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Communication

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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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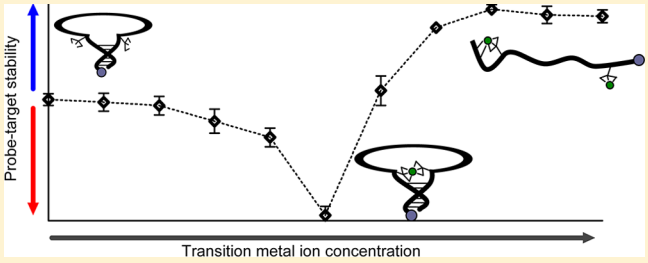
Supporting Information

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-complementary 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 midtransition, 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 nonspecific 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, 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)-dimethoxytrityl-glycidol,[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 nonstem DNA sequence. A bimolecular fluorescence-quenching assay was

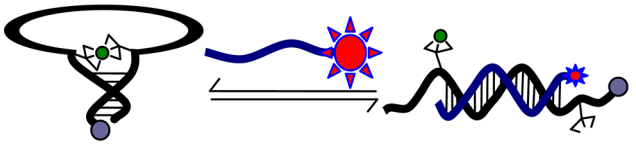


Figure 1. Representative bimolecular fluorescence-quenching assay design for detecting probe hybridization with target DNA. Target hybridization results in stem and chelate dissociation, probe-target duplex formation and fluorescence quenching. Key: Thick black line, probe DNA; blue line, target DNA;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

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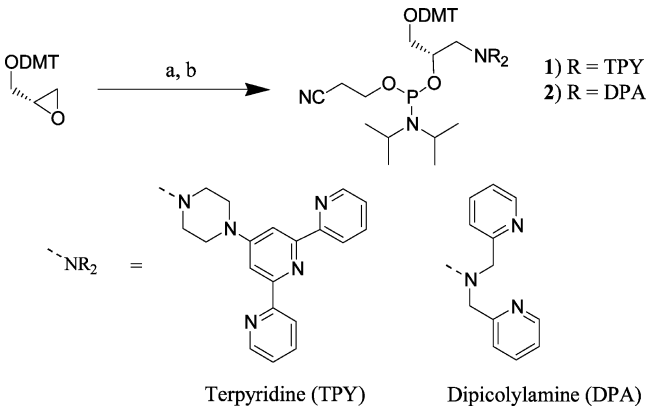
Scheme 1. Synthesis of TPY and DPA Cyanoethyl Phosphoramidites



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

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 substoichiometric (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), nonchelate probe elements (nc), and nonspecific 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 equiv (600 nM) of metal ion (Figure 2, yellow segment). Excess

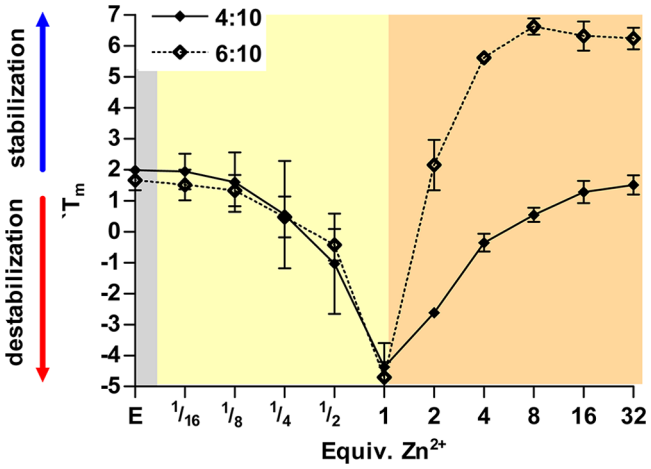


Figure 2. Probe-target stability ($'T_m$) of stemless (**4:10**) and stemmed (**6:10**) TPY-containing probe-target duplexes in the absence of free transition metal-ions, i.e., 5 μM EDTA (*E*), or in the presence of the indicated equivalents of $ZnCl_2$; [probe] = 600 nM, [target **10**] = 100 nM, 10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

$ZnCl_2$ 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 equiv 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+}-

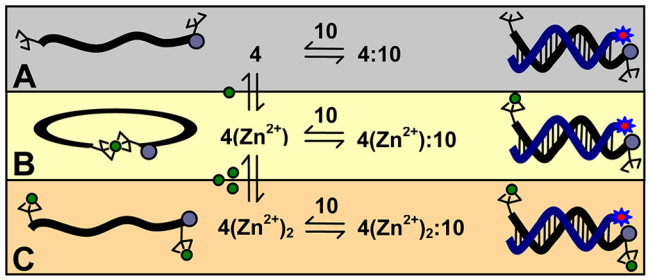


Figure 3. Model of probe **4** and target **10** hybridization with (A) EDTA, (B) 1 equiv of Zn^{2+}, or (C) excess Zn^{2+}. The color coding in each panel designates predominant probe and target species in the corresponding colored segments in Figure 2.

