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(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, Twelfth Floor, Oakland, CA 94607-5200 (US).

(72) Inventors: **NELSON, Hosea, M.**; 10889 Wilshire Blvd., Suite 920, Los Angeles, CA 90095-7191 (US). **GREEN, Sydnee**; 10889 Wilshire Blvd., Suite 920, Los Angeles, CA 90095-7191 (US).

(74) Agent: **HALSTEAD, David, P.** et al.; Foley Hoag LLP, 155 Seaport Boulevard, Boston, MA 02210-2600 (US).

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(54) Title: COMPOSITIONS AND METHODS RELATED TO GOLD-MEDIATED NUCLEIC ACID HYBRIDIZATION

(57) Abstract: The present disclosure provides a gold-containing compound including an aurous gold atom and a nucleic acid strand that forms a toehold-stem-loop structure, in which the aurous gold atom is complexed with a mismatched pair of residues in the stem portion of the nucleic acid strand. The disclosure also provides a gold-containing double-stranded nucleic acid including an aurous gold coordinated between a first nucleobase and a mismatching second nucleobase. Related methods of increasing stability of a nucleic acid duplex, regulating an aurous-gold-catalyzed reaction, detecting an oligonucleotide, and detecting aurous gold in a substance are additionally provided.



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COMPOSITIONS AND METHODS RELATED TO GOLD-MEDIATED NUCLEIC ACID HYBRIDIZATION

RELATED APPLICATION

This application claims a right of priority from and the benefit of an earlier filing date of U.S. Provisional Application No. 62/816,103, filed March 9, 2019, which is hereby incorporated by reference in its entirety.

BACKGROUND

Some of the potential uses of nucleic acids in nanotechnology involve their interactions with transition metals. For example, studies on metal-mediated base pairs (MMBPs) have led to technological advances, and have contributed to our understanding of how metals interact with nucleic acids. Specific applications of specifically incorporated transition metals have included metal sensors and metal-responsive materials. Despite such progress, the field has been limited by its reliance both on a few select metals and on synthesized nucleobase mimics. In particular, Ag(I) and Hg(II) are currently the only metals that have been reported to specifically coordinate to mismatches in canonical DNA.

In parallel, there is a possibility of using transition metals to provide additional reactivity to biological systems. Because the reactions catalyzed by transition metals can be complementary to those of biological catalysts, such a use may expand the range of chemical transformations that can be carried out in biological systems. Despite being hypothetically interesting, achieving such a melding of synthetic chemical systems with native biological processes is practically challenging, both because transition metals tend to be incompatible with biological conditions in general and because reactivity of transition metals tends to be difficult to control under those conditions.

SUMMARY OF THE INVENTION

In one aspect, a gold-containing compound including an aurous gold atom and a nucleic acid construct that forms a toehold-stem-loop structure is disclosed. In this aspect, the nucleic acid construct includes a first part, a second part, a third part, and a fourth part. The four parts are arranged in sequence; preferably, the four parts form a contiguous nucleic acid sequence, i.e., the second part is contiguous with the first part, the third part is contiguous with the second part, and the fourth part is contiguous with the third part. The first part forms a toehold portion of the toehold-stem-loop structure; the second part together with a fourth part of the nucleic acid strand forms a stem portion of the toehold-stem-loop structure by

annealing with the fourth part; the third part forms a loop portion of the toehold-stem-loop structure; and the fourth part together with the second part forms the stem portion. The aurous gold atom is complexed with two nucleic acid residues of the nucleic acid strand, and the two nucleic acid residues form a mismatched pair in the stem portion of the toehold-stem-loop structure.

In some embodiments of the gold-containing compound, the nucleic acid strand is a deoxyribonucleic acid strand, and the two nucleic acid residues include one of the following nucleobase pairs: cytosine-thymine, cytosine-cytosine, cytosine-adenine, thymine-guanine, adenine-guanine, and thymine-thymine. In some embodiments, at least one detectable label is conjugated to the nucleic acid strand.

In another aspect, a method of increasing stability of a nucleic acid duplex having a mismatched nucleobase is disclosed. The method includes contacting the nucleic acid with an aurous gold compound in an aqueous solution.

In some embodiments of this method, the compound is chloro(dimethylsulfide)gold(I). In some embodiments, the solution has a basic pH (e.g., between 7.5 and 10.5). This basic pH can be attained at various times with respect to the contacting step (e.g., before, during, after). In some embodiments, the nucleobase is a cytosine mismatched with a thymine, a cytosine mismatched with a cytosine, a cytosine mismatched with an adenine, a thymine mismatched with a guanine, an adenine mismatched with a guanine, or a thymine mismatched with a thymine.

In another aspect, a method of regulating an aurous-gold-catalyzed reaction, such as an aurous-gold-catalyzed hydroamination, is disclosed. The method includes mixing a reactant with an aurous-gold-containing toehold-stem structured nucleic acid (e.g., a compound as previously described) to obtain a mixture, and adding to the mixture an oligonucleotide that is complementary to a part of the nucleic acid. The nucleic acid of this aspect has in its stem portion a mismatched residue pair that is complexed with the aurous gold, and the addition step allows this aurous gold to catalyze the reaction of the reactant.

In some embodiments of this method, the mismatched residue pair includes a cytosine and a thymine, a cytosine and a cytosine, a cytosine and an adenine, a thymine and a guanine, an adenine and a guanine, or a thymine and a thymine. The oligonucleotide, in some embodiments, is complementary to a part of the nucleic acid that includes at least a part of its toehold portion and a part of its stem portion that is contiguous with that toehold portion.

In another aspect, a method of detecting an oligonucleotide is disclosed. The method includes obtaining an aurous-gold-containing nucleic acid that has a single-stranded toehold

portion and a double-stranded stem portion, contacting the oligonucleotide with the aurous-gold-containing nucleic acid, assaying to determine a degree of completion of a reaction, and detecting the oligonucleotide when the degree passes a threshold. In this aspect, the stem portion includes a mismatched pair of nucleic acid residues complexed with the aurous gold, and the contacting step allows the aurous gold to catalyze the reaction.

In some embodiments, the nucleic acid is a deoxyribonucleic acid. The oligonucleotide may be a ribonucleic acid, such as an mRNA. In some embodiments, the contacting step occurs in a solution, on a solid surface, *ex vivo*, or *in vivo*. The nucleic acid, in some embodiments, also includes a loop portion adjacent to its stem portion. In some embodiments, the mismatched pair includes a cytosine and a thymine, a cytosine and a cytosine, a cytosine and an adenine, a thymine and a guanine, an adenine and a guanine, or a thymine and a thymine.

In yet another aspect, a method of detecting aurous gold in a substance is disclosed. The method includes obtaining a nucleic acid that has a double-stranded portion, contacting the substance with the nucleic acid, assaying to determine a degree of completion of a reaction, and detecting the aurous gold when the degree passes a threshold. In this aspect, the double-stranded portion includes a mismatched nucleobase, and the contacting step allows or disallows the aurous gold to catalyze the reaction. Furthermore, in this aspect the reaction can be the conversion of the double-stranded nucleic acid into a more stable double-stranded nucleic acid-gold complex. Alternatively, the reaction can be an aurous-gold-catalyzed reaction.

In some embodiments of this method, the nucleic acid further includes a loop portion adjacent to its stem portion. The nucleobase, in some embodiments, is a cytosine mismatched with a thymine, a cytosine mismatched with a cytosine, a cytosine mismatched with an adenine, a thymine mismatched with a guanine, an adenine mismatched with a guanine, or a thymine mismatched with a thymine.

In still another aspect, a gold-containing double-stranded nucleic acid is disclosed, which includes an aurous gold coordinated between a first nucleobase and a mismatching second nucleobase. In some embodiments of this aspect, the first nucleobase and the second nucleobase are a cytosine and a thymine, a cytosine and a cytosine, a cytosine and an adenine, a thymine and a guanine, an adenine and a guanine, or a thymine and a thymine.

The provided compounds and methods enable creating a double-helical nucleic acid that has increased stability (e.g., as compared to a double-helical nucleic acid with only a mismatch but no metal, or as compared to a double-helical nucleic acid with a mismatch and

another metal such as Ag(I) or Hg(II)). The use of Au(I) can also avoid the toxic effects caused by analogous use of Ag(I) or Hg(II), a concern especially for *in vivo* applications. In addition, the provided compounds and methods enable modulating the reactivity of the aurous gold, and detecting either aurous gold or a specific oligonucleotide.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Metal-mediated base pairing with pyrimidine mismatches.

Figure 2. Proposed structure of T-Au(I)-C mismatch based on proposed structures of with Hg(II) and Ag(I). Calculated binding energy of each metal through N3 of cytosine and N3 of thymine. All the energy values are Gibb's free energies reported in kcal/mol. Geometries from B3LYP/6-31G(d)LANL2DZ. Energies are dG from B3LP/6311++G(2df,2pd)/SDD in H₂O (SMD model).

Figure 3. Thermal denaturation curves for CT1 (a), TT1 (b), and CC1 (c) duplex with varying equivalents of Au(I) (0, 0.4, 1.0, 1.4, 2.0, 3.0). 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). (•[when ranked from highest to lowest at 50 °C in (c), the first one] 0 eq (Me₂S)AuCl, •[when ranked from highest to lowest at 50 °C in (c), the second one] 0.4 eq (Me₂S)AuCl, •[when ranked from highest to lowest at 50 °C in (c), the third one] 1.0 eq (Me₂S)AuCl, •[when ranked from highest to lowest at 50 °C in (c), the fourth one] 1.4 eq (Me₂S)AuCl, •[when ranked from highest to lowest at 50 °C in (b), the fifth one] 2.0 eq (Me₂S)AuCl, •[when ranked from highest to lowest at 50 °C in (b), the sixth one] 3.0 eq (Me₂S)AuCl, •[when ranked from highest to lowest at 50 °C in (b), the seventh one] 4.0 eq (Me₂S)AuCl).

