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Macrocyclic Metal Complex–DNA Conjugates for Electrochemical Sensing of Single Nucleobase Changes in DNA

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Supporting Information

ABSTRACT: The direct incorporation of macrocyclic cyclidene complexes into DNA via automated synthesis results in a new family of metal-functionalized DNA derivatives that readily demonstrate their utility through the ability of one redox-active copper(II)-containing strand to distinguish electrochemically between all four canonical DNA nucleobases at a single site within a target sequence of DNA.

DNA presents an ideal scaffold for the assembly of nanoscale architectures as a result of its well-understood structure, high programmability, and the ease in which derivatives can be synthesized containing non-natural components.[1] In particular, the incorporation of metal-containing moieties into DNA has become a highly attractive field of study because of the range of potential applications,[2] which include the development of electrochemical sensors[3] and DNA nanotechnology.[4] A convenient way to incorporate functional tags into DNA for such purposes is via solid-phase automated synthesis using phosphoramidite chemistry. As well as being the most direct method, this approach offers precise control over the number and position of groups within a strand. However, one drawback of this method is that the tag must be able to withstand the conditions used in both monomer preparation and automated synthesis. This has meant that examples of metal-containing tags that have been successfully incorporated in this way have been largely limited to robust organometallic (e.g., ferrocene)[5] and transition metal bipyridine moieties.[6] Other approaches to incorporating metal complexes through chemical means are less versatile[7] or less direct, for example requiring the use of additional postsynthesis metalation steps.[4b,8]

On the other hand, organic tags are relatively easy to append to DNA via automated synthesis, which has led to the widespread practice of tagging organic fluorophores to DNA for fluorescence sensing applications. One particular class of DNA probe that has attracted recent interest in the literature is the base-discriminating fluorophores (BDFs);[9] these probes can detect changes at the single nucleobase level in target DNA strands upon hybridization. The detection of such single nucleotide polymorphisms (SNPs) at specific loci within DNA sequences is important for the screening and monitoring of diseases with a genetic component. Analogous electrochemical probes, including those with redox-active metal tags, that could perform a similar function with respect to SNP detection would be attractive due to the prospect of low background interference and their ready incorporation into devices. Some electrochemical SNP sensors are indeed known,[10] but there is a scarcity of appropriate redox-active tags with the required base-discriminating properties that can be directly inserted at any position[11] within a DNA strand.

Herein we report the first example of the direct (i.e., in one-step) incorporation of a macrocyclic transition metal complex into DNA in the form of a Ni(II) or Cu(II) [14]cyclidene group (Figure 1). These redox-active complexes are robust

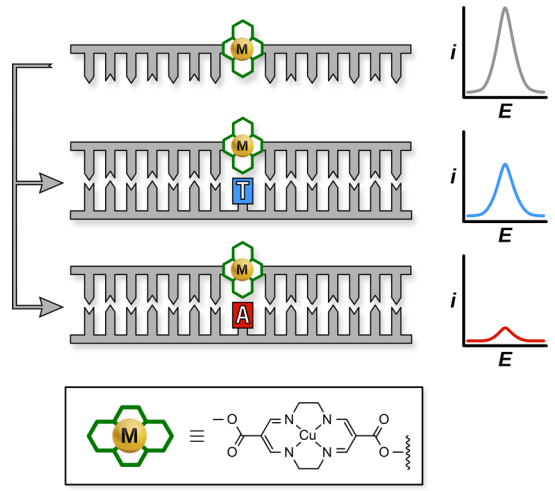

Figure 1. Schematic representation of a modified DNA oligomer used in this study, containing a redox-active copper [14]cyclidene complex, and the effect of hybridization with target DNA strands on its electrochemical properties.

enough to withstand automated synthesis and also more than one incorporation into DNA.[12] Furthermore, the usefulness of the redox properties of the resulting metal-containing DNA strands is readily demonstrated by the Cu systems being capable of distinguishing between different nucleobases at a single locus in a target strand of DNA at physiological pH.