$(TPY)_2$ 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 equiv) 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 equiv of metal ion (Figure 2, yellow segment). Surprisingly, however, excess $ZnCl_2$ beyond 1 equiv not only reversed the destabilization of the probe−target duplex, but also 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 equiv of Zn^{2+} is consistent with assembly

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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

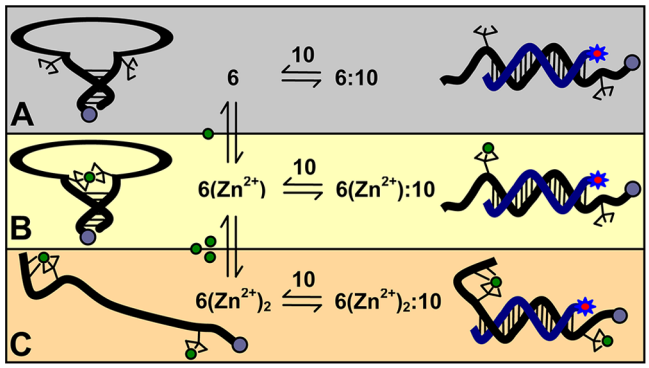

Figure 4. Model of stemmed probe **6** and target **10** hybridization with (A) EDTA, (B) 1 equiv of Zn^{2+}, or (C) excess Zn^{2+}. The color coding in each panel designates predominant probe and target species in the corresponding colored segments in Figure 2.

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 analogue (see Supporting Information). If excess Zn^{2+} (>1 equiv) 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

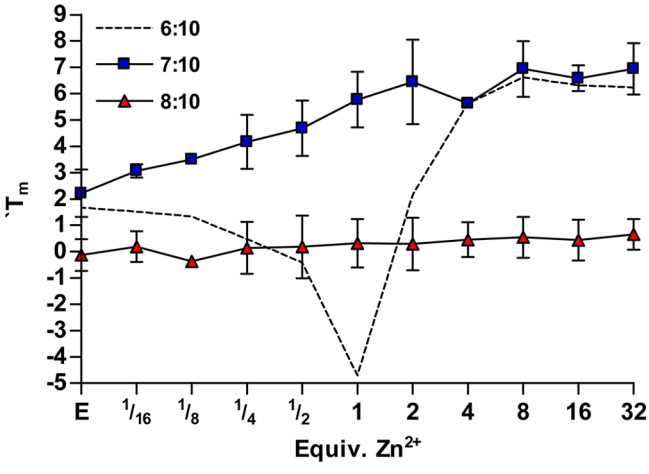

Figure 5. Probe-target duplex stability ('T_m) of monoterpyridine controls (**7**:**10**) and (**8**:**10**) in the absence of free transition metal ions, i.e., 5 μM EDTA (E), or in the presence of the indicated equivalents of $ZnCl_2$.

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 equiv of Zn^{2+}, indicating both TPY motifs are required for stem-stabilization. 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 bimetal 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 interstem 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.[25−34] 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 equiv 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 the 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 nonspecifically 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

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stem-chelate-loop structure may thus have broad utility in life science applications, as well as in the development of DNA-based nanodevices and electronic components, all applications where it may be useful to have reversible upward and downward, metal-dependent control over duplex stability.[1,40]

■ ASSOCIATED CONTENT

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.

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Notes

The authors declare the following competing financial interest(s): All of the authors are current (J.R.M., D.V.X.N., J.A.Z.) or former (A.R.F., S.R.R.) employees of Syntrix Biosystems (Auburn, WA). Syntrix Biosystems manufactures dipicolylamine cyanoethyl phosphoramidite, for commercial sale through Glen Research (Sterling, VA).

