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Organometallic modification confers oligonucleotides new functionalities

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To improve their properties or to introduce entirely new functionalities, the intriguing scaffolds of nucleic acids have been decorated with various modifications, most recently also organometallic ones. While challenging to introduce, organometallic modifications offer the potential of expanding the field of application of metal-dependent functionalities to metal-deficient conditions, notably those of biological media. So far, organometallic moieties have been utilized as probes, labels and catalysts. This Feature Article summarizes recent efforts and predicts likely future developments in each of these lines of research.

1. Introduction

The programmable target recognition based on the simple rules of Watson–Crick base pairing makes nucleic acids attractive scaffolds for diverse applications, ranging from therapeutic agents[1–3] to nanoelectronic components.[4–6] The other properties of nucleic acids, however, leave much to be desired from the point of view of many of these applications. Natural oligonucleotides tend to accumulate in the liver and kidneys and get rapidly excreted in urine.[7–10] They also do not readily penetrate cell membranes and, once inside, are promptly degraded by nucleases.[11,12] The hybridization affinity is insufficient for targets with long double-helical regions, notably miRNAs.[13–16] While strongly chromophoric, oligonucleotides do not provide a signal that would stand out against the background of the intracellular medium. Nucleic acid catalysts (ribozymes and DNAzymes) and aptamers would both benefit from a wider range of functional groups than what is provided by the canonical nucleotides.[17–19] Electron transfer along DNA double helices has been detected but is insufficient to make DNA useful as a molecular wire.[20] All of these issues have been addressed by chemical modifications, with varying degrees of success.[21–23]

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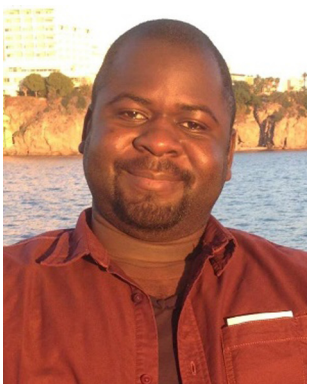


Lange Yakubu Saleh

Lange Yakubu Saleh, born in 1990 in Potiskum, Nigeria, obtained his MSc from Bolu Abant Izzet Baysal University in Turkey in 2016. Currently, he is working towards his PhD under the guidance of Professor Tuomas Lönnberg at the University of Turku, Finland, focusing on organometallic oligonucleotide conjugates as artificial ribonucleases. Lange's other research interests include the preparation of small organic molecules, the solid-supported synthesis of oligonucleotides as well as the reaction kinetics of nucleosides, nucleotides, and oligonucleotides.

1.1. Organic *vs.* metal-carrying modifications

Simple organic modifications are often sufficient for altering the physicochemical properties or enzymatic stability of oligonucleotides. Cellular uptake can be improved by conjugation of hydrophobic groups or cell-penetrating peptides.[24–28] Nuclease resistance, in turn, can be achieved through modification of the sugar or phosphate moieties, such as 2′-*O*-alkylation or replacement by phosphorothioate, respectively.[29,30] Glycoclusters show promise in targeting the liver and similar strategies for other organs, utilizing other conjugate groups, can be envisaged.[31–33] Organic fluorophores are widely employed in oligonucleotide-based hybridization probes and for visualization in biological samples.[34–36] Hybridization affinity can be enhanced through either backbone modifications, such as LNA[37] or PNA, or augmenting the base moieties with expanded stacking surface[38,39] or additional hydrogen bond donors and acceptors.[40–48] Similar strategies have also met with considerable success in increasing the binding affinity and specificity of aptamers.[49–57] Chemical modification of ribozymes and DNAzymes[17,18] has largely focused on biostability but recently rationally designed changes based on high-resolution 3D structures have been shown to also improve the catalytic activity.[58] As long as the modifications are confined to the sugar moieties, such engineered nucleic acid catalysts still usually retain their reliance on metal ion cofactors. Modifications of the base moieties, on the other hand, have afforded a number of metal-independent DNAzymes.[59–62]