The choice of a cyclidene complex for incorporation into DNA satisfied a number of requirements that came from

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analyzing work undertaken on strands containing organic photoactive groups. In particular, as four-coordinate macrocyclic complexes, cyclidenes adopt a planar geometry, a prerequisite for duplex intercalation,[13] and have a similar size to a number of organic fluorophore tags.[14] In fact, the shape of the cyclidene ring closely resembles that of pyrene, which has previously been successfully incorporated into DNA to make a series of photoactive oligonucleotide arrays.[15] In addition, cyclidene complexes are stable, can be synthesized relatively easily, and have a number of interesting properties.[16] In particular, the neutral nickel(II) and copper(II) cyclidene complexes[17] can act as π-donors,[18] form nanoscale structures,[19] and, of particular relevance to this work, possess electrochemical activity through their metal-centered redox couples.[17b,c] Therefore, cyclidene complexes containing these metal atoms were chosen for DNA incorporation. Two structural designs were considered for the metal-modified DNA (Figure 2), one in which the complex acts as a tag and can be considered to be a non-nucleosidic base surrogate (**M-Tag**) and another forming a metal link along the DNA backbone (**M-Link**).

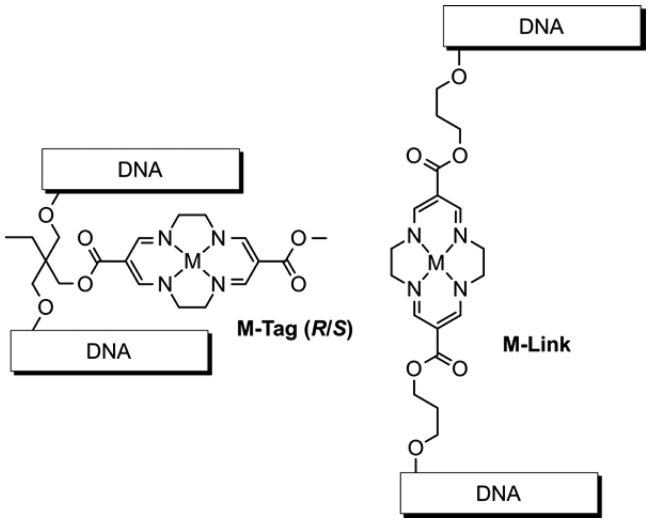

Figure 2. Metal cyclidene complexes (**M** = **Cu** or **Ni**) incoporated into DNA showing tagged (**M-Tag**) (left) and linked (**M-Link**) systems (right).

Both phosphoramidite precursors were made in four steps[18,19] from known methyl ester cyclidenes (see Supporting Information (SI)). These were then incorporated into DNA 15-mer oligonucleotides containing a modification site and base sequence (strands **S1**, Table 1) that would allow comparisons with previous work.[9a] Complementary strands that would present a base change directly opposite the modification site in duplexes were also prepared (strands **S2**, Table 1).

The metal complex incorporations proceeded smoothly and after purification by RP-HPLC, each strand was characterized by analytical HPLC, UV/vis spectroscopy, and electrospray mass spectrometry (see SI). As expected, in the case of the tagged system, two diastereomeric strands were isolated for each metal-functionalized strand, due to the creation of a stereogenic center in the linker group during the synthesis of the monomers. The strand with the longer elution time was assigned the (*R*)-stereochemistry on the basis of a combination of computational models and spectroscopic measurements (see SI). The UV/vis spectra of the modified strands **S1** in phosphate buffer at neutral pH gave a distinct absorption peak at 330 nm for the Ni(II) systems and a shoulder between 300 and 340 nm for the Cu(II) systems. These arise as a result of Soret-like bands that are associated with metal cyclidene complexes.[17b]

The strength of the duplexes formed by mixing equimolar amounts (5 μM) of **S1** and **S2** together was then assessed using variable temperature UV/vis spectroscopy. A selection of the resulting melting points (T_m values) for **Y** = A are presented in Table 2. These show striking differences in stability between the linked and tagged systems. Compared with the unmodified duplex **S1T·S2A**, the linked system is highly destabilizing, having a T_m value of ca. 16 °C lower for either metal. This suggests that the linker geometry is certainly not optimum for a strong interaction with 15-mer target strands. However, in contrast, the tagged sequences do not significantly disrupt the stability of the duplex, having T_m values very close to the unmodified control. Such an effect has been observed previously with similarly sized fluorophore tags[9a,c,14b] and can be explained by intercalation of the tag into the duplex and stacking with adjacent base pairs. This is further supported by the existence of a small red shift (2−3 nm) and some hypochromicity (3−5%) in the Soret band of **Ni-Tag(*R*)** upon hybridization (see SI). Additionally, induced excitonic bands are visible in the CD spectra (see SI), which is consistent with the tags being located within the helical environment of the duplexes.