Figure 4. Computational base-pairing energy of Au(I) ions to 1-methylcytosine. Geometries from B3LYP/6-31G(d)LANL2DZ. Energies are dG from B3LP/6311++G(2df,2pd)/SDD in H₂O (SMD model).

Figure 5. Thermal denaturation curves for CC1 duplex with varying equivalents of Au(I) (0, 1.0, 2.0) at pH a) 5.5 and b) 8.5. 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). (•[when ranked from highest to lowest at 50 °C in (b), the first one] 0 eq (Me₂S)AuCl, •[when ranked from highest to lowest at 50 °C in (b), the second one] 1.0 eq (Me₂S)AuCl, •[when ranked from highest to lowest at 50 °C in (b), the third one] 2.0 eq (Me₂S)AuCl).

Figure 6. CD spectrum of CC2 (5'-CCTTTCCCTCCCTC-3' • 3'-GAGGGACCGAAAGG-5') with varying equivalents of Au(I) (0, 1, 2, 3) (• 0 eq (Me₂S)AuCl, • 1 eq (Me₂S)AuCl, • 2 eq (Me₂S)AuCl, • 3 eq (Me₂S)AuCl).

Figure 7. Thermal denaturation profiles of GT1, AA1, and CA1 in the presence of (Me₂S)AuCl. Relative absorbance, $A_{260nm} = (A_t - A_{15\text{ }^\circ\text{C}}) / (A_{90\text{ }^\circ\text{C}} - A_{15\text{ }^\circ\text{C}})$, vs. temperature (°C) curves for pyrimidine-mismatch-containing oligonucleotides, a) GT1 b) AA1 c) CA1, in the presence of (Me₂S)AuCl. Solutions contained 3.5 μM DNA in buffer containing 0.75 mM sodium phosphate, pH 7, 150 mM NaClO₄ and 1.4 μM (0.4 equiv) 3.5 μM (1 equiv), 4.9 μM (1.4 equiv), 7 μM (2 equiv) or 10.5 μM (3 equiv), (Me₂S)AuCl (60:1 H₂O:MeOH v/v).

Figure 8. 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.

Figure 9. 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 parentheses (Ono 2008 *Chem Commun* 39: 4825-27).

Figure 10. a) Proposed incorporation of Au(I) into DNA hairpin followed by hybridization to expose a coordination site on the metal center. b) Profluorescent BODIPY cyclyzation catalyzed by addition of DNA-Au complex. c) Kinetic model showing initial rates of 5 and 6 versus concentration of BODIPY substrate (7).

Figure 11. a) Hybridization of hairpin containing FRET pair (FAM as donor and Dabcyl 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 10) 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').

DETAILED DESCRIPTION OF THE INVENTION

In some aspects, the present disclosure provides nucleic acids having a double-stranded region with at least one mismatching base pair coordinated by at least one aurous gold atom. In some embodiments, the nucleic acid has a hairpin structure (i.e., has a stem portion and a loop portion, in which the stem portion is the double-stranded region with the mismatching base pair). In some embodiments, the nucleic acid has a toehold-stem-loop structure (i.e., has a loop portion as in a hairpin, has a stem portion as in a hairpin, and has a single-stranded toehold portion extending from one of the strands of the stem portion). In some embodiments, the nucleic acid has two separable strands and at least one single-stranded region (e.g., has a duplex region on one side and an overhang from one of the strands on the other side, has a duplex region in the middle and overhangs on both sides from different strands).

In such nucleic acids, incorporation of an aurous gold can increase the stability of the double-stranded region. In addition, for example when the nucleic acid has a single-stranded region such as a toehold or an overhang, an oligonucleotide having a portion complementary to the single-stranded region of the nucleic acid can displace some of the double-stranded region of the nucleic acid, and by doing so expose the aurous gold (e.g., to act as a catalyst). The ability of aurous gold to increase the stability of a double-stranded nucleic acid and to carry out catalysis when its coordinating nucleobase is displaced allows detection of oligonucleotides and aurous gold, as further described in this disclosure.

Definitions

As used in this specification, “a” and “an” can mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising”, the words “a” and “an”

can mean one or more than one. As used herein, “another” can mean at least a second or more.

The term “toehold-stem-loop” refers to a nucleic acid structure in which there is a double-stranded region (e.g., adopted under physiological conditions) called the stem portion, which on one of its two sides (i.e., linear end when thought of as an aligned sequence pair) extends to the toehold portion and on the other of its two sides extends to the loop portion. The toehold portion either is entirely in single-stranded form or has at least some parts that are in single-stranded form (e.g., some secondary structure or a short hybridized oligonucleotide would not take away the character of the toehold region). Similarly, the loop portion either is entirely in single-stranded form or is partially in single-stranded form (e.g., the two parts that immediately extend from the strands of the stem portion are single-stranded, but at other parts there might be a secondary structure or a short hybridized oligonucleotide).

The term “hairpin” refers to a toehold-stem-loop structure that lacks the toehold portion.

The terms “duplex,” “double-stranded,” and “double-helical” include regions of all nucleic acid structures in which at least two contiguous residues are paired (e.g., through Watson-Crick base pairing or in any other way, including mismatching pairs) with two other contiguous residues. The pairing residues can be part of the same strand (e.g., as it happens in the stem portion of a hairpin, in which the same strand loops back to pair with itself) or they can be part of two strands (e.g., which can be denatured and annealed as in polymerase chain reaction).

The term “contiguous” with respect to nucleic acid parts means that the parts are next to each other in sequence (e.g., for a sequence with residues 1-10, the part with residues 1-5 is contiguous with the part with residues 6-10).

The term “mismatch” includes any nucleobase pairings that are other than adenine-to-thymine (i.e., an adenine forming a Watson-Crick base pair with a thymine, for example where the adenine is a part of an adenosine in RNA or a part of a deoxyadenosine in DNA) and guanine-to-cytosine. When referring to nucleobase pairings herein, a recited particular order of pairing nucleobases includes the reverse as well (e.g., adenine-to-thymine is equivalent to thymine-to-adenine, and guanine-to-cytosine is equivalent to cytosine-to-guanine). In particular, the term mismatch includes the following nucleobase pairings: cytosine-to-thymine, cytosine-to-cytosine, cytosine-to-adenine, thymine-to-guanine,

adenine-to-guanine, and thymine-to-thymine. In some embodiments, the mismatch also includes an adenine-to-adenine pairing.

The term “detectable label” includes all compounds that can be attached (e.g., conjugated) to a nucleic acid and which can provide or modulate a measurable signal (e.g., fluorescence signal). For example, 6'-Carboxyfluorescein (FAM) is a detectable label, and can be used, in some embodiments, together with another label, Dabcyl, which can act as a quencher to decrease the fluorescence emitted by FAM.

The term “aurous-gold-catalyzed reaction” includes all reactions in which aurous gold can act as a catalyst. An example of an aurous-gold-catalyzed reaction is hydroamination.

The term “passing a threshold” means that a measured or observed value is more or less than a threshold value (e.g., a predetermined threshold value). For example, when an assay determines a certain activity by measuring a signal that decreases with that activity, then passing a threshold means being lower than the threshold value. Conversely, when the measured signal positively correlates with activity, passing a threshold means being higher than the threshold value. The threshold value can be set through various methods (e.g., with respect to a control sample, with respect to a calibration curve).

Gold-containing Compounds

In some aspects, the disclosure relates to a nucleic acid with a double-stranded region in which at least one mismatching base pair is coordinated by at least one aurous gold atom. The nucleic acid can be a ribonucleic acid or a deoxyribonucleic acid. In some embodiments, the nucleic acids can have artificial nucleobases (e.g., dNaM) or other non-canonical nucleobases (e.g., inosine, wyosine). In some embodiments, all of the nucleobases in the nucleic acids are canonical. In some embodiments, the double-stranded region of the nucleic acid can be a hybrid (e.g., RNA/DNA hybrid).

The overall structure of the nucleic acid can take various forms, as long as there is at least one double-stranded region with at least one mismatching base pair. For example, the nucleic acid can adopt a hairpin structure, a toehold-stem-loop structure, a duplex with a single-stranded overhang structure, or a duplex with two antiparallel single-stranded overhangs structure.

The mismatches can include any of the following pairings: cytosine-thymine, cytosine-cytosine, cytosine-adenine, thymine-guanine, adenine-guanine, thymine-thymine, and adenine-adenine.

Methods of Increasing Nucleic Acid Stability

In some aspects, the disclosure relates to a method of increasing the stability of a double-stranded region of a nucleic acid that has at least one mismatching pair in its double-stranded region.

The method includes contacting the nucleic acid with an aurous gold, and by doing so obtaining a stabilized nucleic acid in which the mismatching pair is coordinated by the aurous gold atom. The aurous gold can be provided through an aurous gold compound (e.g., chloro(dimethylsulfide)gold(I)). The step of contacting can be achieved through causing the nucleic acid and the aurous gold to be proximal to each other, for example by mixing aqueous solutions of the two with each other. The amount of the aurous gold can be varied (e.g., it can be 1 equivalent, 2 equivalent, 3 equivalent of the duplex, or it can be another integer or non-integer equivalent).

In some embodiments, increasing the pH of the solution of the gold-containing nucleic acid further increases the stability of the nucleic acid. For example, basic pH values (e.g., 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, and any other values greater, by any increment, than 7.0) can be used to confer stability to the gold-containing nucleic acid. In some embodiments, the increase in stability depends on the type of mismatch. The pH of the solution can be made basic at various points: before the contacting step, during the contacting step, or after the contacting step. In some embodiments, the amount of aurous gold mixed with the nucleic acid can be adjusted depending on the pH, on the type of the mismatch, or on both the pH and the type of the mismatch.

