Regulating transition metal catalysis through interference by short RNAs

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Transition metal-catalyzed chemical transformations mediated by biological stimuli would enable the development of a platform for the communication of synthetic chemical systems with native biological processes. However, this remains a challenge, in part due to the incompatibility of many types of transition metal catalysts with biological conditions and the resulting difficulty in controlling their reactivity. Here we report the discovery of a Au(I)-DNA hybrid catalyst that is compatible with biological media and whose reactivity can be regulated by small complementary nucleic acid sequences. The development of this catalytic system was enabled by the discovery of a novel Au(I) metal-mediated base pair. We find that Au(I) binds selectively to double stranded DNA containing C–T mismatches and stabilizes these complexes by 7 °C. In the Au(I)-DNA catalyst’s latent state, the Au(I) ion is sequestered by the mismatch such that it is coordinatively saturated, rendering it catalytically inactive. Upon addition of an RNA or DNA strand that is complementary to the latent catalyst’s oligonucleotide backbone, catalytic activity is induced leading to a 7-fold increase in formation of a fluorescent product, forged through a Au(I)-catalyzed hydroamination reaction. Moreover, we demonstrate that regulation of this abiotic chemical reactivity is highly sequence selective, as 1-nucleotide and 2-nucleotide mismatched complements underperform the perfect complement. Further development of this catalytic system will expand not only the chemical space available to synthetic

biological systems but also allow for temporal and spatial control of transition metal catalysis through gene transcription.

The use of biocatalysis in synthetic chemistry is emerging as a powerful strategy for the construction of complex molecules^{1, 2}. Protein enzymes, utilized in isolated form^{3, 4}, as part of constructed artificial pathways^{5, 6}, or encapsulated within cells programmed to express them^{7, 8}, often form reaction products efficiently and stereoselectively under mild conditions. While many of the recently developed biocatalytic transformations are mechanistically similar to native biochemical processes, several reported systems feature distinctly abiotic transformations, where the products of the reactions arise through a mechanism hitherto unknown in biology. Examples include Ru-catalyzed olefin metathesis reactions in the artificial active site of an evolved streptavidin protein⁹ and Ir- and Fe-catalyzed metal-carbenoid and nitrenoid insertion reactions from evolved P450 enzymes^{10, 11, 12}. In these systems, abiotic chemical reactivity is developed through directed evolution¹³, construction of novel metalloenzymes *via* transmetallation reactions¹⁴, a posttranslational metallation¹⁵, or some combination thereof¹⁶. It is doubtless that as these strategies improve, the availability of protein enzymes that catalyze novel abiotic transformations will advance in unison. However, chemical concepts allowing for control of biocatalytic reactions by biological stimuli, a goal that would lead to advances in synthetic biology and chemical biology, have yet to be explored fully^{17, 18}.

Inspired by current hypotheses concerning the role of ribozymes in biogenesis^{19, 20}, the stimuli-responsiveness of riboswitches and molecular beacons, and the well-established propensity of late transition metals to bind nucleic acids in a sequence selective manner^{21, 22}, our group recently became interested in the development of biocatalytic systems composed of nucleic acids and transition metals that mediate chemical reactions. Here, we envisioned the

application of metal-mediated base pairs²³ (MMBPs) comprised of catalytically relevant transition metal centers and duplex DNA composed exclusively of canonical nucleobases. Specifically, toehold-stem-loop oligonucleotide hairpins could be designed with MMBPs such that the metal reactivity would be regulated through outer sphere, sense/antisense interactions with small RNA/DNA. Here, strand displacement reactions would dictate the coordination sphere of the reactive metal center and influence substrate binding events (**Figure 1**). In this scenario, chemical transformations mediated by transition metal species could then be “controlled” by inherently biological molecules. The successful demonstration of this concept could open the door to genotype-specific transition metal catalysis and the development of new biosynthetic pathways that feature transformations with no biological equivalent. Here we report the initial steps toward achieving this goal. We find that an unprecedented Au(I) MMBP is formed through the treatment of DNA hairpins possessing a C–T mismatch with an appropriate Au(I) precursor. Importantly, the rate at which this organometallic complex mediates an abiotic hydroamination reaction is dramatically increased when exposed to short strands of sequence-complementary DNA or RNA. This study represents an early example of an organometallic catalyst that can be regulated by biological stimuli.

In our initial formulation, we envisioned the design of structured oligonucleotides that possess a sequence specific, transition metal-binding motif. While several transition metal species are known to complex both DNA and RNA through intrastrand or interstrand binding modes, the catalytic activity of these species has been sparsely investigated. This is likely due to the lack of studies concerning catalytically relevant metals. For example, Cisplatin, the privileged anti-cancer Pt(II) complex, binds intrastrand DNA between G–G and G–A bases²⁴. Barton and coworkers have shown that coordinatively-saturated Ru(II) and Rh(III) complexes

intercalate dsDNA at mismatches and abasic sites^{25, 26}. Moreover, Ag(I) and Hg(II) ions are well known to selectively form metal-mediated base pairs between C–C²⁷ and T–T^{28, 29} mismatches, respectively. Here, metal ion intercalation between the canonical bases (C–C and T–T) leads to the formation of base-metal bonds that are energetically similar to hydrogen bonding of matched nucleobases, thus increasing the thermal stability of dsDNA containing mismatches by 2–9 °C³⁰. The reported sequence selectivity of metal binding is exquisite, as primer extension experiments in the presence of these metal ions leads to incorporation of T–T and C–A mismatches into the extended product strand, driven by the formation of stable T–Hg(II)–T and C–Ag(I)–A MMBPs³¹.

a) Metal incorporation and activation controlled through hybridization

b) Gold MMBP and subsequent unveiling of active Au(I) complex

Figure 1. a) Proposed formation of active catalyst from DNA-metal complex. b) Putative binding of Au(I) into C–T mismatch and active Au(I) species formed through hybridization.

The reported Ag- and Hg-mediated base pairs inspired us to consider the construction of a Au-mediated base pair, as Au(I) shares a similar preference for linear coordination geometries. Additionally, in bioactive Au(I) complexes, interactions between the gold center and DNA have been implicated as modes of action towards disease treatment^{32, 33}. Interestingly, the potential for interstrand binding of Au(I) to dsDNA was originally proposed over three decades ago by Blank

and Dabrowiak³⁴. Spectroscopic experiments support the binding of Au(III) and Au(I) to the N7 of guanine and also support the formation of a dimeric Au(II) complex between two cytosine nucleosides^{35, 36, 37, 38}. Despite these theoretical predictions and experimental studies, the formation of a Au(I)-MMBP has not yet been reported. In fact, several early reports attempted their formation unsuccessfully utilizing simple AuCl and AuCl₃ salts^{39, 40}. In our hands, use of these inorganic salts led to the rapid formation of purple precipitates (presumably Au-nanoparticles) when exposed to dsDNA bearing a C–T mismatch and we noted no increase in thermal stability (**SI, Figure S3**).

Results and Discussion

Informed by the homogeneous transition metal catalysis community, we examined different Au(I) precursors^{41, 42}. We were gratified to find that thermal stability analysis of a 14-mer dsDNA possessing a C–T mismatch showed a significant increase in thermal stability (T_m) when exposed to one equivalent of (Me₂S)AuCl (**Figure 2a**), with no apparent formation of Au-nanoparticles. Moreover, titration experiments indicated the incorporation of a single gold ion into the dsDNA (**Figure 2b**). Circular dichroism (CD) experiments support preserved helicity upon exposure to (Me₂S)AuCl, suggesting the formation of a Au-MMBP with uninterrupted helicity and not the formation of complex secondary structures such as G-quadruplexes, which are known to form in the presence of metal ions⁴³ (**SI, Figure S4 and S5**). In addition to photophysical analysis, mass spectrometry studies support incorporation of a single Au(I) ion as the base peak of recorded spectra coincided with the mass of the addition of one gold ion. Importantly, in control studies utilizing a 14-mer containing no mismatch, there were minimal Au-adducts observed (**SI, Figure S7**).