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■ REFERENCES

(1) Shionoya, M., and Tanaka, K. (2004) Artificial metallo-DNA: A bio-inspired approach to metal array programming. *Curr. Opin. Chem. Biol. 8*, 592−597.
(2) McLaughlin, C. K., Hamblin, G. D., and Sleiman, H. F. (2011) Supramolecular DNA assembly. *Chem. Soc. Rev. 40*, 5647−5656.
(3) Frezza, B. M., Cockroft, S. L., and Ghadiri, M. R. (2007) Modular multi-level circuits from immobilized DNA-based logic gates. *J. Am. Chem. Soc. 129*, 14875−14879.
(4) Benes, V., and Castoldi, M. (2010) Expression profiling of microrna using real-time quantitative PCR, how to use it and what is available. *Methods 50*, 244−249.
(5) Aiba, Y., Sumaoka, J., and Komiyama, M. (2011) Artificial DNA cutters for DNA manipulation and genome engineering. *Chem. Soc. Rev. 40*, 5657−5668.
(6) Kole, R., Krainer, A. R., and Altman, S. (2012) RNA therapeutics: Beyond RNA interference and antisense oligonucleotides. *Nat. Rev. Drug Discovery 11*, 125−140.
(7) Bennett, C. F., and Swayze, E. E. (2010) RNA targeting therapeutics: Molecular mechanisms of antisense oligonucleotides as a therapeutic platform. *Annu. Rev. Pharmacol. Toxicol. 50*, 259−293.
(8) De Paula, D., Bentley, M. V., and Mahato, R. I. (2007) Hydrophobization and bioconjugation for enhanced siRNA delivery and targeting. *RNA 13*, 431−456.
(9) Park, S., and Sugiyama, H. (2010) DNA-based hybrid catalysts for asymmetric organic synthesis. *Angew. Chem., Int. Ed. Engl. 49*, 3870−3878.
(10) Silverman, A. P., and Kool, E. T. (2006) Detecting RNA and DNA with templated chemical reactions. *Chem. Rev. 106*, 3775−3789.
(11) Marky, L. A., and Breslauer, K. J. (1987) Calculating thermodynamic data for transitions of any molecularity from equilibrium melting curves. *Biopolymers 26*, 1601−1620.
(12) Tan, Z. J., and Chen, S. J. (2006) Nucleic acid helix stability: Effects of salt concentration, cation valence and size, and chain length. *Biophys. J. 90*, 1175−1190.
(13) Kalek, M., Madsen, A. S., and Wengel, J. (2007) Effective modulation of DNA duplex stability by reversible transition metal complex formation in the minor groove. *J. Am. Chem. Soc. 129*, 9392−9400.
(14) Karlsen, K. K., Jensen, T. B., and Wengel, J. (2009) Synthesis of an unlocked nucleic acid terpyridine monomer and binding of divalent metal ion in nucleic acid duplexes. *J. Org. Chem. 74*, 8838−8841.
(15) Andres, P. R., Lunkwitz, R., Pabst, G. R., Böhn, K., Wouters, D., Schmatloch, S., and Schubert, U. S. (2003) New 4′-functionalized 2,2′:6′,2″-terpyridines for applications in macromolecular chemistry and nanoscience. *Eur. J. Org. Chem.*, 3769−3776.
(16) Johansson (2006) Pd-catalyzed amination of chloro-terpyridine for the preparation of amine-containing ruthenium (II) complexes. *Synthesis*, 2585−2589.
(17) Schlegel, M. K., Zhang, L., Pagano, N., and Meggers, E. (2009) Metal-mediated base pairing within the simplified nucleic acid gna. *Org. Biomol. Chem. 7*, 476−482.
(18) Zhang, L., Peritz, A., and Meggers, E. (2005) A simple glycol nucleic acid. *J. Am. Chem. Soc. 127*, 4174−4175.
(19) Acevedo, O. L., and Andrews, R. S. (1996) Synthesis of propane-2,3-diol combinatorial monomers. *Tetrahedron Lett. 37*, 3911−3934.
(20) Cook, R. M., Lyttle, M., and Dick, D. *Dark quenchers for donor-acceptor energy transfer*, U.S. Patent 7,109,312, 2006.