Renal clearance can be retarded by increasing the size of the molecule. With oligonucleotides, this concept was first realized by immobilization of thiol-functionalized strands onto gold nanoparticles, giving rise to a spherical nucleic acid.[63,64] A metal core is not indispensable, however, and more recently spherical nucleic acids have also been synthesized with various non-metallic cores, such as fullerene or cubic silsesquioxane.[65] Even completely coreless ones have been prepared by dissolving the gold nanoparticle core after assembly of the spherical nucleic acid and cross-linking the constituent strands.[66]

In certain applications, metal complexes offer clear advantages over purely organic modifications. Lanthanide chelates offer longer luminescence lifetime, less concentration quenching and larger Stokes shift than organic fluorophores.[67] Analogously, while a number of redox active organic compounds potentially useful as electrochemical labels have been reported,[68–72] ferrocene is by far the most widely employed moiety for this purpose.[73–77] Artificial ribonucleases bearing a transition metal ion at the catalytic core tend to be more active than their purely organic counterparts.[78–83] For converting DNA into a nanowire, coordination of an array of metal ions appears all but indispensable.[84–88]

1.2. Self-assembly *vs.* covalent metalation

Coordinative complexes between oligonucleotides and metal ions are obtained conveniently through self-assembly provided that the former presents an appropriate high-affinity binding site and the latter is sufficiently labile kinetically. In ribozymes and DNAzymes, such a binding site is formed on folding of the oligonucleotide into a specific tertiary structure, bringing donor atoms from nucleobase and phosphate moieties that are often separated by several residues to converge on a common metal center (Fig. 1(A)).[89,90] Artificial nucleases and oligonucleotides labelled with a radiometal or a fluorescent lanthanide ion, in turn, usually exploit conjugation with high-affinity chelating groups (Fig. 1(B)).[91,92] Long arrays of metal ions have been formed within double-helical DNA through metal-mediated base pairing (Fig. 1(C)), paving the way to

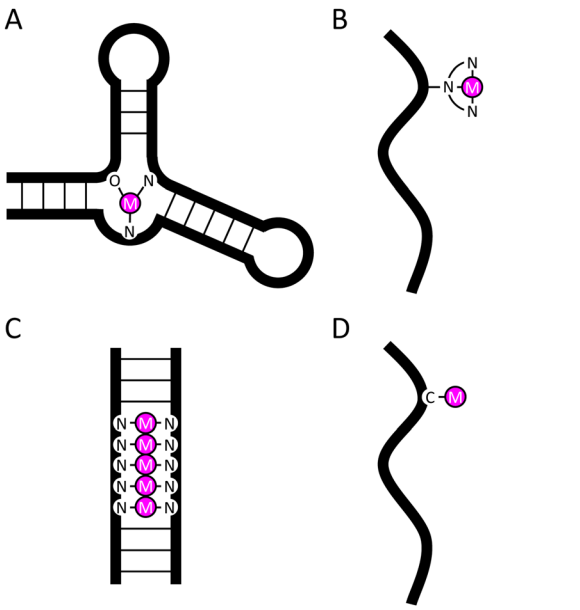


Fig. 1 Metal ion binding (A) at a site provided by folding of an aptamer or a catalytic oligonucleotide into its active conformation; (B) by a high-affinity chelating group conjugated to an oligonucleotide; (C) along the axis of a double-helical nucleic acid through metal-mediated base pairing and (D) through a carbon–metal bond at a natural or modified residue within an oligonucleotide.