Methods of Regulating an Aurous-gold-catalyzed Reaction

In some aspects, the disclosure relates to a method of regulating an aurous-gold-catalyzed reaction. The method, in some embodiments, includes mixing a reactant with a nucleic acid that has in a double-stranded region a mismatching base pair coordinated by an aurous gold, and adding to that mixture an oligonucleotide that causes one of the strands in the double-stranded region to be displaced. This can be accomplished, for example, when the nucleic acid has a toehold-stem-loop structure by choosing an oligonucleotide that is in part complementary to the toehold portion and in part complementary to the stem portion of the nucleic acid. In general, any nucleic acid and oligonucleotide combination can be used as long as the addition of the oligonucleotide causes the region of the nucleic acid with the mismatching base pair to unwind. Various details for designing and manipulating nucleic acids structures, as used and as applicable here, can be found in the literature (e.g., B. Yurke et

al., A DNA-fuelled molecular machine made of DNA, *Nature* 406: 605-08 (2000); S.J. Green et al., DNA hairpins: fuel for autonomous DNA devices, *Biophys J.* 91(8): 2966-75 (2006); Y. Guo et al., Recent advances in molecular machines based on toehold-mediated strand displacement reaction, *Quantitative Biology* 5(1): 25-41 (2017)).

The displacement of one of the strands at the mismatch position exposes the aurous gold, and allows it to catalyze a reaction. In this way, the reactivity of the aurous gold can be controlled (e.g., as a binary on/off switch, or as a more graduated spectrum of activity levels). In some embodiments, addition of a yet another oligonucleotide (e.g., one that is complementary to the initially added oligonucleotide) can sequester the initial oligonucleotide, and by doing so again deactivate the aurous gold. In some embodiments, this can be repeated cyclically (e.g., active Au(I) → inactive Au(I) → active Au(I) → inactive Au(I)).

Many additional variations of these methods can be implemented. For example, it is also possible to have a very long nucleic acid with periodic (e.g., regular or irregular) mismatches that complex gold; so that a single catalyst “molecule” has multiple catalytic metal ions.

Methods of Detecting an Oligonucleotide or Aurous Gold

In some aspects, the disclosure relates to a method of detecting an oligonucleotide or aurous gold. For example, referring to the oligonucleotide or aurous gold collectively as the test sample for convenience, one can obtain a gold-containing nucleic acid as previously described, and then contact that nucleic acid with the test sample to allow the aurous gold to catalyze a reaction. The degree of completion of the reaction can be measured and used to detect the presence or amount of the test sample.

In some embodiments, one can use a control (e.g., standard, negative, positive, or a combination of some or all of standard, negative, and positive) to determine a threshold value for the degree of completion, and then the measured degree of completion can be determined to pass (e.g., be less than or greater than depending on the assay system used) that threshold value. In some embodiments, instead of a singular control, a series of controls can be used to obtain a calibration curve to use in determining the threshold value.

The test sample, in some embodiments, is mRNA. In some embodiments, the nature of the used nucleic acid and the tested oligonucleotide are different (e.g., one is DNA, and the other is RNA). In some embodiments, the contacting step occurs in solution, on a solid surface, *ex vivo*, or *in vivo*.

When the test sample is for aurous gold, the reaction can be the conversion of the double-stranded region of the nucleic acid into a more stable double-stranded region (e.g., because of incorporation of the aurous gold). Stability of double-stranded nucleic acids can be estimated or compared through a measurement of their melting temperatures (e.g., through ultraviolet–visible spectroscopy, circular dichroism spectroscopy, differential scanning calorimetry, fluorescence anisotropy, nuclear magnetic resonance). In some embodiments, the reaction can be an aurous-gold-catalyzed reaction (e.g., a saturating amount of a double-stranded nucleic acid with a specific mismatching pair would inhibit the reaction, which inhibition can vary depending on the type of mismatch and the level of pH, and those parameters can be used to increase the accuracy of the detection methods).

These methods of detecting gold can also be adopted for fishing the gold out of a solution or other substance by having the gold form a complex between two nucleobases—the first one from a single-stranded nucleic acid sequence that could hybridize to the second one from a complementary sequence on a solid support. This can allow recovering the gold. After the recovery, the gold complex can be removed and moved to a new solution that discourages that hybridization. The solid support can then be removed or recycled. This method, or its variations, can allow regenerating the gold. Alternatively, gold can be on a solid support during the full process: before, after, and during the reaction. Throughout these methods, gold can be used or treated as a catalyst (e.g., as a catalyst, it can be moved from solution to solution, recovered, concentrated, regenerated, etc.).

Additionally, a system can be set up in which the nucleic acid has nearly complementary ends that have a mismatch or two (e.g., 1, 2, 3, 4 mismatches), such that it only hybridizes in the presence of aurous gold. In such a system, at least one detectable label (e.g., a FRET pair—a donor fluorophore and a quencher) can be attached to the ends of the nucleic acid to signal when the ends come close together.

When the test sample is for an oligonucleotide (i.e., the method is for detecting an oligonucleotide in the test sample), the inactive form of the aurous-gold-catalyst may result in a low level of activity (e.g., less than 10% or less than 5% yield of a fluorescent product that is created through the catalyzed reaction), whereas the active form of the aurous-gold-catalyst may result in a high level of activity (e.g., greater than 40% or 50% yield of a fluorescent product that is created through the catalyzed reaction). Such a system can be used to detect small nucleic acids, such as mRNA.

In some embodiments, this catalysis can also be performed on paper. As an example, a DNA-Au complex and a profluorescent molecule can be applied to a paper and then dried

down. After application of a complementary sequence (e.g., an invading sequence), the paper may become fluorescent due to the gold-catalyzed reaction of the profluorescent molecule. This type of a system can allow for simple and inexpensive testing of biological samples for small molecules and nucleic acids (e.g., mRNA).

EXAMPLES

Example 1: A Highly Stabilizing Au(I) Metal-Mediated Base Pair Between Cytosine—Cytosine Mismatches

Past studies of the interactions between transition metals and DNA have led to the development of metal-based therapeutics as well as advances in DNA based nanotechnology. Specifically, the discovery of metal-mediated base pairs (MMBP), metal-coordination complexes incorporated into DNA base pairs, have fueled many recent advances in new technologies and have enhanced our understanding of metal-DNA interactions. The site-specific incorporation of transition metals has led to the development of new metal sensors, the improvement of charge transfer through DNA, and the design of metal-responsive materials. However, there is a limited scope of transition metals that can be incorporated into oligonucleotides, and most MMBPs rely on the synthesis of base mimics. Therefore, the exploration of new metals that can be incorporated into canonical bases is important for further developments of metalloDNA based nanotechnology and therapeutics.

Currently, Ag(I) and Hg(II) are the only two metals that have been reported to coordinate specifically to canonical DNA mismatches (**Figure 1**). Au(I), a metal sharing similar coordination and size to Ag(I) and Hg(II), has been shown to exhibit similar coordination and size to these metals, making it an ideal candidate for further investigating transition metal interactions with oligonucleotides. Additionally, there is significant precedence for Au(I) as a medically relevant transition metal for treatment of diseases such as rheumatoid arthritis and cancer, leading to the extensive investigation of Au(I) and DNA interactions over the last four decades. The possibility of interstrand binding of Au(I) to double stranded DNA was originally proposed over three decades ago by Blank and Dabrowiak. Further spectroscopic experiments suggested Au(III) and Au(I) binding to N7 of guanine as well as a dimeric Au(II) complex between two cytosine nucleosides. However, despite numerous theoretical predictions and experimental data showing Au(I) interactions with nucleobases, the coordination of Au(I) within a DNA mismatch has never been reported. This study represents the first example of Au(I) incorporation into pyrimidine

mismatches within a DNA duplex, facilitating the formation of the most stable metal-mediated base pair between canonical bases to date. We probed the formation of this highly stabilizing Au(I) metal-mediated base pair using thermal stability measurements, density functional theory (DFT) calculations, mass spectrometry (MS), and circular dichroism (CD) spectroscopy.

In order to characterize Au(I) binding, we performed thermal denaturation studies to identify changes in the thermal stabilities of the mismatch-containing duplexes in the presence of Au(I). Common Au(I) complexes favor the coordination of one neutral donating ligand and one anionic ligand in a linear geometry.¹⁵ Therefore, we hypothesized that binding between N3 of cytosine and the deprotonated N3 of thymine would be favorable. These interactions, in addition to the coordination geometries proposed for T—Hg(II)—C (1) and T—Ag(I)—C (2) MMBPs, led to the proposed structure of the T—Au(I)—C (3) metal mediated base pair shown in **Figure 2**.^{17,18} Furthermore, crystal structure and NMR studies that show binding of Au(I) to deprotonated N3 of 1-methylthymidine and N3 of cytosine are consistent with this structure.^{14,16}

Table 1. T_m values for matches and pyrimidine mismatches.

entry	duplex		T_m (°C) with n equiv of (Me ₂ S)AuCl			ΔT_m (°C)
			$n = 0$	$n = 1$	$n = 2$	
1	5'-d(CCT TTC TCT CCC TC) 3'-d(GGA AAG ACA GGG AG)	CG1	55.6 ± 0.3	60.0 ± 0.5	—	4.4 ± 0.6 ^a
2	5'-d(CCT TTC TAT CCC TC) 3'-d(GGA AAG AIA GGG AG)	AT1	52.4 ± 0.3	53.8 ± 0.3	—	1.4 ± 0.4
3	5'-d(CCT TTC TCT CCC TC) 3'-d(GGA AAG AIA GGG AG)	CT1	40.6 ± 0.7	47.3 ± 1.1	51.3 ± 0.6	10.7 ± 1.4
4	5'-d(CCT TTC TTT CCC TC) 3'-d(GGA AAG AIA GGG AG)	TT1	43.0 ± 0.4	53.2 ± 0.5	60.9 ± 0.7	17.9 ± 0.9
5	5'-d(CCT TTC TCT CCC TC) 3'-d(GGA AAG ACA GGG AG)	CC1	38.3 ± 0.9	35.2 ± 0.8 67.1 ± 1.4 ^b	71 ± 1	33 ± 3

^aIncrease in CG1 due to known favorable interactions between gold, cytosine, and guanine.¹²⁻¹⁴ ^bTwo values reported due to biphasic melting.