Table 1. Oligonucleotides Synthesized (where X = T, M-Tag or M-Link and Y = A, C, T, G)

Oligonucleotide	Sequence
S1	5′-TGGACTCXCTCAATG-3′
S2	3′-CATTGAGYGAGTCCA-5′

Table 2. Melting Temperatures (T_m) of Unmodified (X = T, Y = A) and Metal Cyclidene-Modified (X = M-Link or M-Tag, Y = A) Duplexes, [DNA] = 5 μM in 10 mM Phosphate Buffer (pH 7), 100 mM NaCl, 0.5 °C/min

S1, X =	**S2**, Y = A, T_m/°C[a,b] (ΔT_m/ °C)[c]
T	55
Ni-Link	39 (−16)
Ni-Tag(*R*)	50 (−5)
Cu-Link	38.5 (−16.5)
Cu-Tag(*R*)[d]	50 (−5)

[a]Average of at least three measurements after annealing. [b]T_m values were calculated from the first derivative of the 260 nm melting curve. [c]ΔT_m values calculated relative to the unmodified duplex **S1T·S2A**. [d]Data for the **Cu-Tag(*S*)** isomer are presented in the SI.

Cyclic voltammetry studies on the metal-modified strands **S1** revealed redox activity for the Cu species only over the accessible potential range in phosphate buffer (up to 0.65 V vs Ag/AgCl). As noted previously for simple cyclidene complexes in organic solvents,[17c] these processes at $E_{1/2}$ = 0.444 V for **Cu-Tag(*R*)** (Figure 3) and $E_{1/2}$ = 0.423 V for **Cu-Link** were ascribed to the Cu(II)/Cu(III) redox couple. In each case, the peak separation, ΔE_p, was ∼60 mV with the peak current proportional to the square root of the scan rate, indicating electrochemical reversibility (SI). The electrochemical output was found to be unchanged after multiple electrochemical

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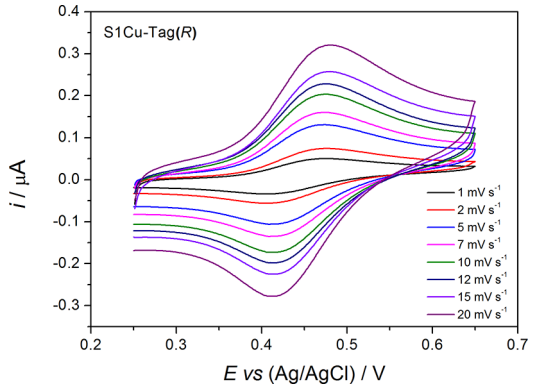

Figure 3. Cyclic voltammograms (1−20 mV s^{-1}) for strand **Cu-Tag(*R*)**. [DNA] = 50 μM in 10 mM Tris-HCl buffer (pH 7), 1 M NaCl, 293 K. CVs for **Cu-Tag(*S*)** and **Cu-Link** provided in the SI.

cycles and leaving for 24 h in phosphate buffered saline solution. This chemical and electrochemical stability compares favorably with other redox-tagged systems and reflects the stability of neutral cyclidene complexes to hydrolysis and metal ion removal.[18,19] Interestingly, no redox behavior was observed for the Ni counterparts within the accessible potential window, with this explained by the loss of an electron from a d^8 square-planar Ni(II) center being much more unfavorable (see SI).

The effect of duplex formation on the redox properties of these strands was then probed using square wave voltammetry. Upon addition of target strand **S2A**, duplex formation was evidenced by a decrease in current (−58% and −29% for **Cu-Tag(*R*)** and **Cu-Link**, respectively).[20] This can be explained by slower diffusion kinetics of larger species to and from the working electrode. However, due to the ability of the cyclidene moiety within the **Cu-Tag** strands to interact with the duplex through intercalation (*vide supra*), further studies were then undertaken on this system to assess its propensity to electrochemically sense single nucleobase changes (i.e. SNPs) in target DNA. The results (Figure 4) clearly show differences in redox current outside of experimental error when the base directly opposite the tag site in **S2** is changed.