Tuomas Lönnberg

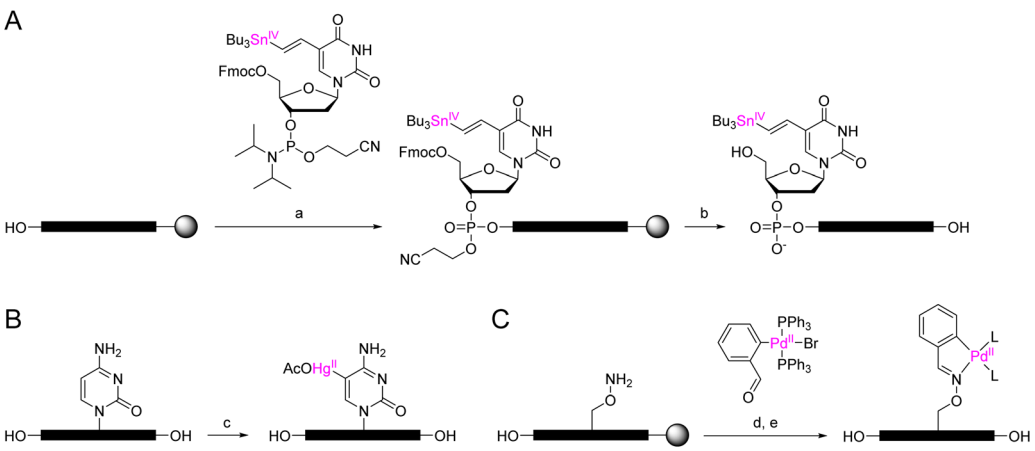
Tuomas Lönnberg obtained his PhD at the University of Turku, Finland, under supervision of Dr Satu Mikkola. He then spent two years as a JSPS Post-Doctoral Fellow in the group of Professor Makoto Komiyama at the University of Tokyo. After returning to the University of Turku in 2008, he has held various research and teaching positions. He was appointed assistant professor of organic chemistry in 2016, associate professor in 2021 and full professor in 2023. He is interested in organometallic oligonucleotides, phosphate-transfer reactions of nucleic acids and oligonucleotide functionalization through dynamic combinatorial chemistry.

rational design and bottom-up synthesis of well-defined nanowires.[93–95] A complementary approach to self-assembly for functionalizing oligonucleotides with metal ions is covalent metalation, *i.e.* using a stable organometallic bond to "pin down" a metal ion at a predetermined site (Fig. 1(D)).[96–100] The merits and limitations of this approach are discussed below.

1.2.1. Preparation. The main obstacle to the use of oligonucleotides bearing organometallic modifications lies undoubtedly with challenges in their synthesis and purification. The most attractive approach would be coupling of organometallic building blocks by conventional phosphoramidite strategy on an automated synthesizer (Scheme 1(A)). However, organometallic moieties introduced in this way have so far been limited to ones having the metal center coordinatively saturated, such as ferrocene[101,102] or tetraalkyltin.[103] Another possibility is post-synthetic covalent metalation of an oligonucleotide containing an appropriate reactive site (Scheme 1(B)), analogous to coordinative metalation of an oligonucleotide furnished with a high-affinity chelating group. With Hg(II), the reactive site can be a single electron-rich aromatic carbon atom,[104–106] whereas with platinum group metals an additional directing ligand is required.[107–109] In some cases such metalations are reasonably high-yielding with a small excess of the appropriate metal salt[110] but more commonly dozens of equivalents are needed, leading to a tedious chromatographic separation of the metalated and unmetalated oligonucleotides and the excess metal salt. The third option is attachment of a previously synthesized organometallic moiety to an appropriately modified oligonucleotide by one of the established conjugation chemistries (Scheme 1(C)), such as peptide coupling,[111–115] Michael addition,[116] oximation[117,118] or azide–alkyne cycloaddition.[119,120] The advantage of this approach is that the conditions used for the preparation of the organometallic moiety do not need to be compatible with those of oligonucleotide synthesis. Depending on the intended application, the relatively bulky linkages resulting from most conjugation reactions may be seen as a limitation.

1.2.2. Control over the site of metalation. In the case of relatively simple systems, such as short oligonucleotides featuring a single chelating ligand, self-assembly through coordinative interactions reliably affords the desired metal-bearing species. Even heterobimetallic assemblies making use of different preferences of the two metal ions in metal-mediated base pairing are possible.[121] With larger and more complex systems, the probability of unexpected coordination increases. For example, the self-assembly of dodecamer DNA oligonucleotides and Ag(I) ions into long nanowires containing four different Ag(I)-mediated base pairs and bulged-out adenine bases[94] was impressive but hardly predictable based on what was known about Ag(I)-mediated base pairing at the time. In ribozymes and DNAzymes the location of any metal ion binding sites, as well as their preferences for a given metal ion, ultimately stem from directed evolution rather than rational design.