Thermal denaturation experiments show that the thermal stability of the cytosine-thymidine mismatched duplex, **CT1**, increases by 10.7 °C upon addition of two equivalents of Au(I) (**Table 1, entry 3**). This metal mediated base pair appears to incorporate approximately one Au(I) with moderate increases with additional Au(I) ions due to nonspecific binding, as shown by thermal stability titration.¹ The addition of one equivalent of Au(I) leads to an increase in 7 °C with **CT1** compared to the duplex with no metal. This

increase is significant and not unlike common T_m changes associated with many metal mediated base pairs.³⁻⁸

Unlike the addition of Au(I) to **CT1**, the addition of Au(I) to **CC1** and **TT1** mismatched duplexes exhibit higher thermal stability than control Watson-Crick AT and GC matched duplexes (**entries 4-5**). The addition of one equivalent of Au(I) to **TT1** increases the thermal stability by 10.2 °C (**entry 4**). This change in thermal stability is similar to reported T-Hg(II)-T metal mediated base pair containing a single T-T mismatch.^{5,6} The addition of excess Au(I), up to three equivalents, results in increased thermal stability. This is suggestive that it may be possible to incorporate multiple Au(I) atoms per mismatch, similar to Ag(I) incorporation into 4-thiothymine¹⁹

In the case of **CC1**, we observe a large increase in thermal stability with the addition of two equivalents of Au(I), while the addition of excess Au(I) has minimal effect (**entry 5**). The biphasic properties of the thermal melting curves indicate direct formation of a doubly incorporated MMBP, behaving similarly to previously reported Ag(I) complexes.^{7,18} The formation of a single Au(I) incorporated C-C mismatch does not appear to be present (**Figure 3a**). When 0.4, 1.0, and 1.4 equivalents of Au(I) are graphed to fit a biphasic curve, there are two T_m transitions. The early transition thermal stability corresponds to a mismatch duplex containing no gold ions, whereas the second transition has a very large thermal stability increase, likely due to incorporation of two gold ions. This preference for binding of two Au(I) ions can be explained in part to the known aurophillic interaction between gold atoms.²⁰

To the best of our knowledge, this is the largest increase in thermal stability from a metal mediated base pair containing canonical bases. Many other examples contain highly engineered bases and/or covalent bond formation.^{7,21} Additionally, this exceptionally large increase in thermal stability is unique to Au(I). Other metals including Ni, Co, Fe, and Cu, which have been previously reported to interact with nucleic acids, as well as Au(III) species and more stabilized Au(I) precursors did not affect the thermal stability of any of the pyrimidine mismatched duplexes.^{sir}

To elucidate potential binding modes of Au(I) to a C—C mismatch, the base-pairing energy of various 1-methylcytosine—Au(I) ions were calculated. **Figure 4** depicts the structures and corresponding base pairing energies (BE) of various possible 1-methylcytosine complexes with either one or two Au(I) ions. Complexes incorporating a single Au(I) ion have modest binding energies with the N3-Au(I)-N3 complex (**4**) preferred over all calculated possibilities. The lack of hydrogen bonding in the mono-Au complex allows for additional

metal coordination without an energetic cost associated with breaking of the highly stabilizing hydrogen bonds present in canonical base pairs. Addition of a second Au(I) ion can then be incorporated either between the 4-amino nitrogen (**5**) or the 2-keto oxygen (**6**), with a preference for the later by nearly 10 kcal/mol. Displacement of a proton from a single 4-amino group results in a more stable complex (**7**) and this effect is significantly more pronounced in the neutral, doubly deprotonated complex (**8**).

We hypothesized that the formation of cationic complex (**7**) and neutral complex (**8**), resulting from the loss of one or two protons, would be highly pH dependent. Specifically, deprotonation of the exocyclic amines of cytosine, and binding to Au(I), should be favored under basic conditions. In turn, acidic conditions should disfavor this binding and result in loss of Au(I) binding. In fact, at pH 5.5 the increase in thermal stability is only 12.6 °C (**Figure 5a**), a loss of 20.4 °C of stabilization present at pH 7. Under basic conditions, at pH 8.5, the increase in thermal stabilization is 34 °C (**Figure 5b**), exhibiting no significant change from thermal stability at neutral pH 7. This is consistent with the formation of the cationic complex **6** (**Figure 4**), stabilized by the highly negative backbone of DNA. Previous NMR and IR studies suggest that Au(I) binds both N3 and the carbonyl of free cytidine.^{13,14} Therefore, the stability of the complex exhibiting this mode of binding would not be dramatically affected under basic conditions. Additionally, the known protonation of N3 of cytosine under acidic conditions is consistent with our result, as protonation would inhibit binding of Au(I), decreasing the stabilization effect.²²

Highly specific metal mediated base pairs, such as thymine-Hg(II)-thymine, have been shown to form dimer complexes with poly(thymine) sequences, such as 5'-TTTTT-3' (**T5**).⁶ Based on the large increase in thermal stability, we proposed that poly(cytosine) sequences would also dimerize in the presence of Au(I) ions. Mass spectrometry experiments indicate formation of a dimer in the presence of 5'-CCCCC-3' (**C5**), and 5 equivalents of Au(I), 1 equivalent per cytosine, wherein 5, 6, and 7 Au(I) atoms are coordinated. In the case of the **T5** sequence, only the mass for the single stranded sequence was observed as well as some nonspecific binding to the single stranded sequence. In the presence of equimolar **T5** and **C5** sequences, only the mass corresponding to **C5** dimers is observed (**Figure 5**). This is suggestive of specific binding to cytosine-cytosine mismatches with a binding affinity strong enough to hold together a sequence of complete mismatches. Further, in the presence of **G5** (5'-GGGGG-3'), and **C5** there is a preference for formation of the **C5** dimers, suggesting that this new MMBP is favored over the natural CG base pair.

While our experiments indicated the formation of a highly stabilized new base pair, we sought to detect any structural changes to the DNA duplex upon addition of Au(I), as it is known that metal binding can cause changes to its helical structure.¹² CD experiments show minimal change in the ellipticity profiles of duplexes containing a single CC, Ti', or CT mismatch both before and after the addition of Au(I) (SI), suggesting that a single pyrimidine mismatch does not induce a significant deformation of the duplex (**Figure 6**). However, duplexes containing multiple CC mismatches show slight changes in spectrum upon addition of Au(I) (**Figure 6**).

Upon addition of Au(I) to a double C-C mismatched DNA duplex, CC2, the positive peak of the CD spectrum decreases in intensity and is shifted to a higher energy wavelength. In addition, the minimal changes in the CD spectrum show that it is unlikely to be forming a complex secondary structure, such as a G-quadruplex, in the presence of the cationic Au(I) atom.

Over the last few decades, there have been many reports of MMBP and much research has been surrounding making these types of metal-DNA complexes useful as sensors, nanowires, and other devices for nanotechnology. However, only two metals have been previously shown to bind the bases of natural DNA mismatches. In this paper, we report on the first metal-mediated base pair containing Au(I) atoms with a C—C mismatch. This highly stabilizing MMBP contains two Au(I) per mismatch and to the best of our knowledge, is the highest reported T_m increase for a MMBP composed of naturally occurring DNA bases. This new discovery can give insight into potential toxicity of Au(I) or potential interactions of gold cancer therapeutics. The addition of a new metal-mediated base pair can be incorporated into DNA structures and may lead to new discoveries of nanowires and structures or as an interesting catalytic scaffold.

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Example 2: Thermal Stability Measurements

General Procedure for Sample Preparation for Thermal Stability Measurements

Solutions contained 3.5 RM DNA in buffer containing 0.75 mM sodium phosphate, pH 7, 150 mM NaClO₄ and 0 μM (0 eq) or 3.5 FAM (1 eq) of (Me₂S)AuCl (60:1 H₂O:MeOH v/v). DNA and buffer were heated at 90 °C for 10 minutes then allowed to cool to room temperature over 45 minutes. After the solution was cooled, (Me₂S)AuCl (210 FIM 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 2. Sequences used in thermal denaturation experiments:

Sequence Name	Strand 1	Strand 2
GT1	5'-GAG GGA AG AAA GG-3'	5'-CCT TTC TTT CCC TC-3'
AA1	5'-GAG GGA AAG AAA GG-3'	5'-CCT TTC TAT CCC TC-3'
CA1	5'-GAG GGA AG AAA GG-3'	5'-CCT TTC TAT CCC TC-3'

Table 3. Thermal denaturation values for GT1 sequence

Thermal Denaturation – CT1			
[Au]	T_m (°C)	ΔT_m (°C)	Error
0 equiv	44.6	--	0.5
1 equiv	54.0	10.5	0.8
2 equiv	55.1	10.7	0.4

Table 3. Thermal denaturation values for AA1 sequence

Thermal Denaturation – CT1			
[Au]	T_m (°C)	ΔT_m (°C)	Error
0 equiv	40.7	--	1.5
1 equiv	50.6	9.9	0.8
2 equiv	55.6	14.9	1.1

Table 4. Thermal denaturation values for CA1 sequence

Thermal Denaturation – CT1			
[Au]	T_m (°C)	ΔT_m (°C)	Error
0 equiv	39.7	--	0.4
1 equiv	49.1	9.4	0.8
2 equiv	64.8	25.1	0.8

Melting temperatures calculated from the thermal denaturation profiles of pyrimidine-mismatch-containing oligonucleotides in the presence of various concentrations of (Me₂S)AuCl. Melting temperatures and error calculated using the sigmoidal dose-response feature in GraphPad Prism 7.0c.

Example 3: Regulating transition metal catalysis through interference by short RNAs

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 catalysts 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 8**). 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 towards 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.

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 CT mismatch and we noted no increase in thermal stability (**SI-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 T_m (thermal stability) when exposed to one equivalent of (Me₂S)AuCl (**Figure 9a**), with no apparent formation of Au-nanoparticles. Moreover, titration experiments indicated the incorporation of a single gold ion into the dsDNA (**Figure 9b**). 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-4** and **SI-5**). 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 (**SI-7**).

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 9c**). This behavior is consistent with binding of gold to the acidic, endocyclic N3 of thymine (**Figure 9d**). 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 9d**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 T–Au(I)–C MMBP is 14 kcal/mol more stable than a Hg-MMBP²⁷ (**1**) and 30 kcal/mol more stable than a Ag-MMBP^{27, 46} (**2**), supporting our experimental observations (**Figure 9d**).