Experimental and computational studies were carried out to elucidate potential binding modes. The ligation of gold to the mismatch sequence is highly pH dependent, with a thermal stability T_m increase of 5 °C at pH 5.5 and 22 °C at pH 8.5, compared to 7 °C at neutral pH (**Figure 2c**). This behavior is consistent with binding of gold to the acidic, endocyclic N3 of thymine (**Figure 2d**). Moreover, previous crystallographic and NMR studies of nucleoside-Au complexes support binding of the metal center to the deprotonated N3 of *I*-methylthymidine and the N3 of cytosine^{35, 36, 37}. This is congruent with common Au(I) complexes that favor linear coordination of one neutral donating “L-type” ligand and one anionic “X-type” ligand^{44, 45}. This binding mode was further investigated by density functional theory (DFT) studies, leading to the proposed structure of the T–Au(I)–C metal-mediated base pair (**3**) shown in **Figure 2d**³⁸. The proposed binding of the Au(I) center between the N3 of cytosine and the deprotonated N3 of thymine is energetically favorable and consistent with the proposed structures of previously reported T–Hg(II)–C (**1**) and T–Ag(I)–C (**5**) MMBPs²⁷. In addition, computational analyses of these metal-DNA complexes suggest that a Au(I) MMBP is 14 kcal/mol more stable than a Hg(II) MMBP²⁷ (**1**) and 30 kcal/mol more stable than a Ag(I) MMBP^{27, 46} (**2**), supporting our experimental observations (**Figure 2d**).

Figure 2. a) UV melting curve of duplex **CT1** (5' – CCT TTC TTT CCC TC – 3' • 5' – GAG GGA CAG AAA GG – 3') with varying amounts of (Me₂S)AuCl, (●) 0 equiv (Me₂S)AuCl, (●) 0.4 equiv (Me₂S)AuCl, (●) 1.0 equiv (Me₂S)AuCl, (●) 1.4 equiv (Me₂S)AuCl, (●) 2.0 equiv (Me₂S)AuCl, (●) 3.0 equiv (Me₂S)AuCl, (●) 4.0 equiv (Me₂S)AuCl at pH 7.0. b) T_m versus equivalents of gold ion (calculated by accounting for non-specific binding) c) UV melting curve at pH 5.5 with no gold (◇) and 1 equivalent of (Me₂S)AuCl (◇) and pH 8.5 with no gold (●) and 1 equivalent of (Me₂S)AuCl (●). All T_m curves normalized using equation $A/A_0 = (A - A_{\min}) / (A_{\max} - A_{\min})$ where A is absorbance, $A_{\min}$ is minimum absorbance (at 15 °C), and $A_{\max}$ is maximum absorbance (at 90 °C). d) Proposed structure of T-Au(I)-C mismatch based on calculated complexes of Hg(II) and Ag(I) with C-T mismatch. Calculated binding energy of each metal through N3 of cytosine and N3 of thymine where dR = Me. Gibb's free energies reported in kcal/mol calculated with B3LYP-D3(BJ)/SDD-6-311++G(2df,2pd)SMD(H₂O)//B3LYP-D3(BJ)/LANL2DZ-6-31G(d)/SMD(H₂O). Energies are ΔG from B3LYP/6-311++G(2df,2pd)/SDD in H₂O (SMD model). ΔT_m reported curves in SI and previously reported values in parentheses²⁷.

Having successfully created a sequence-specific transition metal binding motif through formation of a Au(I)-mediated base pair, we moved forward with investigations of its catalytic properties. We hypothesized that the hairpin containing a C-T mismatch in the stem (**4**) would bind one equivalent of Au(I) to form latent DNA-Au complex **5** (**Figure 3a**). Upon exposure to a short, complementary DNA or RNA sequence, strand displacement would interfere with the Au-base bonds of the stable MMBP, forming an active complex **6**. We assessed the catalytic activity of this complex using pro-fluorophore **7**, which cyclizes to form fluorescent BODIPY **8** through

a Au-catalyzed hydroamination reaction, allowing for high throughput sequence optimization using a simple plate reader (**Figure 3b**).

We were pleased to find that treatment of latent DNA-Au catalyst **5** with 1 equivalent of a complementary short oligo (**cCTH5**) increases the fluorescence of a solution containing pro-fluorophore **7** by approximately 700% (**Figure 3c**). Kinetics based on fluorescence show that the initial rates of active complex **6** are higher than complex **5**. Interestingly, both putative complexes have similar substrate affinity ($K_M = 16 \mu\text{M}$), suggesting that this affinity is inherent to the Au(I) center. However, the initial rates are dramatically increased upon addition of the complement strand, wherein more Au(I) is accessible, resulting in a higher effective catalyst concentration. The background fluorescence induced by latent catalyst **5** may be due to unbound Au(I) or Au(III) formed through decomposition of precatalyst **5** in solution⁴⁷. In thermal stability studies, we determined that Au(III) does not bind C–T mismatches, but does catalyze the formation of fluorophore **8** (SI-3).

Figure 3. a) Proposed incorporation of Au(I) into DNA hairpin followed by hybridization to expose a coordination site on the metal center. b) Profluorescent BODIPY cyclization catalyzed by addition of DNA-Au complex. c) Kinetic model showing initial rates of **5** and **6** versus concentration of BODIPY substrate (**7**) with calculated $V_{\max}$ (μMmin^{-1}) and K_M (μM).

To further support that observed reactivity was due to the modulation of the Au(I)-mediated base pair in a sequence-specific manner, we carried out further control experiments. First, we wanted to confirm that a hairpin bearing a Au-mediated base pair could undergo strand-displacement reactions, analogous to canonical structured oligonucleotides, despite the inclusion of strong Au-base bonds. A FRET probe was designed to probe this key elementary step. Fluorescein was appended to the 5' end of hairpin **5** and a distal quencher dabcyI was appended to the 3' end, forming FRET probe **9** (**Figure 4a**). Here, strand displacement with a complementary sequence would yield fluorescent duplex **10**⁴⁸. Gratifyingly, the addition of complement to either probes containing a Au(I) MMBP or a mismatch without Au(I) resulted in an increase in fluorescence due to spatial separation between the donor and quencher (**Figure 4b**). This result confirms that stabilization of the duplex due to strong Au(I) base bonds does not hinder strand displacement reactions.

Additionally, exposure of a solution of (Me₂S)AuCl and a hairpin containing no DNA mismatches (a G–C base pair instead of a C–T mismatch in complex **5**) to the complement strand (**cCTH5**) did not result in an increase of fluorescence (**Figure 4c**). This suggests that the formation of a precatalytic complex, such as complex **5**, is dependent on the presence of a mismatch. Moreover, when the complementary strand (**cCTH5**) was replaced with non-complementary, purine-rich, random DNA sequence **R1**, significant activation of the latent DNA-Au catalyst **5** does not occur, as minimal increase in fluorescence is observed (**Figure 4d**). In sequence-selectivity experiments, the highest increase in fluorescence is exhibited in the presence of the exact complementary sequence (**cCTH5**), whereas addition of complements possessing single (1-nt) or double (2-nt) mismatches (**TMs/3s/5s** and **TMd/3d/5d**, respectively) result in lower fluorescence intensity, with the 2-nt mismatched sequence **TMd** resulting in the

lowest levels of fluorescence (**Figure 4d**). This further supports our hypothesis that complement hybridization is required to form the active catalyst.

