the self-complementary stem.