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 10a**). 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 10b**).

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 **5** by approximately 700% (**Figure 10c**). 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, 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).

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 11a**). Here, strand displacement with a complementary strand 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 11b**). 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 11c**). 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 11d**). 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. 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 **R1** and **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 11e**). 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.

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 11f**). 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.

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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 for 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, $A_{260nm} = (A_t - A_{15^\circ C}) / (A_{90^\circ C} - A_{15^\circ C})$, 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 **10** in ethanol was added resulting in a final solution containing (1:1:0.02 H₂O:EtOH:(CH₃)₂O). Reactions contain 10 μM DNA hairpin, 10 μM complement sequence, 250 mM NaClO₄, and 40 μM BODIPY **10**. 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 **11** 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).

INCORPORATION BY REFERENCE

Each publication and patent mentioned herein is hereby incorporated by reference in its entirety. In case of conflict, the present application, including any definitions herein, will control.

EQUIVALENTS

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification and the following claims. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

CLAIMS

What is claimed is:

1. A gold-containing compound comprising
a nucleic acid strand that forms a toehold-stem-loop structure, wherein the nucleic acid strand comprises
a first part that forms a toehold portion of the toehold-stem-loop structure;
a second part that together with a fourth part of the nucleic acid strand forms a stem portion of the toehold-stem-loop structure by annealing with the fourth part, wherein the second part is contiguous with the first part;
a third part that forms a loop portion of the toehold-stem-loop structure, wherein the third part is contiguous with the second part; and
the fourth part that together with the second part forms the stem portion, wherein the fourth part is contiguous with the third part; and
an aurous gold atom complexed with two nucleic acid residues of the nucleic acid strand, wherein the two nucleic acid residues form a mismatched pair in the stem portion of the toehold-stem-loop structure.
2. The gold-containing compound of claim 1, wherein the nucleic acid strand is a deoxyribonucleic acid strand, and wherein the two nucleic acid residues comprise a nucleobase pair selected from cytosine-thymine, cytosine-cytosine, cytosine-adenine, thymine-guanine, adenine-guanine, and thymine-thymine.
3. The gold-containing compound of claim 1 or 2, further comprising at least one detectable label conjugated to the nucleic acid strand.
4. A method of increasing stability of a nucleic acid duplex having a mismatched nucleobase, the method comprising contacting the nucleic acid with an aurous gold compound in an aqueous solution.
5. The method of claim 4, wherein the compound is chloro(dimethylsulfide)gold(I).
6. The method of claim 4 or 5, wherein the solution has a pH between 7.5 and 10.5.

7. The method of any one of claims 4-6, wherein the nucleobase is a cytosine mismatched with a thymine, a cytosine mismatched with a cytosine, a cytosine mismatched with an adenine, a thymine mismatched with a guanine, an adenine mismatched with a guanine, or a thymine mismatched with a thymine.

8. A method of regulating an aurous-gold-catalyzed reaction, the method comprising mixing a reactant with an aurous-gold-containing toehold-stem structured nucleic acid (e.g., a compound of any one of claims 1-3) to obtain a mixture, wherein the nucleic acid has in its stem portion a mismatched residue pair that is complexed with the aurous gold; and adding to the mixture an oligonucleotide that is complementary to a part of the nucleic acid, thereby allowing the aurous gold to catalyze the reaction of the reactant.

9. The method of claim 8, wherein the mismatched residue pair comprises a cytosine and a thymine, a cytosine and a cytosine, a cytosine and an adenine, a thymine and a guanine, an adenine and a guanine, or a thymine and a thymine.

10. The method of claim 8 or 9, wherein the oligonucleotide is complementary to a part of the nucleic acid that includes at least a part of its toehold portion and of its stem portion that is contiguous with said toehold portion.

11. The method of any one of claims 8-10, wherein the reaction is aurous-gold catalyzed hydroamination.

12. A method of detecting an oligonucleotide, the method comprising obtaining an aurous-gold-containing nucleic acid that has a single-stranded toehold portion and a double-stranded stem portion, wherein the stem portion comprises a mismatched pair of nucleic acid residues complexed with the aurous gold; contacting the oligonucleotide with the aurous-gold-containing nucleic acid, thereby allowing the aurous gold to catalyze a reaction; assaying to determine a degree of completion of the reaction; and detecting the oligonucleotide when the degree passes a threshold.

13. The method of claim 12, wherein the oligonucleotide is an mRNA.

14. The method of claim 12 or 13, wherein the contacting step occurs in a solution.
15. The method of claim 12 or 13, wherein the contacting step occurs on a solid surface.
16. The method of any one of claims 12 to 15, wherein the contacting step occurs *ex vivo*.
17. The method of any one of claims 12 to 15, wherein the contacting step occurs *in vivo*.
18. The method of any one of claims 12-17, wherein the nucleic acid further comprises a loop portion adjacent to its stem portion.
19. The method of any one of claims 12-18, wherein the mismatched pair comprises a cytosine and a thymine, a cytosine and a cytosine, a cytosine and an adenine, a thymine and a guanine, an adenine and a guanine, or a thymine and a thymine.
20. The method of any one of claims 12-19, wherein the nucleic acid is a deoxyribonucleic acid, and wherein the oligonucleotide is a ribonucleic acid.
21. A method of detecting aurous gold in a substance, the method comprising obtaining a nucleic acid that has a double-stranded portion, wherein the double-stranded portion comprises a mismatched nucleobase;
contacting the substance with the nucleic acid, and allowing the aurous gold to catalyze a reaction;
assaying to determine a degree of completion of the reaction; and
detecting the aurous gold when the degree passes a threshold.
22. The method of claim 21, wherein the nucleic acid further comprises a loop portion adjacent to its stem portion.
23. The method of claim 21 or 22, wherein the nucleobase is a cytosine mismatched with a thymine, a cytosine mismatched with a cytosine, a cytosine mismatched with an adenine, a thymine mismatched with a guanine, an adenine mismatched with a guanine, or a thymine mismatched with a thymine.

24. A gold-containing double-stranded nucleic acid, comprising an aurous gold atom coordinated between a first nucleobase and a mismatching second nucleobase.
25. The nucleic acid of claim 24, wherein the first nucleobase and the second nucleobase are a cytosine and a thymine, a cytosine and a cytosine, a cytosine and an adenine, a thymine and a guanine, an adenine and a guanine, or a thymine and a thymine.