Biological systems contain many potential ligands for metal binding, leading to difficulties developing biocompatible complexes and controlling abiotic metal reactivity under physiological conditions. Specifically, Au(I) and Au(III) have been shown to readily form nanoparticles in the presence of small biomolecules, such as short nucleic acids, amino acids, and sugars⁴⁹. To assess the stability and reactivity of complex **5** under biological conditions, we exposed our catalyst system to mixtures of nucleic acids and biologically relevant solutions. We were pleased to find that the addition of a mixture of random sequence **R1** and complement **cCTH5** to latent catalyst **5**, led to a 1.5-fold increase in fluorescence, a significant increase in reactivity when compared to complex **5** alone (**Figure 4e**). In addition, complex **6** showed significant catalytic activity under conditions containing urine or saliva solutions, with nearly a 2-fold and 4-fold increase in yield respectively. This result is especially remarkable due to the fact that these solutions contain albumin, an enzyme with a considerable amount of sulfur containing residues⁵⁰, and urea, a small molecule well known to denature DNA⁵¹. These examples suggest that Au(I) binding to hairpin **4** protects the metal ion from non-productive binding to nucleic acids, proteins, and small biomolecules. These findings suggest that control of the reactivity of species such as latent catalyst **5** can be achieved in biologically relevant environments.

a) Turn on probe for hybridization of complement to hairpin sequence with and without gold(I)

b) FRET probe with and without Au(I)

c) Hairpin containing G-C (GCH5) instead of T-C (TCH5)

d) Mismatch selectivity with 1-nt and 2-nt mismatched complements

e) Reaction under various biological conditions

f) RNA complement (RcCth5) and DNA complement (cCth5)

Name	Sequence (5' to 3')
cCth5	GAT GTC GTG CTG AAA AC
R1	GAG GGA TAG AAA GG
TMd	GAG ATC GTG CTG AAA AC
5d	GAT GTC GGG ATG AAA AC
3d	GAT GTC GTG CTG AAA GA
5s	GAT GTC GTG ATG AAA AC
TMs	GAT ATC GTG CTG AAA AC
3s	GAT TGC GTG CTG AAA AA

Figure 4. a) Hybridization of hairpin containing FRET pair (FAM as donor and DabcyI as quencher) and fluorescence increase corresponding to hybridization. b) Fluorescence intensity of FRET probe with and without Au(I). c) Control experiment with hairpin sequence containing G-C match instead of C-T mismatch (GCH5 : 5' – CGT GCT GTT TTC AGC ACG ACA TC – 3'). d) Mismatch selectivity of hairpin with completely mismatched sequence R1 and 1-nt and 2-nt mismatches (TMs, 5s, 3s, TMd, 5d, and 3d). e) Fluorescence intensity of complex 5 and complex 6 (Figure 3) without any additive and with added nucleic acids, R1, and biological fluids, urine and saliva f) Fold increase in yield following the addition of RcCth5 (5' – GAU GUC GUG CUG AAA AC – 3').

In living organisms, it is known that small RNA sequences selectively bind to complementary nucleotide acids and promote nucleotide degradation *via* the recruitment of active enzymes. These systems in which small RNA sequences modulate gene expression have been utilized as biological tools and therapeutics⁵². We envisioned an analogous system in which our DNA-Au complex could be regulated by small RNA sequences, ultimately controlling the chemical reactivity of transition metal DNAzymes. Gratifyingly, the addition of a short

complementary RNA strand (**RcCTH5**) to latent Au-DNA catalyst **5** results in nearly a 600% increase in yield, akin to the DNA-based complement (**cCTH5**) (**Figure 4f**). This increase in fluorescence, likely due the formation of a hybrid DNA-RNA complex, demonstrates that there is potential for the use of this type of system to perform catalytic reactions in response to gene transcription.

Conclusion

The synergy of synthetic catalysts and biomolecules remains underdeveloped in chemistry and biology. There are several potential applications for such biocommunicative organometallic species, including the development of chemical biology probes capable of signal amplification through catalyst turnover, the ability to treat diseases through gene-specific cytotoxic reactions and catalytic formation of therapeutics in targeted cells, the construction of artificial biosynthetic pathways possessing abiotic chemical transformations, and the catalytic modification of biomolecules. This work represents an early proof-of-principle study where the innately abiotic reactivity of a transition metal catalyst can be regulated through interactions with native biological molecules such as DNA and RNA.

References

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1. Hudlicky, T & Reed, J. W. Applications of biotransformations and biocatalysis to complexity generation in organic synthesis. *Chem. Soc. Rev.* **38**, 3117–3132 (2009).
 2. Koeller, K. M. & Wong, C. H. Enzymes for chemical synthesis. *Nature* **409**, 232–240 (2001).
 3. Huang, F. C. et al. Preparation of (R)- and (S)-mevalonic acids. *J. Am. Chem. Soc.* **97**, 4144–4145 (1975).
 4. Whitesides, G. M. & Wong C.-H. Enzymes as catalysts in synthetic organic chemistry. *Angew. Chem., Int. Ed.* **24**, 617–718 (1985).
 5. Fessner, W.-D. & Walter, C. “Artificial metabolisms” for the asymmetric one-pot synthesis of branched-chain saccharides. *Angew. Chem., Int. Ed.* **31**, 614–616 (1992).
 6. Schrittwieser, J. H., Vilkogne, S., Hall, M. & Kroutil, W. Artificial biocatalytic linear cascades for preparation of organic molecules. *Chem. Rev.* **118**, 270–348 (2018).
 7. Mihovilovic, M. D. et al. Asymmetric Baeyer–Villiger Oxidations of 4-Mono- and 4,4-disubstituted cyclohexanones by whole cells of engineered *Escherichia coli*. *J. Org. Chem.* **66**, 733–738 (2001).

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8. Wachtmeister, J. & Rother, D. Recent advances in whole cell biocatalysis techniques bridging from investigative to industrial scale. *Curr. Opin. Biotechnol.* **42**, 169–177 (2016).
 9. Jeschek, M. et al. Directed evolution of artificial metalloenzymes for in vivo metathesis. *Nature* **537**, 661–665 (2016).
 10. Key, H. M., Dydio, P., Clark, D. S. & Hartwig, J. F. Abiological catalysis by artificial haem proteins containing noble metals in place of iron. *Nature* **534**, 534–537 (2016).
 11. Dydio, P., Key, H. M., Hayashi, H., Clark, D. S. & Hartwig, J. F. Chemoselective, enzymatic C-H bond amination catalyzed by a cytochrome P450 containing an Ir(Me)-PIX cofactor. *J. Am. Chem. Soc.* **139**, 1750–1753 (2017).
 12. Coelho, P. S., Brustad, E. M., Kanna, A. & Arnold, F. H. Olefin Cyclopropanation via Carbene Transfer Catalyzed by Engineered Cytochrome P450 Enzymes. *Science* **339**, 307–310 (2013).
 13. Chen, K. & Arnold, F. H. Enzyme engineering for nonaqueous solvents: random mutagenesis to enhance activity of Subtilisin E in polar organic media. *Nat. Biotechnol.* **9**, 1073–1077 (1991).
 14. Dydio P. et al. An artificial metalloenzyme with the kinetics of native enzymes. *Science* **354**, 102–106 (2016).
 15. Wilson, M. E. & Whitesides, G. M. Conversion of a protein to a homogeneous asymmetric hydrogenation catalyst by site-specific modification with a diphosphinerhodium(I) moiety. *J. Am. Chem. Soc.* **100**, 306–307 (1978).
 16. Illie, A. & Reetz, M. T. Directed evolution of artificial metalloenzymes. *Isr. J. Chem.* **55**, 51–60 (2015).
 17. Boersma, A. J., Klijn, J. E., Feringa, B. L. & Roelfes, G. DNA-based asymmetric catalysis: sequence-dependent rate acceleration and enantioselectivity. *J. Am. Chem. Soc.* **130**, 11783–11790 (2008).
 18. Prusty, D. K., Kwak, M., Wildeman, J. & Herrmann, A. Modular assembly of a Pd catalyst within a DNA scaffold for the amplified colorimetric and fluorimetric detection of nucleic acids. *Angew. Chem., Int. Ed.* **51**, 11894–11898 (2012).
 19. Joyce, G. F. RNA evolution and the origins of life. *Nature* **338**, 217–224 (1989).
 20. Higgs, P. G. & Lehman, N. The RNA world: molecular cooperation at the origins of life. *Nat. Rev. Genet.* **16**, 7–17 (2015).
 21. Barton, J. K. Metal/Nucleic Acid Interactions. *Bioinorganic Chemistry*. University Press, **455** (1994).
 22. Zhou, W., Saran, R. & Liu, J. Metal sensing in DNA. *Chem. Rev.* **117**, 8272–8325 (2017).
 23. Takezawa, Y. & Shionoya M. Metal-mediated DNA base pairing: alternatives to hydrogen-bonded Watson-Crick base pairs. *Acc. Chem. Res.* **45**, 2066–2076 (2012).
 24. Pil, P. M. & Lippard, S. J. Specific binding of chromosomal protein HMG1 to DNA damaged by the anticancer drug cisplatin. *Science* **256**, 234–237 (1992).
 25. Boynton, A. N., Marcelis, L. & Barton, J. K. $[\text{Ru}(\text{Me}_4\text{phen})_2\text{dppz}]^{2+}$, a light switch for DNA mismatches. *J. Am. Chem. Soc.* **138**, 5020–5023 (2016).
 26. Kisko, J. L. & Barton, J. K. Recognition of DNA base pair mismatches by a cyclometalated Rh(III) intercalator. *Inorg. Chem.* **39**, 4942–4949 (2000).
 27. Ono, A. et al. Specific interactions between silver(I) ions and cytosine–cytosine pairs in DNA duplexes. *Chem. Commun.* **39**, 4825–4827 (2008).
 28. Miyake, Y. et al. Mercury^{II}-mediated formation of thymine–Hg^{II}–thymine base pairs in DNA duplexes. *J. Am. Chem. Soc.* **128**, 2172–2173 (2006).