A rationalization of these results comes from considering the size of the base opposite the tag, with the larger purine bases bringing about a larger decrease in current. The most marked change is when A is compared with T (58% decrease in current compared with 25%), the A−T transversion being an important mutation in various cancers.[21] It is noteworthy that the (*S*)-isomer of **Cu-Tag** does not give significant differences between A and T (see SI) which suggests that subtle effects related to the precise position of the cyclidene tag within the duplex are responsible for these differences. In particular, the ability of the tag to partially displace the base opposite and thus bury itself further into the duplex would be expected to impede electron transfer between the metal center and the electrode surface. Such a decrease in electron transfer rate is evidenced through cyclic voltammetry by a trend toward more marked increases in peak separation (ΔE_p) for those systems with higher current depletions (see SI). Further T_m results support this hypothesis, with notably higher values for the pyrimidine target strands **S2C** (54 °C) and **S2T** (55 °C) than for the purine systems **S2G** (50 °C) and **S2A** (50 °C). Taken together, these results indicate that the cyclidene tag can accommodate a smaller pyrimidine alongside it within the base-stack, which stabilizes the duplex overall. However, if a larger purine base is opposite,

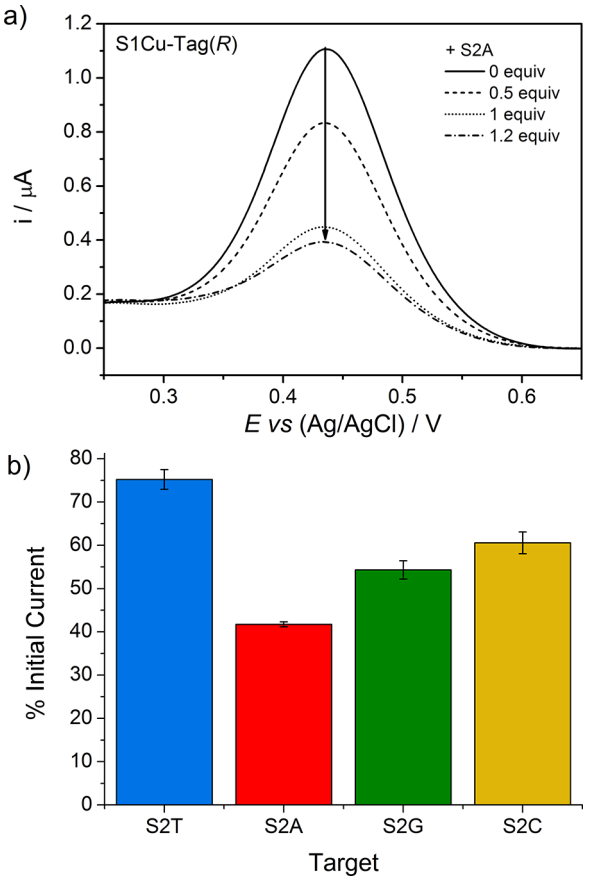

Figure 4. (a) Square wave voltammetry current changes for **Cu-Tag(*R*)** bound to increasing molar equivalents of **S2A**. (b) Percentage current change at i_{max} for duplexes with each of the four canonical bases opposite tag (1 mol equiv of **S2**). Error bars represent the SEM, [DNA] = 50 μM in 10 mM tris-HCl buffer (pH 7), 100 mM NaCl, 293 K.

the tag inserts itself more deeply, largely at the expense of that base, which, in the case of the (*R*)-isomer, results in both a lower T_m value and slower electron transfer kinetics.

In conclusion, we have presented stable oligonucleotides incorporating copper or nickel cyclidene complexes that are remarkably compatible with well-established automated DNA synthesis methodology. The **Cu-Tag** systems demonstrate stable electrochemical activity and can sense DNA, with one isomer giving a change in redox current that depends on the identity of the nucleobase opposite the tag. This new approach to electrochemical SNP sensing builds on other examples of organic[10c,d,11] and metal-based[10a,b] redox-active probes designed for this task, with these systems having the potential to offer a new generic sensing platform in which surface-immobilized probes could target SNPs in biological samples, for example those amplified by PCR. The particular attraction of these cyclidene-based systems is that they are both readily accessible and versatile, containing metal tags that may be positioned at any position within a strand, being not restricted to modification of a particular nucleobase or to tagging at the ends of strands. These facets, coupled with their rich electrochemical and spectroscopic properties, hold much promise for their further study within the area of metal-based DNA nanotechnology.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b11319.

Full synthesis and characterization details, including X-ray crystallography, spectroscopy, and electrochemistry (PDF)

Crystallographic data (CIF)

Crystallographic data (CIF)

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Notes

The authors declare no competing financial interest.

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