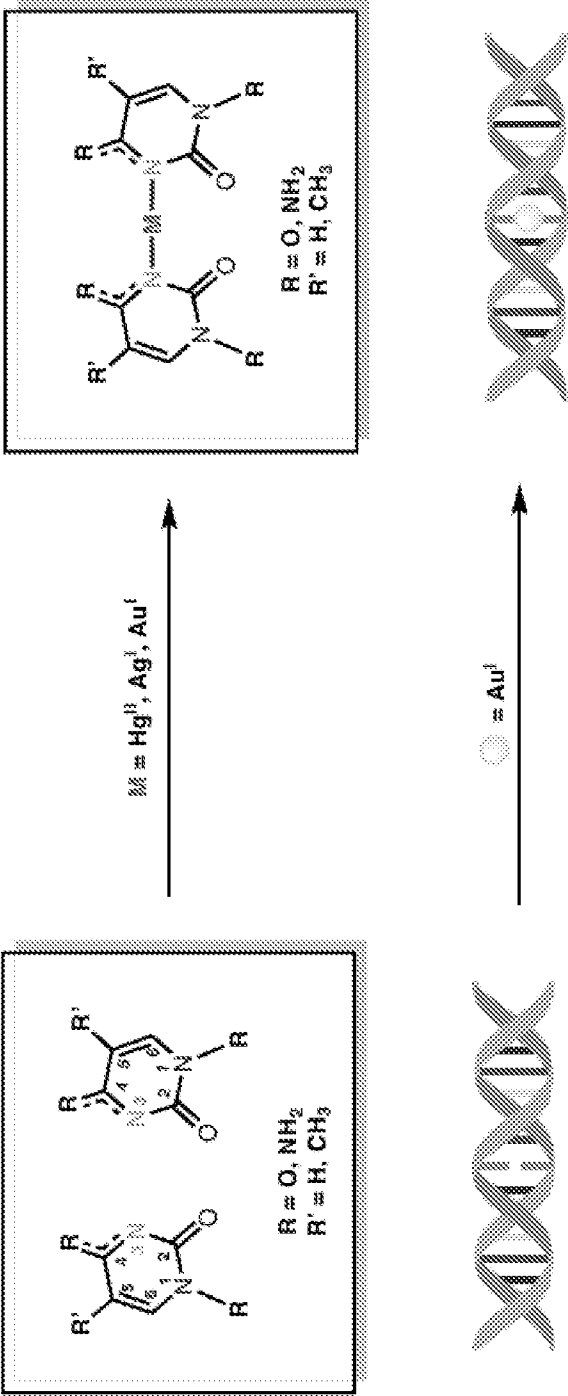


Figure 1

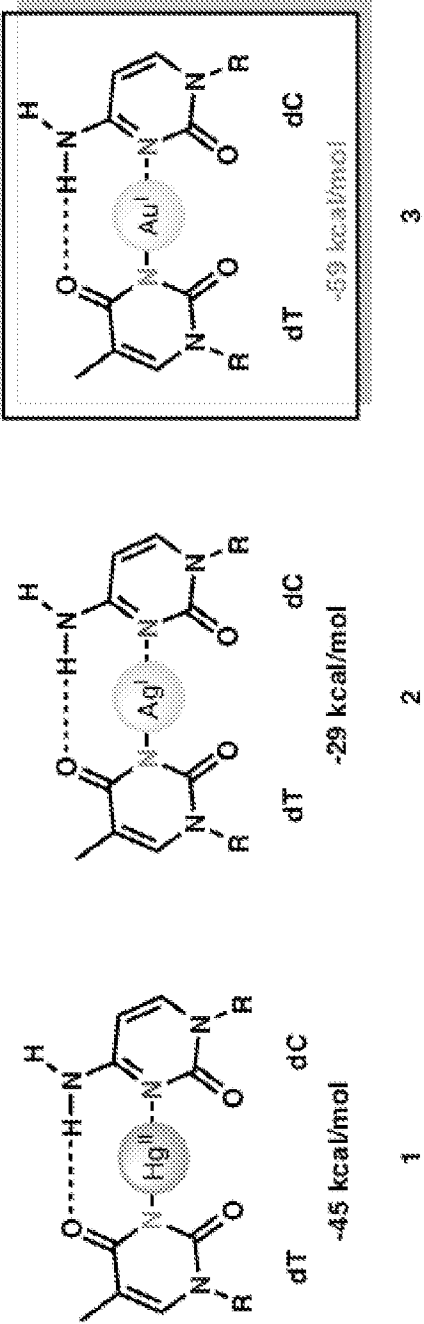


Figure 2

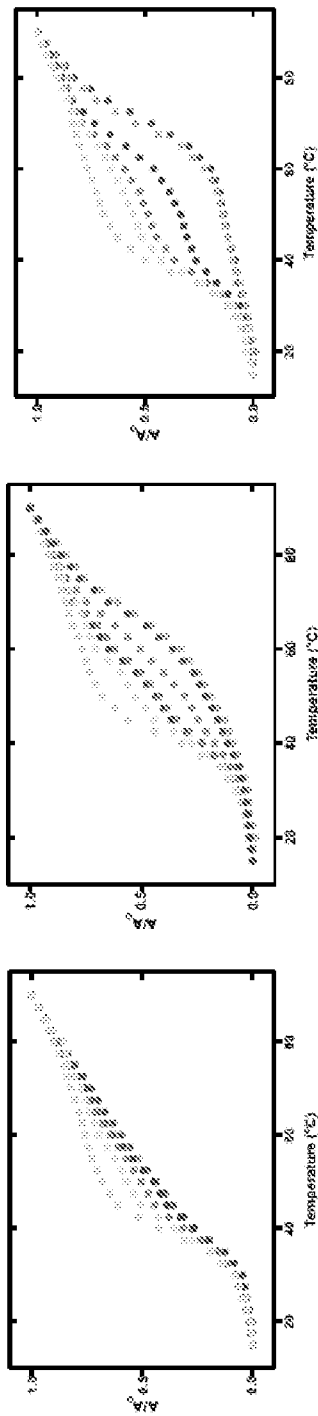


Figure 3

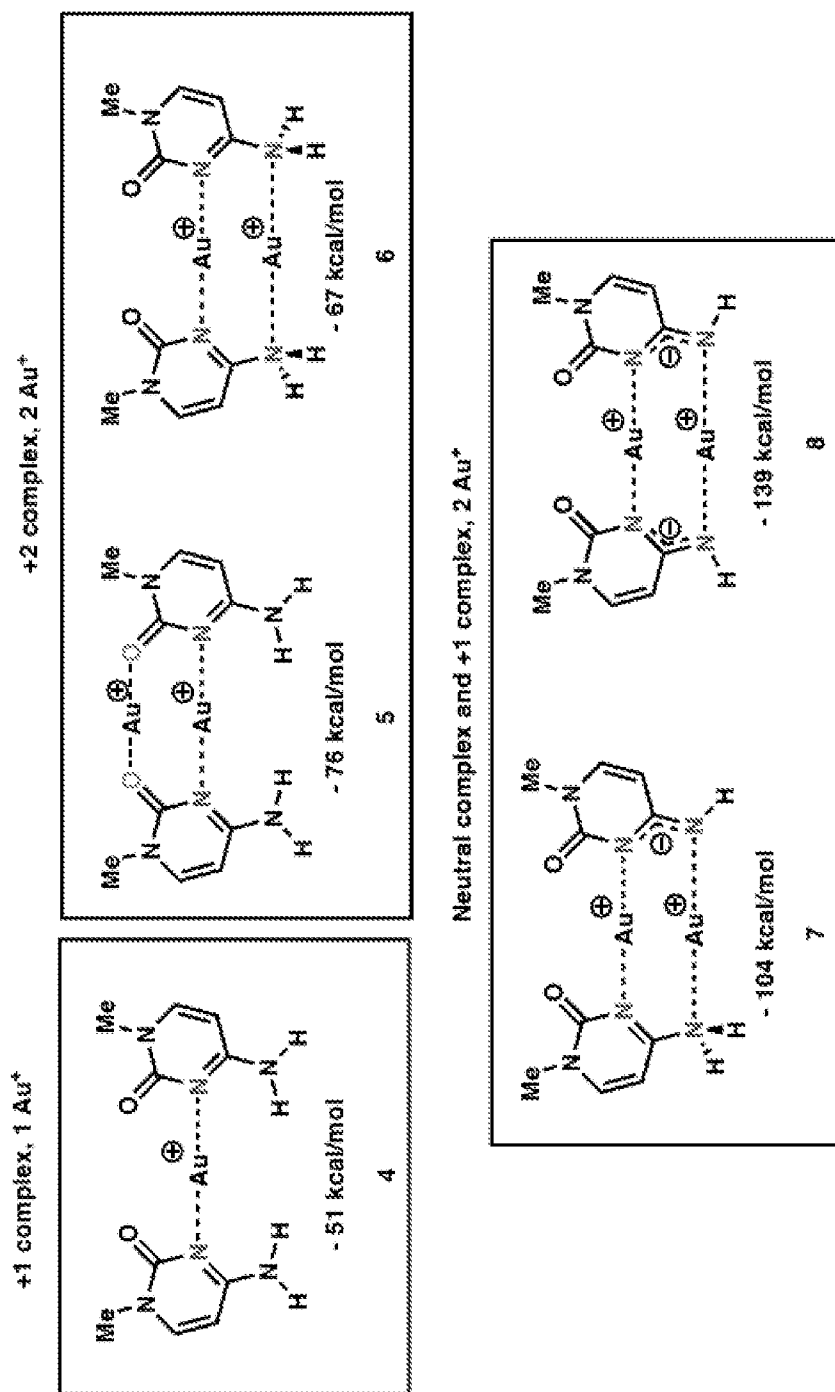