Metalation strategies involving coupling of a previously synthesized organometallic species offer unparalleled control over the site of metalation as long as the oligonucleotide presents a single "handle" with unique reactivity, such as the 5′-hydroxy group in Scheme 1(A) or the aminooxy group in Scheme 1(C). Ligand-directed cyclopalladation can also be carried out with high site-selectivity as the necessary arrangement of an aromatic carbon atom and a proximal soft Lewis base is not found on natural nucleic acids. Similarly, Ag(0) has been deposited exclusively on aldehyde-bearing DNA by a Tollens reaction but the precise structure and extent of metalation remained obscure.[122] With mercuration, the situation is different because the C5 atoms of both cytosine and uracil bases readily react with various Hg(II) salts.[104] Some artificial nucleobase analogues[110] are much more reactive than the natural pyrimidine bases but even so cytosines should be replaced by 5-methylcytosines and uracils by thymines to prevent off-target mercuration. This approach has been successful with short oligonucleotides[105,110,123–127] but has never been tested with very long ones.



Scheme 1 Preparation of oligonucleotides bearing organometallic modification through (A) automated synthesis with organometallic phosphoramidite building blocks,[103] (B) post-synthetic metalation of an oligonucleotide in solution[105] and (C) on-support conjugation with a previously synthesized organometallic complex.[117] *Reagents and conditions*: (a) conventional phosphoramidite coupling with 3-chloroperoxybenzoic acid as the oxidant and no capping step; (b) NH_3, $MeNH_2$, H_2O, 55 °C, 30 min; (c) $Hg(OAc)_2$, H_2O, 60 °C, 16 h; (d) CH_2Cl_2, 25 °C, 12 h; (e) NH_3, $MeNH_2$, H_2O, 65 °C, 10 min.

1.2.3. Stability, available coordination sites and steric factors. With the exception of kinetically very inert metal ions, such as Pt(II) and Ru(II), coordination complexes are prone to dissociation under dilute and metal-deficient conditions. An important example of such conditions are those prevailing inside a cell. The stability of a coordination complex can be dramatically increased by forming a multidentate chelate and the utility of this approach has been demonstrated in a number of biological applications, especially those that tolerate the metal ion being coordinatively saturated. Illustrative examples include therapeutic oligonucleotides labeled with a radiotracer, typically an azacrown chelate of a radioactive metal ion.[128] Applications where the metal ion needs to have at least one vacant coordination site for binding to a target present a more challenging case. As a rare and remarkable example, artificial DNAzyme and PNAzyme ribonucleases relying on coordination of Zn(II) have reached potentially useful levels of catalytic activity at Zn(II) concentrations close to those prevailing inside malaria-infected red blood cells.[129–131] However, it should be pointed out that Zn(II) is the most abundant transition metal ion in cells and utilizing any other metal ion in the same way would be much more difficult. Finally, some applications place strict demands not only on the number of available coordination sites but also on the geometry of the complex. One such application is metal-mediated base pairing, where the coordination geometry of the metal ion needs to be either linear or planar to be accommodated within the base stack of a double-helical nucleic acid.[132] Compared to coordinative complexes that typically achieve sufficiently high affinity only through chelate formation, organometallic complexes have the advantage that even a single carbon–metal bond may be enough, leaving the metal center largely exposed and thus giving more freedom in the design of any additional interactions.

1.2.4. Selection of suitable metals. Essentially any metal ion is amenable to formation of a coordinative complex and an appropriate ligand in each case can be designed following the principles of preferred coordination geometry and hard and soft Lewis acids and bases. Organometallic compounds are also known for a number of metals but few are sufficiently stable hydrolytically to be useful as modifications of oligonucleotides. So far, organometallic oligonucleotide conjugates have been reported for cobalt,[133–136] iron,[101] mercury,[106] palladium,[96] platinum[119] and tin.[103,137,138] While this list is likely to expand, it is clear that compared to the coordinative counterparts, modification of oligonucleotides with organometallic complexes is much more limiting in terms of the repertoire of suitable metals.

1.2.5. Metal-responsive switching. Metal coordination can be used to induce a change in the secondary structure of an oligonucleotide. Such metal-responsive molecular switches are analogous to *e.g.* pH- or photoresponsive switches but rely on orthogonal triggering conditions. Applications include sensors for metal ions,[139–141] as well as metal-responsive aptamers, allosteric DNAzymes and molecular machines.[142–146] Obviously, covalent bonding of the metal ion to the oligonucleotide effectively rules out this kind of metal-responsive switching but the complementary approach of adding a competing ligand has been moderately successful with organomercury oligonucleotides.[105,123,147]