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29. Kondo, J. et al. Crystal structure of metallo DNA duplex containing consecutive Watson–Crick–like T–Hg^{II}–T base pairs. *Angew. Chem., Int. Ed.* **53**, 2385–2388 (2014).
30. Ono, A., Torigo, H., Tanaka, Y. & Okamoto, I. Binding of metal ions by pyrimidine base pairs in DNA duplexes. *Chem. Soc. Rev.* **40**, 5855–5866 (2011).
31. Urata, H., Yamaguchi, Y., Funai, T., Matsumura, Y., & Wada, S. Incorporation of thymine nucleotides by DNA polymerases through T–Hg^{II}–T base pairing. *Angew. Chem., Int. Ed.* **49**, 6516–6519 (2010).
32. Ott, I. On the medicinal chemistry of gold complexes as anticancer drugs. *Coord. Chem. Rev.* **253**, 1670–1681 (2009).
33. Tieckink, E. R. Gold derivatives for the treatment of cancer. *Crit. Rev. Oncol. Hematol.* **42**, 225–248 (2002).
34. Blank, C. E. & Dabrowiak, J. C. Absorption and circular dichroism studies of a gold(I)–DNA complex. *J. Inorg. Biochem.* **21**, 21–29 (1984).
35. Hadjiliadis, N., Pneumatikakis, G. & Basosi, R. Gold complexes of purine and pyrimidine nucleosides. *J. Inorg. Biochem.* **14**, 115–126 (1981).
36. Bressan, M., Ettorre, R. & Rigo, P. NMR studies of the interaction between cytosine and gold complexes. *J. Magn. Reson.* **26**, 43–47 (1977).
37. Faggiani, R., Howard-Lock, H. E., Lock, C. J. L. & Turner, M. A. The reaction of chloro(triphenylphosphene)gold(I) with 1–methylthymine. *Can J Chem* **65**, 1568–1575 (1987).
38. Rosopulos, Y., Nagel, U. & Beck, W. Metal complexes with biologically important ligands, XXXIV. allyl–Palladium(II) and triphenylphosphane gold(I) complexes with nucleobases and nucleosides. *Chem. Ber.* **118**, 931–942 (1985).
39. Zimmerman, N., Meggers, E. & Schultz, P. G. A novel silver(I)-mediated DNA base pair. *J. Am. Chem. Soc.* **124**, 13684–13685 (2002).
40. Wang, J.-B., Wu, Q.-Q., Min, Y.-Z., Liu, Y.-Z. & Song, Q.-H. A novel fluorescent probe for Au(III)/Au(I) ions based on an intramolecular hydroamination of a Bodipy derivative and its application to bioimaging. *Chem. Commun.* **5**, 744–746 (2012).
41. Fremont, P., Scott, N. M., Stevens, E. D. & Nolan, S. P. Synthesis and structural characterization of N-heterocyclic carbene gold(I) complexes. *Organometallics* **24**, 2411–2418 (2005).
42. Barnard, P. J., Baker, M. V., Berners-Price, S. J., Skelton, B. W. & White, A. H. Dinuclear gold(I) complexes of bridging bidentate carbene ligands: synthesis, structure and spectroscopic characterization. *J. Chem. Soc., Dalton Trans.* **7**, 1038–1047 (2004).
43. Bhattacharyya, D., Arachchilage, G. M. & Basu, S. Metal cations in G-quadruplex folding and stability. *Front Chem*, **4**, doi:10.3389/fchem.2016.00038 (2016).
44. Schwerdtfeger, P., Hermann, H. L. & Schmidbaur, H. Stability of the gold(I)–phosphine bond. A comparison with other group 11 elements. *Inorg. Chem.* **42**, 1334–1342 (2003).
45. Caravjal, M. A., Novoa, J. J. & Alvarez, S. Choice of coordination number in d¹⁰ complexes of group 11 metals. *J. Am. Chem. Soc.* **126**, 1465–1477 (2004).
46. Urata, H., Yamaguchi, E., Nakamura, Y. & Wada, S. I. Pyrimidine–pyrimidine base pairs stabilized by silver(I) ions. *Chem. Commun.* **47**, 941–943 (2011).
47. Kumar, M., Jasinski, J., Hammond, G. B. & Zu, B. Alkyne/alkene/allene–induced disproportionation of cationic gold(I) catalyst. *Chem. Eur. J.* **20**, 3113–3119 (2014).

-
48. Marras, S. A. E., Kramer, F. R. & Tyagi, S. Efficiencies of fluorescence resonance energy transfer and contact—mediated quenching in oligonucleotide probes. *Nucleic Acids Res.* **30**, e122 (2002).
49. Kharissova, O. V., Dias, H. V. R., Kharisov, B. I., Pèrez, B. O. & Pèrez, V. M. J. The greener synthesis of nanoparticles. *Trends Biotechnol.* **31**, 240–248 (2013).
50. Carter, D. C. & Ho, J. X. Structure of serum albumin. *Adv. Protein Chem.* **45**, 153–176 (1994).
51. Herskovits, T. T. Nonaqueous solutions of DNA; denaturation by urea and its methyl derivatives. *Biochemistry* **2**, 335–340 (1963).
52. McManus, M. T. & Sharp, P. A. Gene silencing in mammals by small interfering RNAs. *Nat. Rev. Genet.* **3**, 737–747 (2002).

Methods

Thermal stability measurements. Solutions of 3.5 mM mismatch-containing DNA were prepared in 0.75 mM phosphate buffer, pH 7.0, and 150 mM NaClO₄. Samples were annealed at 90 °C in a heating block for 10 minutes and allowed to cool to room temperature over 30 minutes. One equivalent (3.5 mM) of (Me₂S)AuCl was added in methanol such that the solution contained 60:1 H₂O:MeOH (v/v). Melting temperature profiles were recorded using HP-8453 spectrophotometer with HP-89090A Peltier temperature controller from 15–90 °C at 5 °C min⁻¹ with a hold time of 1 min. Relative absorbance at 260 nm, $A_{260nm} = (A_t - A_{15\text{ }^\circ\text{C}}) / (A_{90\text{ }^\circ\text{C}} - A_{15\text{ }^\circ\text{C}})$, vs. temperature(°C) curves were fitted using GraphPad Prism 7.0c

Fluorescent DNA-Au(I) hydroamination reactions. Samples for catalysis were prepared by heating the buffered DNA solution without metal at 90 °C in a heating block for 10 minutes. Solutions were cooled to room temperature over 30 minutes. Then one equivalent (2.38 μl, 420 μM stock solution) of (Me₂S)AuCl as a solution in acetone was added. Following the addition of the gold solution, the complement sequence was added and the solution was allowed to incubate at room temperature for 5 minutes. Then a solution of BODIPY **7** in ethanol was added resulting in a final solution containing (1:1:0.02 H₂O:EtOH:(CH₃)₂CO). Reactions contain 10 μM DNA hairpin, 10 μM complement sequence, 250 mM NaClO₄, and 40 μM BODIPY **7**. Positive control contains no DNA. Negative control contains no DNA nor (Me₂S)AuCl. No complement reactions: 0 equivalents of complement sequence added to reaction. 1 equivalent of complement: 1 equivalent (10 μM) complement sequence added to reaction. Progress of reactions was determined by fluorescence intensity. All fluorescence values reported in arbitrary units (AU). A standard curve containing various concentrations of BODIPY **8** was used to calculate a yield of product (60 μM, 30 μM, 15 μM, 7.5 μM, 3.25 μM, 1.63 μM, and 0.81 μM)

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Author Contributions H. M. N. conceived of project and designed experiments. S. A. G., H. R. M., and N. J. C. designed and conducted experiments. T. R. B. designed and conducted computations. H. M. N., S. A. G., and H. R. M. prepared the manuscript.