Figure 4

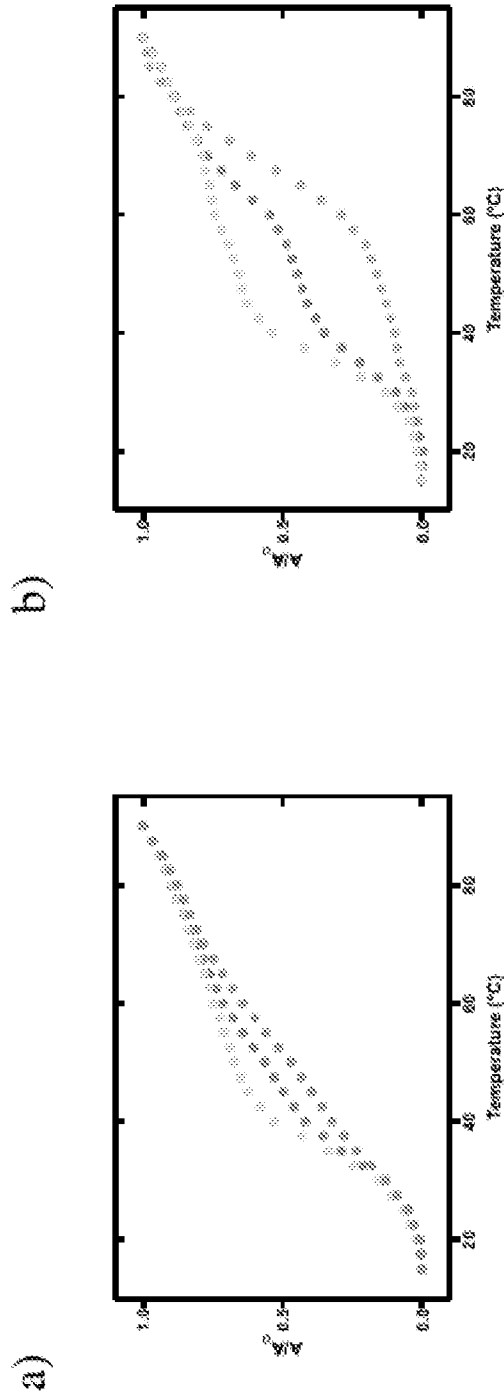


Figure 5

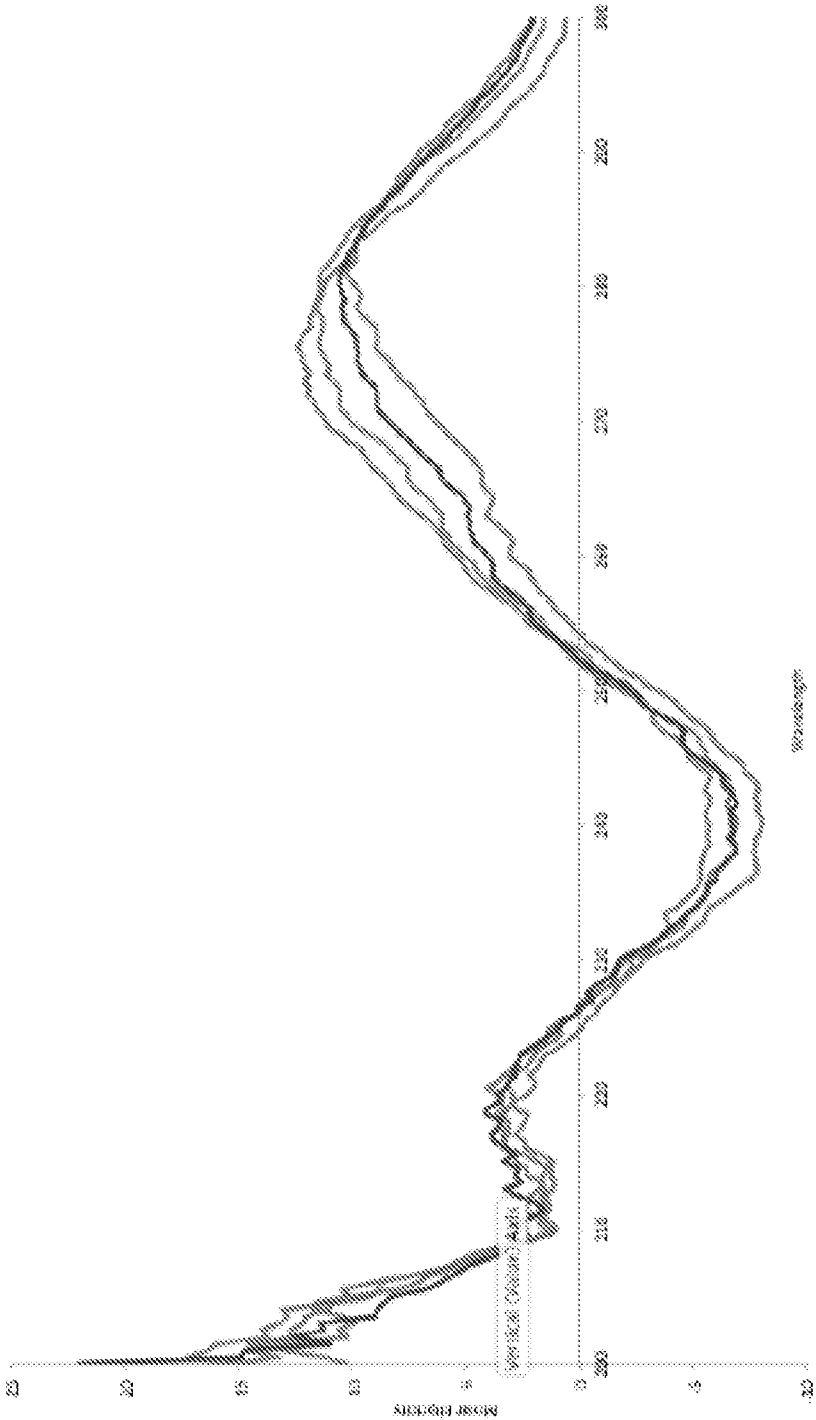
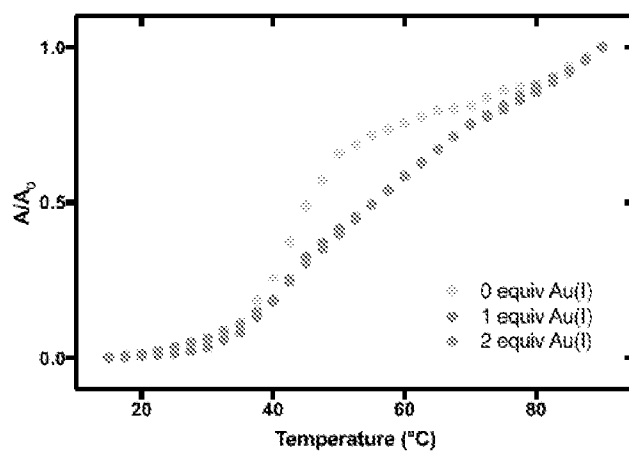
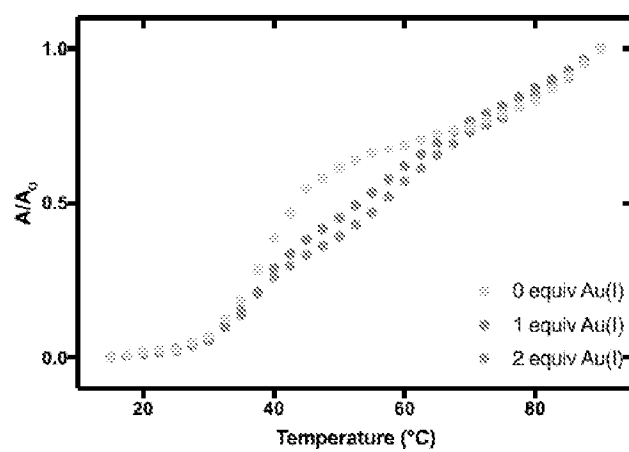