(21) Freville, F., Richard, T., Bathanay, K., and Moreau, S. (2006) Targeting of single-stranded oligonucleotides through metal-induced cyclization of short complementary strands. *Helv. Chim. Acta 89*, 2958−2973.
(22) Bonnet, G., Tyagi, S., Libchaber, A., and Kramer, F. R. (1999) Thermodynamic basis of the enhanced specificity of structured DNA probes. *Proc. Natl. Acad. Sci. U. S. A. 96*, 6171−6176.
(23) Morgan, J. R., Lyon, R. P., Maeda, D. Y., and Zebala, J. A. (2008) Snap-to-it probes: Chelate-constrained nucleobase oligomers with enhanced binding specificity. *Nucleic Acids Res. 36*, 3522−3530.
(24) Goritz, M., and Kramer, R. (2005) Allosteric control of oligonucleotide hybridization by metal-induced cyclization. *J. Am. Chem. Soc. 127*, 18016−18017.
(25) Yang, R., Jin, J., Long, L., Wang, Y., Wang, H., and Tan, W. (2009) Reversible molecular switching of molecular beacon: Controlling DNA hybridization kinetics and thermodynamics using mercury(ii) ions. *Chem. Commun.*, 322−324.
(26) Meggers, E., Holland, P. L., Toman, W. B., Romesberg, F. E., and Schultz, P. G. (2000) A novel copper-mediated DNA base pair. *J. Am. Chem. Soc. 122*, 10714−10715.
(27) Weizman, H., and Tor, Y. (2001) 2,2′-bipyridine ligandoside: A novel building block for modifying DNA with intra-duplex metal complexes. *J. Am. Chem. Soc. 123*, 3375−3376.
(28) Atwell, S., Meggers, E., Spraggon, G., and Schultz, P. G. (2001) Structure of a copper-mediated base pair in DNA. *J. Am. Chem. Soc. 123*, 12364−12367.
(29) Clever, G. H., Kaul, C., and Carell, T. (2007) DNA–metal base pairs. *Angew. Chem., Int. Ed. Engl. 46*, 6226−6236.
(30) Müller, J. (2008) Metal-ion-mediated base pairs in nucleic acids. *Eur. J. Inorg. Chem.*, 3749−3763.
(31) Zhang, L., and Meggers, E. (2005) An extremely stable and orthogonal DNA base pair with a simplified three-carbon backbone. *J. Am. Chem. Soc. 127*, 74−75.
(32) Heuberger, B. D., Shin, D., and Switzer, C. (2008) Two watson-crick-like metallo base-pairs. *Org. Lett. 10*, 1091−1094.
(33) Ono, A., and Togashi, H. (2004) Highly selective oligonucleotide-based sensor for mercury(II) in aqueous solutions. *Angew. Chem., Int. Ed. Engl. 43*, 4300−4302.
(34) Ehrenschwender, T., Barth, A., Puchta, H., and Wagenknecht, H. A. (2012) Metal-mediated DNA assembly using the ethynyl linked terpyridine ligand. *Org. Biomol. Chem. 10*, 46−48.
(35) Kim, Y., Yang, C. J., and Tan, W. (2007) Superior structure stability and selectivity of hairpin nucleic acid probes with an l-DNA stem. *Nucleic Acids Res. 35*, 7279−7287.

(36) Grossmann, T. N., Roglin, L., and Seitz, O. (2007) Triplex molecular beacons as modular probes for DNA detection. *Angew. Chem., Int. Ed. Engl. 46*, 5223−5225.
(37) Dubertret, B., Calame, M., and Libchaber, A. J. (2001) Single-mismatch detection using gold-quenched fluorescent oligonucleotides. *Nat. Biotechnol. 19*, 365−370.
(38) Crey-Desbiolles, C., Ahn, D. R., and Leumann, C. J. (2005) Molecular beacons with a homo-DNA stem: Improving target selectivity. *Nucleic Acids Res. 33*, e77.
(39) Bourdoncle, A., Estevez Torres, A., Gosse, C., Lacroix, L., Vekhoff, P., Le Saux, T., Jullien, L., and Mergny, J. L. (2006) Quadruplex-based molecular beacons as tunable DNA probes. *J. Am. Chem. Soc. 128*, 11094−11105.
(40) Tanaka, K., Tengeiji, A., Kato, T., Toyama, N., and Shionoya, M. (2003) A discrete self-assembled metal array in artificial DNA. *Science 299*, 1212−1213.