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Regulating transition metal catalysis through interference by short RNAs

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1. Materials and Methods

Unless stated otherwise, reactions were performed in flame-dried glassware under an atmosphere of nitrogen. Benzene, THF, dichloromethane, and dimethylformamide were degassed and dried in a JC Meyer solvent system. SilicaFlash P60 silicagel (230–400 mesh) was used for flash chromatography. NMR spectra were recorded on a Bruker AV-300 (1H), Bruker AV-400 (1H, 13C), Bruker DRX-500 (1H), and Bruker AV-500 (1H, 13C). 1H NMR spectra are reported relative to CDCl₃ (7.26 ppm). All oligonucleotides were purchased through Integrated DNA Technology with standard desalting unless otherwise specified. Samples for thermal denaturation, mass spectrometry studies, and catalysis were prepared by heating the buffered DNA solution without metal at 90 °C in a heating block for 10 minutes then cooled to room temperature for 30 minutes. Once cool, the metal solution was added. In thermal denaturation experiments, all absorbances were measured at 260 nm using HP-8453 spectrophotometer with HP-89090A Peltier temperature controller from 15–90 °C at 5 °C min⁻¹ with a hold time of 1 min. Relative absorbance, $A_{260nm} = (A_t - A_{15^\circ C}) / (A_{90^\circ C} - A_{15^\circ C})$, vs. temperature (°C) curves were fitted using GraphPad Prism 7.0c. Fluorescence experiments were recorded on a Tecan Infinite M1000 Pro plate reader with the following conditions: 480 nm excitation, 510 nm emission, 8 mm excitation and emission bandwidth, 50 flashes with a frequency of 400 Hz, and a 10 ms delay time. Fluorescence data was collected on a JASCO-J715 CD spectrophotometer with a scan rate of 20 nm/min from 200 nm to 300 nm with 3 accumulations. Mass spectrometry data was collected on a Thermo Scientific Q Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer using negative ionization mode.

2.1 Thermal Stability Measurements

General Procedure for Sample Preparation for Thermal Stability Measurements

Solutions contained 3.5 μM DNA in buffer containing 0.75 mM sodium phosphate, pH 7, 150 mM NaClO_4 and 0 μM (0 eq) or 3.5 μM (1 eq) of $(\text{Me}_2\text{S})\text{AuCl}$ (60:1 $\text{H}_2\text{O}:\text{MeOH}$ v/v). DNA and buffer were heated at 90 $^\circ\text{C}$ for 10 minutes then allowed to cool to room temperature over 45 minutes. After the solution was cooled, $(\text{Me}_2\text{S})\text{AuCl}$ (210 μM stock solution in methanol) was added and the resulting solution was incubated at room temperature for 5 min before performing experiments. The reported data is based on 3 trials and graphed on GraphPad Prism 7.0c.

Table S1. Sequences used in thermal denaturation experiments:

Sequence Name	Strand 1	Strand 2
CT1	5'-GAG GGA C AG AAA GG-3'	5'-CCT TTC T TT CCC TC-3'
AT1	5'-GAG GGA A AG AAA GG-3'	5'-CCT TTC T TT CCC TC-3'
CG1	5'-GAG GGA C AG AAA GG-3'	5'-CCT TTC T GT CCC TC-3'

Figure S1. Duplex mismatch sequences with and without gold.

Thermal denaturation profiles of CT1, AT1, and CG1 in the presence of $(\text{Me}_2\text{S})\text{AuCl}$

a) CT1

b) AT1

c) CG1

Figure S2. Relative absorbance, $A_{260nm}=(A_t-A_{15^\circ C})/(A_{90^\circ C}-A_{15^\circ C})$, vs. temperature ($^\circ\text{C}$) curves for pyrimidine-mismatch-containing oligonucleotides, a) CT1 b) AT1 c) CG1, in the presence of $(\text{Me}_2\text{S})\text{AuCl}$. Solutions contained $3.5\ \mu\text{M}$ DNA in buffer containing $0.75\ \text{mM}$ sodium phosphate, pH 7, $150\ \text{mM}$ NaClO_4 and $1.4\ \mu\text{M}$ ($0.4\ \text{equiv}$) $3.5\ \mu\text{M}$ ($1\ \text{equiv}$), $4.9\ \mu\text{M}$ ($1.4\ \text{equiv}$), $7\ \mu\text{M}$ ($2\ \text{equiv}$) or $10.5\ \mu\text{M}$ ($3\ \text{equiv}$), $(\text{Me}_2\text{S})\text{AuCl}$ ($60:1\ \text{H}_2\text{O}:\text{MeOH}$ v/v).

Table S2. Thermal denaturation values for CT1 sequence

Thermal Denaturation – CT1			
[Au]	T_m ($^\circ\text{C}$)	ΔT_m ($^\circ\text{C}$)	Error
0 equiv	40.6	--	0.7
0.4 equiv	42.7	2.1	1.0
1 equiv	47.3	6.7	1.1
1.4 equiv	50.7	10.1	0.6
2 equiv	51.3	10.7	0.6
3 equiv	53.2	12.6	1.8

Table S3. Thermal denaturation values for control sequences AT1 and GC1

Thermal Denaturation – AT1 and GC1				
Sequence	0 equiv T_m ($^\circ\text{C}$)	1 equiv T_m ($^\circ\text{C}$)	ΔT_m ($^\circ\text{C}$)	Error
AT1	52.4	53.8	1.4	0.4
GC1	55.6	60.0	4.4	0.6

Melting temperatures calculated from the thermal denaturation profiles of pyrimidine-mismatch-containing oligonucleotides in the presence of various concentrations of $(\text{Me}_2\text{S})\text{AuCl}$. Melting temperatures and error calculated using the sigmoidal dose-response feature in GraphPad Prism 7.0c.

Thermal denaturation profiles of pyrimidine-mismatch-containing oligonucleotides in the presence of various Au precursors

Figure S3. Relative absorbance, $A_{260nm} = (A_t - A_{15^\circ C}) / (A_{90^\circ C} - A_{15^\circ C})$, vs. temperature ($^\circ C$) curves for pyrimidine-mismatch-containing oligonucleotides, CT1, in the presence of $(Me_2S)AuCl$, $AuCl_3$ or $(Ph_3P)AuCl$. Solutions contained $3.5 \mu M$ DNA in buffer containing 0.75 mM sodium phosphate, pH 7, 150 mM $NaClO_4$ and $3.5 \mu M$ (1 equiv) of Au precursor: $(Me_2S)AuCl$, $AuCl_3$ or $(Ph_3P)AuCl$ ($60:1$ $H_2O:MeOH$ v/v).

Table S4. Thermal denaturation values of CT1 with different gold precursors.

Thermal Denaturation – CT1			
Gold Precursor	T_m ($^\circ C$)	ΔT_m ($^\circ C$)	Error
No Au	40.6	—	0.7
$(Me_2S)AuCl$	47.3	6.7	1.1
$(Ph_3P)AuCl$	39.3	0.9	0.6
$AuCl_3$	41.2	0.6	1.2

Melting temperatures calculated from the thermal denaturation profiles of pyrimidine-mismatch-containing oligonucleotides in the presence of 1 equivalent of various gold precursors. Melting temperatures and error calculated using the sigmoidal dose-response feature in GraphPad Prism 7.0c.