Figure 6

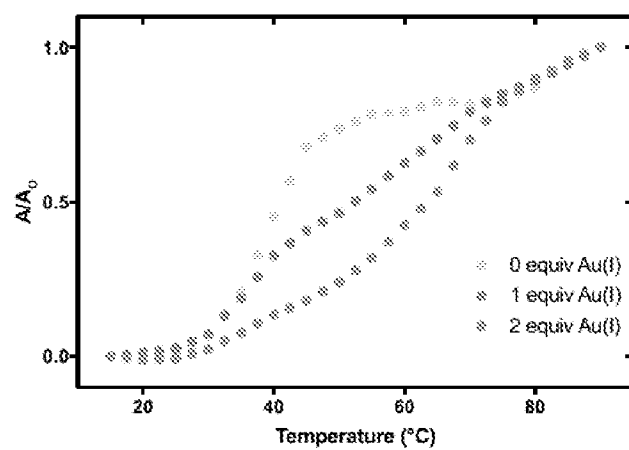
a) GT1



b) AA1



d) CA1

**Figure 7**

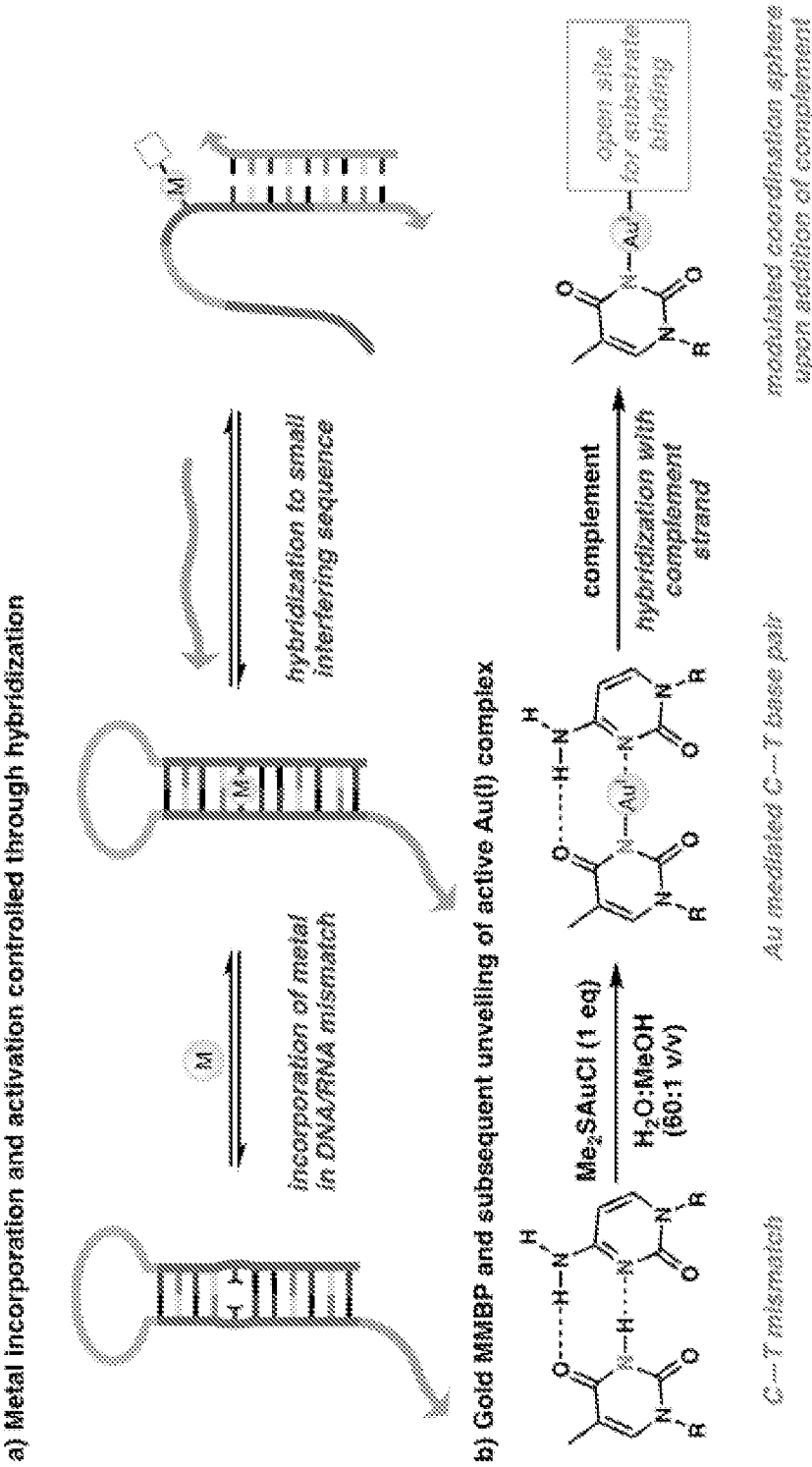


Figure 8

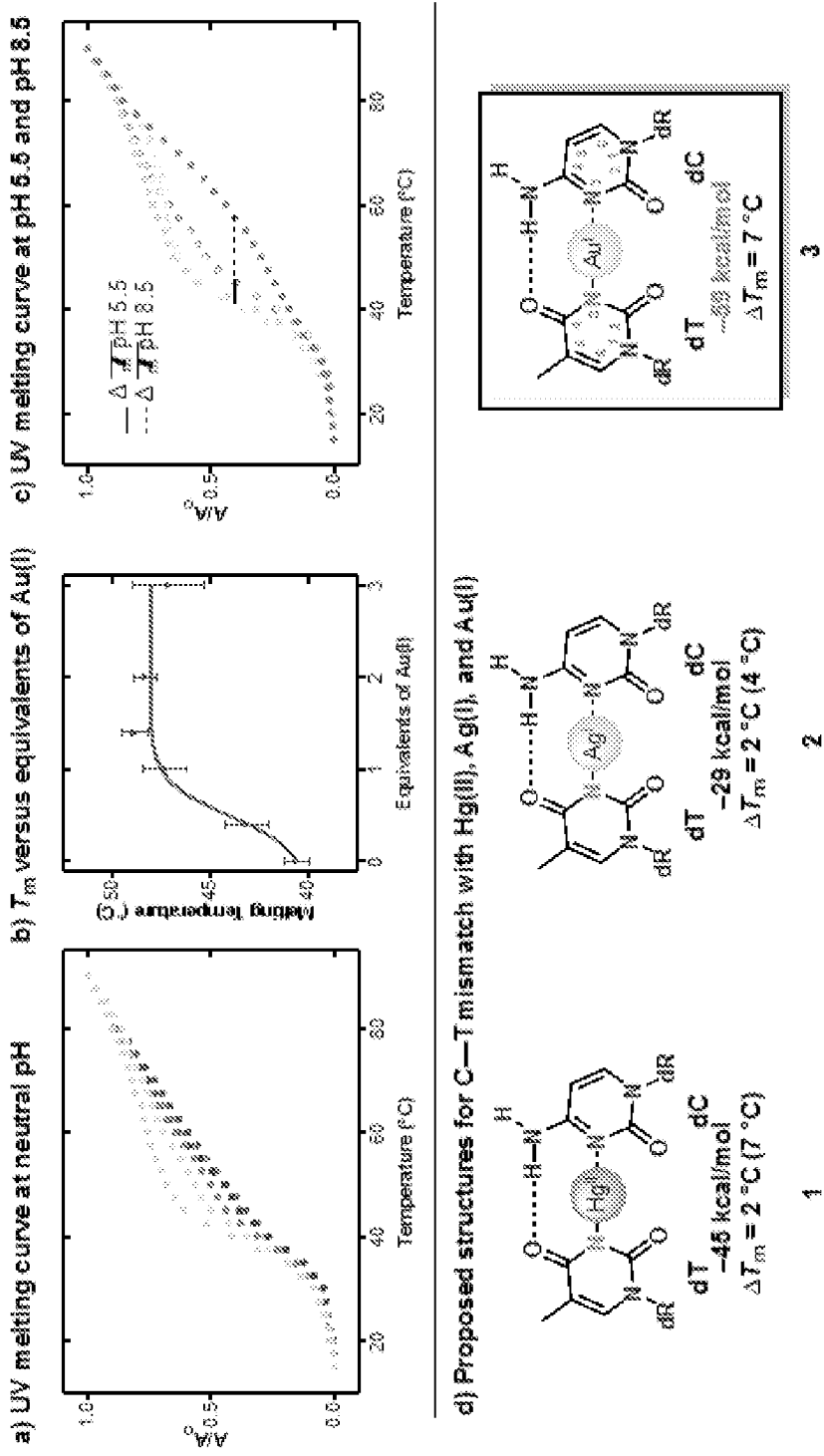


Figure 9

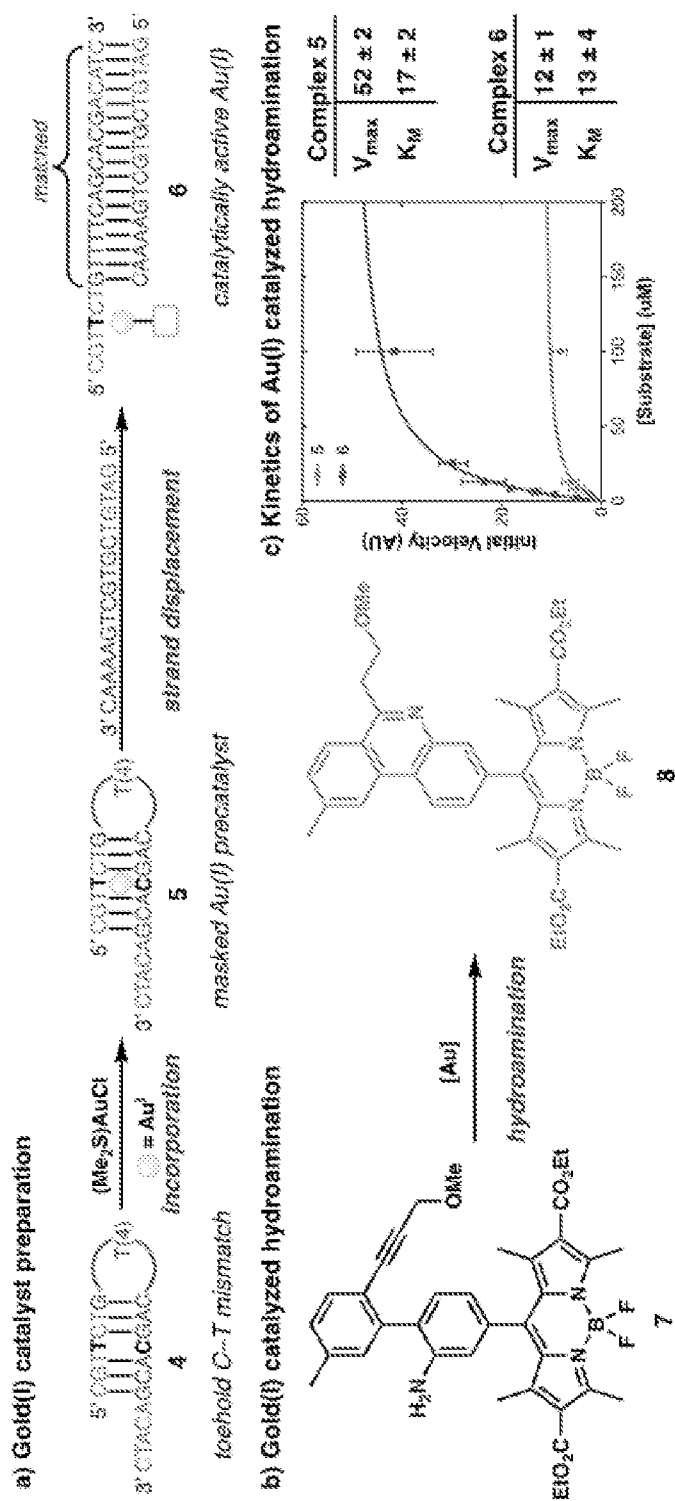


Figure 10

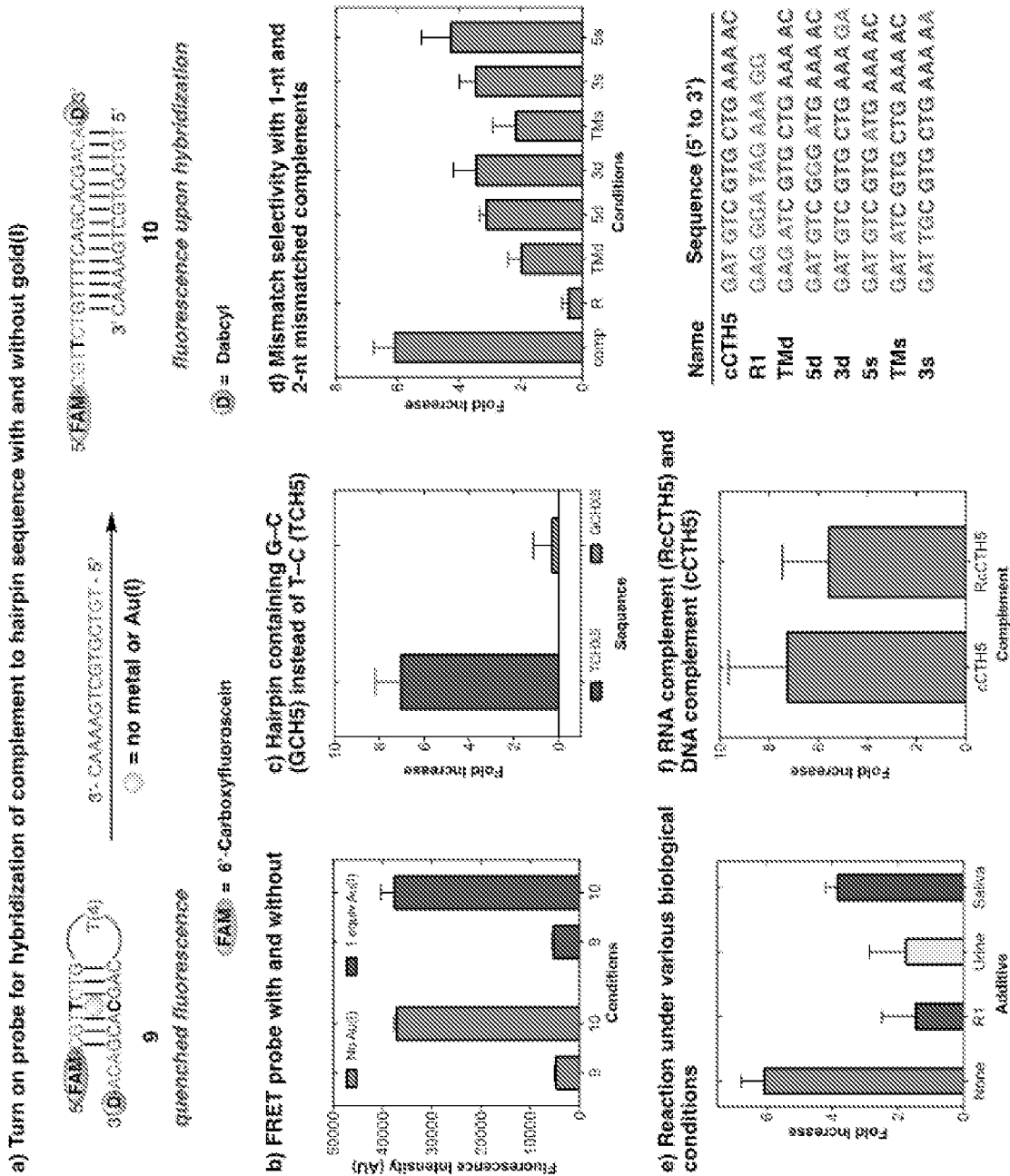


Figure 11