2. Metal-mediated base pairing of organometallic nucleobase surrogates

While metal-mediated base pairing of organometallic nucleobase analogues was first reported with small molecule model compounds already in the mid-1990s,[148] to the best of our knowledge we remain the only group to study such interactions within oligonucleotides. Initially, these studies were motivated by the desire to enhance the hybridization affinity of therapeutic oligonucleotides through metal coordination. Increased melting temperatures have indeed been observed for many short double-[105,108,110,125,127,149–151] and triple-helical[123,147] oligonucleotides incorporating a single organometallic residue and in one case a palladacyclic splice-switching oligonucleotide modestly outperformed its unmodified counterpart in a human cell line.[152] The connection between these two observations, however, remains uncertain. As higher hybridization affinity often comes at the cost of lower sequence selectivity, we were surprised to find that in some cases an organometallic nucleobase surrogate not only increased the melting temperature of a double helix but also robustly discriminated between canonical nucleobases.[110,125,127] Inspired by these results, the focus of our studies on oligonucleotides bearing organometallic base modifications has recently been on potential applications in single nucleotide polymorphism (SNP) genotyping.[153]

2.1. Hg(II)-mediated base pairing

The T–Hg(II)–T homo base pair was the first metal-mediated base pair reported and arguably the most thoroughly investigated one.[154–157] Coordination of Hg(II) between the N3 atoms of two thymine residues results in a structure with similar dimensions as the canonical Watson–Crick base pairs,[155] serving as a good starting point for the design of other Hg(II)-mediated base pairs. Given the relative ease of mercuration of the C5 atom of pyrimidine bases,[104] 5-mercuricytosine was a natural candidate to be tested first.[105] In the *syn* conformation of pyrimidine nucleosides, C5 assumes the position normally occupied by N3 and thus in a double helix points the Hg(II) ion towards the opposite nucleobase, analogous to the T–Hg(II)–T base pair. This first organometallic nucleoside within an oligonucleotide was later followed by several artificial C-nucleoside analogues retaining the same position of the Hg(II) ion but varying the other substituents or expanding the aromatic ring system.

Fig. 2 summarizes the melting temperatures of various 11-mer double-helical oligodeoxyribonucleotides featuring a single organomercury nucleobase in the middle of one strand and any of the four canonical nucleobases in the middle of the other strand, the rest of the sequences being identical in all cases. For reference, respective results on duplexes with thymine in place of the organometallic nucleobase are also

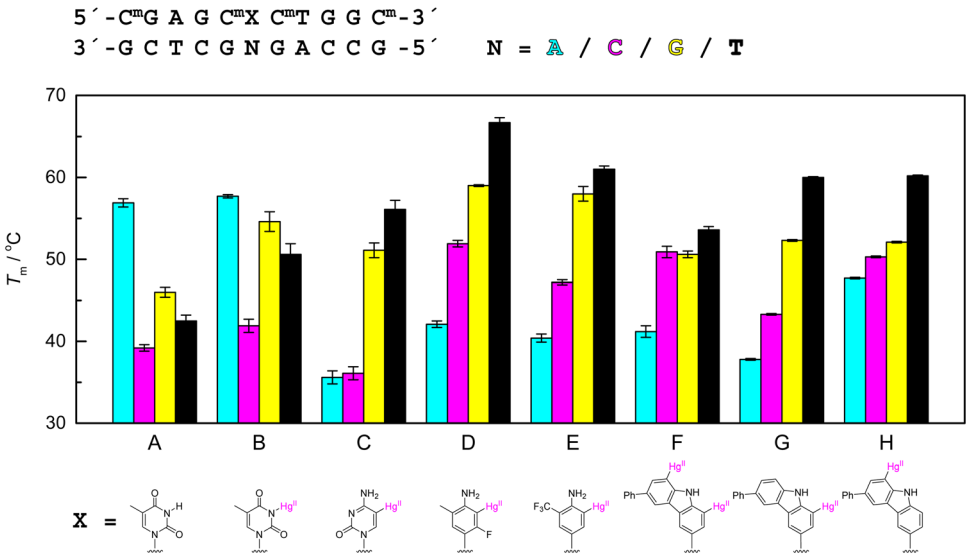


Fig. 2 UV melting temperatures of 11-mer oligodeoxyribonucleotide duplexes pairing either (A) thymine through hydrogen bonding, (B) thymine through Hg(II) coordination, (C) 5-mercurycytosine,[105] (D) 3-fluoro-2-mercuri-6-methylaniline,[110] (E) 2-mercuri-6-trifluoromethylaniline,[127] (F) 1,8-dimercuri-6-phenylcarbazole,[124] (G) 1-mercuri-6-phenylcarbazole or (H) 8-mercuri-6-phenylcarbazole[125] with adenine (cyan), cytosine (magenta), guanine (yellow) or thymine (black). For experimental conditions, see the original publications.