2.2 Circular Dichroism (CD)

General Procedure for Sample Preparation for Circular Dichroism Spectroscopy

Solutions contained 3.5 μM DNA in buffer containing 0.75 mM sodium phosphate, pH 7, 150 mM NaClO_4 and 0 μM (0 equiv) or 3.5 μM (1 equiv) of $(\text{Me}_2\text{S})\text{AuCl}$ (60:1 $\text{H}_2\text{O}:\text{MeOH}$ v/v). DNA and buffer were heated at 90 $^\circ\text{C}$ for 10 minutes then allowed to cool to room temperature over 45 minutes. After the solution was cooled, 5 μl of $(\text{Me}_2\text{S})\text{AuCl}$ (210 μM stock solution in methanol) was added and the resulting solution was incubated at room temperature for 5 min before performing experiments. The reported data is based on 3 trials and graphed on GraphPad Prism 7.0c.

Table S5. Sequences used in circular dichroism experiments denaturation experiments.

Sequence Name	Strand 1	Strand 2
CT1	5'-GAG GGA CAG AAA GG-3'	5'-CCT TTC TTT CCC TC-3'
CTH	5'-CGT TCT GTT TTC AGC ACG-3'	N/A
TAH	5'-CGT TCT GTT TTC AGA ACG-3'	N/A

Figure S4. CD spectra of CT1 duplex in the presence of 0 equiv (0 eq) or 1 equiv (1 eq) $(\text{Me}_2\text{S})\text{AuCl}$.

Figure S5. CD spectra of CTH1 hairpin in the presence of 0 equiv (0 eq) or 1 equiv (1 eq) Me_2SAuCl .

Figure S6. CD spectra of TAH hairpin in the presence of 0 equiv (0 eq) or 1 equiv (1 eq) Me_2SAuCl .

2.3 Mass Spectrometry

Figure S7. Mass spectrometry of CT1 sequence a) without $(\text{Me}_2\text{S})\text{AuCl}$, and b) with 1 equivalent $(\text{Me}_2\text{S})\text{AuCl}$ ($5 \mu\text{M}$). Solutions contained $5 \mu\text{M}$ CT1, 50 mM NH_4OAc in 4:1 $\text{H}_2\text{O}:\text{MeOH}$ v/v.

3. DNA-Au(I) Hydroamination Reactions

3.1 General Procedure

Samples for catalysis were prepared by heating the buffered DNA solution without metal at 90 °C in a heating block for 10 minutes. After 10 minutes, the solutions were cooled to room temperature over 30 minutes. Once cool, the metal solution was added. Following metal addition, the complement sequence was added and the solution was allowed to equilibrate for 10 minutes before adding it to a solution of BODIPY **7** in ethanol resulting in a final solution containing (1:1:0.02 H₂O:EtOH:(CH₃)₂CO). Reactions contain 10 μM DNA hairpin, 10 μM complement sequence, 250 mM or 62.5 mM NaClO₄ and 40 μM BODIPY **7**. All other conditions/concentrations vary depending on experiment. Positive control contains no DNA. Negative control contains no DNA nor (Me₂S)AuCl. No comp (0 eq): 0 equivalents of complement sequence added to reaction. Comp (1 eq): 1 equivalent (10 μM) complement sequence added to reaction. Progress of reactions was determined by fluorescence intensity. All fluorescence values reported in arbitrary units. Fold increase in fluorescence was calculated based on equation $FI = (F_{1eq} - F_{0eq}) / (F_{0eq})$. Yields were calculated using a standard curve of product BODIPY **8** at known concentrations ranging from 3.25 μM to 60 μM, this was then fit to a line in excel and the equation was used to calculate the yield of fluorescent product for each reaction.

Figure S8. Gold catalyzed hydroamination reaction¹.

Table S6. Sequences used in catalysis experiments.

Sequence Name	Sequence
CTH5	5'-CGT TCT GTT TTC AGC ACG ACA TC-3'
cCTH5	5'-GAT GTC GTG CTG AAA AC-3'
cCTH5-5s	5'-GAT GTC GTG ATG AAA AC-3'
cCTH5-5d	5'-GAT GTC GGG ATG AAA AC-3'
cCTH5-3s	5'-GAT GTC GTG CTG AAA AA-3'
cCTH5-3d	5'-GAT GTC GTG CTG AAA GA-3'
cCTH5-TMs	5'-GAT ATC GTG CTG AAA AC-3'
cCTH5-TMd	5'-GAG ATC GTG CTG AAA AC-3'
ReCTH5	5'-GAU GUC GUG CUG AAA AC-3'
CGH5	5'-CGT GCT GTT TTC AGC ACG ACA TC-3'

3.2 Fluorescence Data

Figure S9. Fluorescence increase under standard conditions reported in general procedure.

Figure S10. Calculated percent yield of standard conditions based on standard curve.

Figure S11. Percent yield increase over 5 hours of standard conditions.

Figure S12. Percent yields of product with perfect complement (cCTH5) and various mismatched complements (R, TMd, 5d, 3d, TMs, 3s, 5s) at 250 mM sodium perchlorate.

Figure S13. Percent yields of product with the addition perfect complement (cCTH5) and various mismatched complements (R, TMd, 5d, 3d, TMs, 3s, 5s) at 62.5 mM sodium perchlorate.

Preparation of experiments containing biologically relevant fluids

Solutions contain 10 μM DNA hairpin, 10 μM complement sequence, and 62.5 mM NaClO_4 and 40 μM BODIPY 7 with 10 μM R1, or 30 % solutions of synthetic saliva (Artificial Saliva for Medical and Dental Research purchased through Pickering Laboratories) or synthetic urine (300 mM urea, 60 mM KCl, 128 mM NaCl, 30 mM Na_3PO_4 , 15 mM creatine, and 1 μM albumin powder).

4. Synthesis

4.1 Synthesis of BODIPY derivative (7)

3,5-dimethyl-4-(ethoxycarbonyl)pyrrole (SI-1): Synthesized according to reported literature. NMR spectra match those reported in literature².

4-bromo-3-nitrobenzaldehyde (SI-2): Synthesized from 4-bromobenzaldehyde according to reported literature. NMR spectra match those reported in literature³.

2-bromo-4-methylaniline (SI-3): Synthesized from 4-methylaniline according to reported literature. NMR spectra match those reported in literature⁴.

2-bromo-4-methyl-iodobenzene (SI-4): Synthesized according to reported literature. NMR spectra match those reported in literature⁴.

3-(2-bromo-4-methylphenyl)prop-2-yn-1-ol (SI-5): Synthesized from 2-bromo-4-methyliodobenzene according to reported literature. NMR spectra match those reported in literature¹.

2-bromo-1-(3-methoxyprop-1-ynyl)-4-methylbenzene (SI-6): Synthesized from 3-(2-bromo-4-methylphenyl)prop-2-yn-1-ol according to reported literature. NMR spectra match those reported in literature¹.

2'-(3-methoxyprop-1-yn-1-yl)-5'-methyl-2-nitro-[1,1'-biphenyl]-4-carbaldehyde (SI-7): Synthesized from 4-bromobenzaldehyde according to reported literature. NMR spectra match those reported in literature¹.

2,8-bis(ethoxycarbonyl)-5,5-difluoro-10-(2'-(3-methoxyprop-1-yn-1-yl)-5'-methyl-2-nitro-[1,1'-biphenyl]-4-yl)-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (SI-8): Synthesized according to reported literature. NMR spectra match those reported in literature¹.

2,8-bis(ethoxycarbonyl)-5,5-difluoro-10-(6-(2-methoxyethyl)-9-methylphenanthridin-3-yl)-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (SI-9): Synthesized according to reported literature. NMR spectra match those reported in literature¹.

4.2 ^1H NMR of Compounds SI-1 to SI-9

^1H NMR of **SI-2** (CDCl_3 , 400 MHz)

^1H NMR of SI-6 (CDCl_3 , 400 MHz)

^1H NMR of SI-7 (CDCl_3 , 400 MHz)

¹H NMR of **SI-8** (CDCl₃, 400 MHz)

¹H NMR of **SI-9** (CDCl₃, 400 MHz)

5. Computation

5.1 General

All density functional theory (DFT) calculations were performed using Gaussian 09 revision D.01⁵. Geometry optimizations and frequency calculations were performed using the functional B3LYP with the 6-31G(d) basis set for all non-metals and the LANL2DZ effective core potential for Au, Ag, and Hg, in implicit water solvent using the SMD continuum solvation model^{6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16}. Single-point energy corrections were calculated with the 6-311++G(2df,2pd) basis set for all non-metals and the SDD effective core potential for Au, Ag, and Hg, in water solvation (SMD)^{17, 18}. Dispersion corrections using D3(BJ), consisting of the D3 method developed by Grimme along with the Becke–Johnson damping scheme, were applied to all calculations^{19, 20, 21, 22}. Gibbs free energies were calculated with vibrational entropy contributions using the GoodVibes python script, in which the vibrational frequencies are corrected by a quasi-harmonic approximation for entropy with a free-rotor description for frequencies below 100 cm⁻¹, as proposed by Grimme^{23, 24}. Normal mode analysis of the harmonic vibrational frequencies confirmed that optimized structures are minima (no imaginary frequency). All 3D rendering of stationary points was generated using CYLview²⁵. *1*-methylthymine and *1*-methylcytosine were used as the model nucleobases for all calculations.

Figure S14. Optimized structures of the C-T metal mediated base pairs showing key bond lengths and structural differences. A) Au(I) b) Hg(II) c) Ag(I).

5.2 Energies

For the following tables, **E** corresponds to the electronic energy, **ZPE** to the zero-point energy correction, **H** to enthalpy, **T•S** entropy, **T•qh-S** quasi-harmonic corrected entropy, **G(T)** uncorrected Gibbs energy, and **qh-G(T)** to the quasi-harmonic Gibbs energy (calculated using a standard state of 1 atmosphere of pressure and at 298.15K). Values are given in atomic units. **SPC** indicates single point corrected energy. Overall calculation method:

B3LYP-D3(BJ)/SDD-6-311++G(2df,2pd)/SMD(H₂O)//B3LYP-D3(BJ)/LANL2DZ-6-31G(d)/SMD(H₂O)

Table S7. Optimization level SCF energies in addition to ZPE and entropy corrections of all pertinent species used for this study.

Optimization/frequency calculation method: B3LYP-D3(BJ)/LANL2DZ-6-31G(d)/SMD(H₂O)

Structure	E	ZPE	T•S	T•qh-S
Thymine anion	-492.994207	0.129584	0.042885	0.042603
Ag(I)	-146.859656	0	0.018966	0.018966
Au(I)	-135.581849	0	0.019831	0.019831
Cytosine	-434.286064	0.126526	0.040988	0.04083
C–Ag–T	-1073.01804	0.260984	0.073707	0.06972
C–Au–T	-1062.71006	0.261439	0.074086	0.069948
C–Hg–T	-969.862223	0.26139	0.075102	0.070725
Hg(II)	-153.186096	0	0.019867	0.019867

Table S8. Single-point corrected energies of all pertinent species used for this study.

Single-point correction method: B3LYP-D3(BJ)/SDD-6-311++G(2df,2pd)/SMD(H₂O)

Structure	E (SPC)	H (SPC)	G(T) (SPC)	qh-G(T) (SPC)
Thymine anion	-493.196659	-493.057693	-493.10058	-493.100297
Ag (I)	-146.859656	-146.857296	-146.87626	-146.876262
Au (I)	-135.581849	-135.579489	-135.59932	-135.59932
Cytosine	-434.4576	-434.322427	-434.36342	-434.363258
C–Ag–T	-1074.59864	-1074.316016	-1074.3897	-1074.385735
C–Au–T	-1063.36986	-1063.086825	-1063.1609	-1063.156773
C–Hg–T	-1080.95165	-1080.668803	-1080.7439	-1080.739528
Hg (II)	-153.186096	-153.183736	-153.2036	-153.203603

Cartesian Coordinates

(Optimized with B3LYP-D3(BJ)/LANL2DZ-6-31G(d)/SMD(H₂O) using Gaussian 09.)

Thymine anion

Charge: -1

Cartesian coordinates

C	-2.414779	1.359558	0.000000
H	-3.245782	0.658200	-0.000191
N	-1.170421	0.593075	-0.000064
C	0.022763	1.270509	-0.000035
H	-0.057990	2.353203	-0.000069
C	1.214358	0.627998	0.000011
C	2.528455	1.351197	-0.000004
H	2.381609	2.436510	-0.000102
H	3.131224	1.088701	0.878976
H	3.131284	1.088551	-0.878898
C	1.185032	-0.834793	0.000103
O	2.271400	-1.484509	0.000099
N	-0.012506	-1.486605	0.000024
C	-1.184796	-0.817398	-0.000060
O	-2.298494	-1.402699	-0.000069
H	-2.474584	1.992553	-0.891191
H	-2.474709	1.992216	0.891424

There are no imaginary frequencies.

Cytosine

Charge: 0

Cartesian coordinates

N	-1.115249	0.437812	-0.000083
C	-0.261991	1.497645	-0.000089
H	-0.718707	2.480411	-0.000174
C	1.084911	1.316368	-0.000010
H	1.767646	2.156352	0.000043
C	1.551525	-0.034435	0.000063
N	2.875662	-0.280302	0.001019
H	3.209191	-1.234170	-0.002130
H	3.546055	0.475125	-0.002437
N	0.723631	-1.083996	-0.000041
C	-0.619870	-0.884709	-0.000032
O	-1.442415	-1.820405	-0.000091
C	-2.565220	0.627692	-0.000052
H	-3.005760	0.169114	-0.888436
H	-3.005672	0.169545	0.888604
H	-2.777866	1.696908	-0.000286

There are no imaginary frequencies.

C-Au-T

Charge: 0

Cartesian coordinates

N	-4.378615	-0.733764	-0.033827
C	-4.967433	0.483764	0.124306
H	-6.049947	0.488334	0.157695
C	-4.231310	1.619327	0.230948
H	-4.698214	2.586973	0.356153
C	-2.814246	1.500613	0.174167
N	-2.040502	2.583111	0.273221
H	-2.462499	3.494787	0.390520
N	-2.236706	0.285671	0.017664
C	-2.991569	-0.863387	-0.096668
O	-2.480884	-1.978953	-0.248369
C	-5.174275	-1.959088	-0.163899
H	-4.979144	-2.427430	-1.130846
H	-4.912713	-2.657481	0.633301
H	-6.228373	-1.694893	-0.089925
Au	-0.162668	0.050221	-0.032583
C	4.498962	-2.649810	0.266887
H	4.242879	-3.291770	-0.579323
N	3.865925	-1.337164	0.122919
C	4.617279	-0.190476	0.040713
H	5.690377	-0.337472	0.080368
C	4.059829	1.038407	-0.080093
C	4.871489	2.297614	-0.163861
H	5.941728	2.074009	-0.124526
H	4.667779	2.841969	-1.093936
H	4.631524	2.978993	0.661470
C	2.609663	1.128629	-0.113952
O	2.012830	2.225130	-0.205091
N	1.895778	-0.054906	-0.039247
C	2.479092	-1.290485	0.073484
O	1.818994	-2.344129	0.135200
H	4.164356	-3.129167	1.189846
H	5.579040	-2.509478	0.301769
H	-1.029583	2.507545	0.230246

There are no imaginary frequencies.