included. As expected, with the unmodified duplexes the highest melting temperature was observed with adenine opposite to thymine and all of the mispairs were destabilizing by more than 10 °C (Fig. 2(A)). The addition of 1 equivalent of Hg(II) to the samples led to a significant increase in the melting temperatures of the duplexes containing a T–G or a T–T mispair, consistent with formation of a Hg(II)-mediated base pair (Fig. 2(B)).[105] The T–A-containing duplex was still the most stable one and only the T–C mispair led to a sufficiently different (lower) melting temperature to allow reliable SNP identification. In other words, coordinative Hg(II)-mediated base pairing offered no real advantage over Watson–Crick base pairing in this case.

At monomer level, 5-mercuricytidine pairs weakly with adenosine and cytidine and strongly with any nucleoside that can undergo deprotonation on coordination of Hg(II).[105] Hybridization properties of the respective 11-mer oligodeoxyribonucleotide reflected these preferences, the most stable duplexes being the ones with thymine or guanine opposite to the organometallic residue (Fig. 2(C)). Pairing with adenine or cytosine, on the other hand, was even more destabilizing than any of the mispairs between canonical nucleobases. Thus a hybridization probe based on Hg(II)-mediated base pairing of 5-mercuricytosine would be able to distinguish reliably between guanine and adenine or thymine and cytosine but not between adenine and cytosine. Comparable (but opposite) discrimination of thymine and cytosine through Ag(I)-mediated base pairing with imidazophenanthroline has been reported within model sequences relevant for the development of breast or pancreatic cancer.[158,159]

Interestingly, replacing the cytosine ligand with a 3-fluoro-6-methylaniline ligand while retaining the position of the mercuri substituent *ortho* to the amino substituent markedly increased the melting temperature of all of the corresponding duplexes (Fig. 2(D)).[110] Furthermore, the duplex placing cytosine opposite to the organometallic residue was stabilized considerably more than the others, leading to discrimination between all canonical nucleobases in the order of A < C < G < T and with at least 5 °C difference between the melting temperatures. Comparable results have been obtained exploiting coordinative Ag(I)-mediated base pairing between cytosine and any of the canonical nucleobases but in that case, two probes were needed for reliable discrimination, one with α and the other one with β anomer of the cytidine residue at the recognition site.[160] Removal of the fluoro substituent from the Watson–Crick face and replacing the methyl group with a trifluoromethyl group did not change the stability order although the melting temperature of the duplex pairing the organometallic residue with thymine decreased somewhat (Fig. 2E).[127] Encouraged by these results, we employed 3-fluoro-2-mercuri-6-methylaniline in a molecular beacon-type Förster resonance energy transfer (FRET) probe and were gratified to find a clear correlation between fluorescence emission and melting temperature for each of the nucleobases at the polymorphic site.[126]

Qualitatively, the stability order of A < C < G < T for the organometallic Hg(II)-mediated base pairs discussed above (Fig. 2(C)–(E)) can be explained in terms of the preference of Hg(II) for anionic ligands and the better fit of the smaller pyrimidine bases within the double-helical environment (Fig. 3). With guanine and thymine, hydrogen bonding between the oxo and amino substituents may provide additional stabilization. To explore Hg(II)-mediated base pairing beyond this paradigm, we expanded the aniline ring to a carbazole ring, with Hg(II) ions at both carbon atoms *ortho* to the nitrogen atom.[124] Melting temperatures of duplexes placing adenine or cytosine opposite to this dimercurated residue (Fig. 2(F)) were