C-Ag-T

Charge: 0

Cartesian coordinates

N	-4.583339	-0.373602	-0.022519
C	-4.902011	0.952041	-0.021406
H	-5.960012	1.184236	-0.031036
C	-3.944725	1.915448	-0.010222
H	-4.202483	2.966330	-0.011374
C	-2.582588	1.490348	0.002445
N	-1.589931	2.385518	0.011266
H	-1.816618	3.371125	0.006318
N	-2.279080	0.176178	0.006957
C	-3.248520	-0.787194	-0.000814
O	-2.978103	-1.998861	0.010190
C	-5.620294	-1.408771	-0.028739
H	-5.474156	-2.074250	-0.881574
H	-5.574433	-1.992386	0.893612
H	-6.592008	-0.922127	-0.107380
C	5.133636	-2.033646	-0.024864
H	4.951309	-2.690677	-0.878274
N	4.170555	-0.932325	-0.021435
C	4.585753	0.375895	-0.021954
H	5.660139	0.521171	-0.034576
C	3.717310	1.417785	-0.009268
C	4.159548	2.850968	-0.010892
H	5.251345	2.923277	-0.020148
H	3.771684	3.384759	-0.887189
H	3.787060	3.381472	0.874018
C	2.298332	1.106620	0.004517
O	1.423707	2.014464	0.012680
N	1.927392	-0.214605	0.009264
C	2.812853	-1.247172	0.001092
O	2.456111	-2.443394	0.011921
H	5.049162	-2.618409	0.894810
H	6.138478	-1.617436	-0.097859
H	-0.603741	2.111840	0.015014
Ag	-0.203710	-0.501887	0.017515

There are no imaginary frequencies.

C-Hg-T

Charge: +1

Cartesian coordinates

N	-4.621843	-0.106421	0.004889
C	-4.874372	1.234588	0.011567
H	-5.919917	1.516686	0.016125
C	-3.874673	2.154211	0.012746
H	-4.081703	3.216220	0.019017
C	-2.533142	1.666445	0.006846
N	-1.492115	2.497867	0.010521
H	-1.658181	3.495632	0.014697
N	-2.302278	0.338915	-0.002323
C	-3.309649	-0.581955	-0.007267
O	-3.085797	-1.803256	-0.021286
C	-5.709116	-1.089595	-0.002950
H	-5.675772	-1.676280	-0.923822
H	-5.608761	-1.758994	0.853473
H	-6.656711	-0.555170	0.057608
C	5.301765	-1.743523	0.006493
H	5.151756	-2.412083	-0.843954
N	4.286932	-0.688607	-0.000016
C	4.636508	0.640146	-0.002963
H	5.702805	0.835566	-0.004483
C	3.720267	1.640972	-0.004570
C	4.091476	3.094487	-0.008337
H	5.178232	3.220178	-0.007360
H	3.686096	3.604081	-0.890919
H	3.683985	3.608795	0.870535
C	2.315877	1.266139	-0.004598
O	1.396602	2.123052	-0.009873
N	2.020968	-0.072847	0.001172
C	2.950133	-1.069204	0.009518
O	2.639475	-2.276269	0.023808
H	5.241612	-2.324767	0.930162
H	6.284527	-1.278501	-0.067178
H	-0.522969	2.163689	0.002879
Hg	-0.174242	-0.663274	-0.002082

There are no imaginary frequencies.

6. References

1. Wang, J., Wu, Q., Min, Y., Liu, Y. & Song, Q. A novel fluorescent probe for Au(III)/Au(I) ions based on an intramolecular hydroamination of a Bodipy derivative and its application in bioimaging. *Chem. Commun.* **48**, 744–746 (2012).
2. Aoyagi, Y. et al. Efficient synthesis of pyrroles and 4,5,6,7-tetrahydroindoles via palladium-catalyzed oxidation of hydroxyenamines. *Tetrahedron* **62**, 8533–8538 (2006).
3. Huang, C. L., Weng, T. & Chen, F. C. Synthesis of biflavonyl ethers. *J. Heterocycl. Chem.* **7**, 1189–1190 (1970).
4. Lv, J., Liu, Q., Tang, J., Perdih, F. & Kranjc, K. A facile synthesis of indolo[3,2,1-*jk*]carbazoles via palladium-catalyzed intramolecular cyclization. *Tetrahedron Lett.* **53**, 5248–5252 (2012).
5. Frisch, M. J. et al. Gaussian 09, Revision D.01, Gaussian, Inc., Wallingford CT, (2010).
6. Becke, A. D. Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **98**, 5648–5652 (1993).
7. Lee, C., Yang, W. & Parr, R. G. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B.* **37**, 785–789 (1988).
8. Becke, A. D. A new mixing of Hartree-Fock and local density-functional theories. *J. Chem. Phys.* **98**, 1372–1377 (1993).
9. Ditchfield, R., Hehre, W. J. & Pople J. A. Self-consistent molecular-orbital methods. IX. An extended Gaussian-type basis for molecular-orbital studies of organic molecules. *J. Chem. Phys.* **54**, 724–728 (1971).
10. Hehre, W. J., Ditchfield, R. & Pople J. A. Self-consistent molecular orbital methods. XII. Further extensions of Gaussian-type basis sets for use in molecular orbital studies of organic molecules. *J. Chem. Phys.* **54**, 2257–2261 (1972).
11. Hariharan, P. C. & Pople, J. A. The influence of polarization functions on molecular orbital hydrogenation energies. *Theor. Chem. Acc.* **28**, 213–222 (1973).
12. Hay, P. J. & Wadt, W. R. Ab initio effective core potentials for molecular calculations. Potentials for K to Au including the outermost core orbitals. *J. Chem. Phys.* **82**, 299–310 (1985).
13. Roy, L. E., Hay, P. J. & Martin, R. L. Revised Basis Sets for the LANL Effective Core Potentials. *J Chem Theory Comput* **2008**, 4, 1029–1031.
14. Ehlers, A. W. et al. A set of *f* polarization functions for pseudopotential basis sets of the transition metals Sc–Cu, Y–Ag, and La–Au. *Chem Phys Lett* **208**, 111–114 (1993).
15. Marenich, A. V., Cramer, C. J. & Truhlar, D. G. Universal Solvation Model Based on Solute Electron Density and on a Continuum Model of the Solvent Defined by the Bulk Dielectric Constant and Atomic Surface Tensions. *J. Phys. Chem. B* **113**, 6378–6396 (2009).
16. Ribeiro, R. F., Marenich, A. V., Cramer, C. J. & Truhlar, D. G. Use of Solution-Phase Vibrational Frequencies in Continuum Models for the Free Energy of Solvation. *J. Phys. Chem. B.* **115**, 14556–14562 (2011).
17. Dolg, M., Wedig, U., Stoll, H. & Preuss, H. Energy-adjusted ab initio pseudopotentials for the first row transition elements. *J. Chem. Phys.* **86**, 866–872 (1987).

-
- 18.** Andrae, D., Häußermann, U., Dolg, M., Stoll, H. & Preu, H. Ab initio energy-adjusted pseudopotentials for the noble gases Ne through Xe: Calculation of atomic dipole and quadrupole polarizabilities. *Theor. Chem. Acc.* **77**, 123–141 (1990).
- 19.** Grimme, S., Antony, J., Ehrlich, S. & Krieg, H. A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. *J. Chem. Phys.* **132**, 154104 (2010).
- 20.** Johnson, E. R., & Becke, A. D. A post-Hartree-Fock model of intermolecular interactions. *J. Chem. Phys.* **123**, 024101 (2005).
- 21.** Johnson, E. R. & Becke, A. D. A post-Hartree-Fock model of intermolecular interactions: Inclusion of higher-order corrections. *J. Chem. Phys.* **124**, 174104 (2006).
- 22.** Grimme, S., Ehrlich, S. & Goerigk, L. Effect of the Damping Function in Dispersion Corrected Density Functional Theory. *J Comput Chem* **32**, 1456–1465 (2011).
- 23.** Grimme, S. Supramolecular Binding Thermodynamics by Dispersion-Corrected Density Functional Theory. *Chem. Eur. J.* **18**, 9955 (2012).
- 24.** Funes-Ardoiz, I. & Paton, R. S. GoodVibes: *GoodVibes 2.0.2* DOI: 10.5281/zenodo.595246 (2016).
- 25.** C. Y. Legault, CYLView, 1.0b (Université de Sherbrooke, 2009); www.cylview.org.