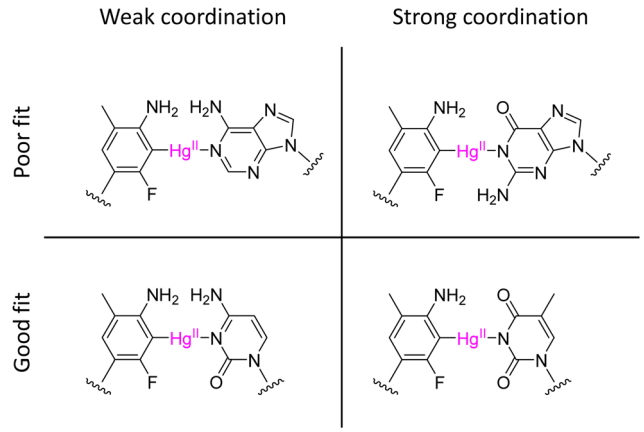


Fig. 3 Hg(II)-mediated base pairs between 3-fluoro-2-mercuri-6-methylaniline and the canonical nucleobases. The strongest pairs are formed with guanine and thymine (with concomitant deprotonation) and the geometrically most compatible ones with cytosine and thymine.

comparable to those of the respective duplexes incorporating one of the aniline derivatives (Fig. 2(D) and (E)) but with guanine and, especially, thymine considerable destabilization was observed. In principle, the 1,8-dimercuri-6-phenylcarbazole should be able to form the same kind of Hg(II)-mediated base pairs with guanine and thymine as depicted in Fig. 3 but the large drops in melting temperature suggested a different binding mode. In the case of thymine, DFT optimization yielded a dinuclear base pair with each of the Hg(II) ions coordinated to one of the oxo substituents of thymine, rather than the usual N3. Such a base pair is stronger than one having only one of the Hg(II) ions coordinated to N3 but less compatible with the geometry of the double helix, consistent with the observed decrease in melting temperature. A dinuclear Hg(II)-mediated base pair has also been reported to form between thymine and 1, N^6-ethenoadenine within a parallel-stranded double helix.[161,162]

The carbazole *C*-nucleoside bearing a Hg(II) ion only at the C1 atom (Fig. 2(G)) structurally resembles its aniline counterparts (Fig. 2(D) and (E)) so it was not unexpected to once again observe a similar pattern of hybridization preferences for the corresponding modified oligonucleotide.[125] Curiously, switching the Hg(II) from C1 to C8 had no effect on pairing with guanine or thymine but pairing with adenine and cytosine became much more favorable. The most interesting results, however, were obtained with duplexes placing either 2- or 4-thiothymine opposite to the organometallic residue.[125] All of the organomercury carbazole derivatives showed very high affinity towards these rare nucleobases and the C1- and C8-monomercurated ones preferred pairing with 2- and 4-thiothymine, respectively.

2.2. Pd(II)-mediated base pairing

The very first metal-mediated base pair between artificial nucleoside analogues featured Pd(II) as the bridging metal ion.[163] Like Pt(II), Pd(II) also exhibits a high affinity towards nitrogen donor atoms of natural nucleic acids.[164] The square planar coordination geometry of Pd(II) should be compatible with base stacking within a double helix, at least as long as steric crowding of the ligands is kept to a minimum.[165] Finally, aromatic rings bearing suitable directing ligands undergo cyclopalladation under conditions tolerated by nucleic acids, providing relatively easy access to oligonucleotides furnished with organopalladium modifications. All of these factors make Pd(II) a promising candidate for metal-mediated base pairing between organometallic oligonucleotides and their natural counterparts. Compared to Hg(II), the realization of this promise has, however, proven much more challenging.

Our first organopalladium oligonucleotide featured a palladacyclic phenylpyridine C-nucleoside analogue (Fig. 4(A)) in the middle of the same sequence as the one used with Hg(II)-mediated base pairing (see above).[108] UV melting studies on corresponding duplexes revealed a problem encountered since with most duplexes incorporating a putative Pd(II)-mediated base pair, namely multiphasicity of the melting curves. In the best case the analysis of such curves yields two melting temperatures, with the higher one presumably associated with dissociation of the two strands. Often, however, the overlapping of several melting events prevents reliable determination of the melting temperatures altogether. Furthermore, while Pd(II) is kinetically orders of magnitude more labile than Pt(II),[166] in many cases hysteresis between the denaturation and renaturation curves is observed,[150,151] indicating that when these

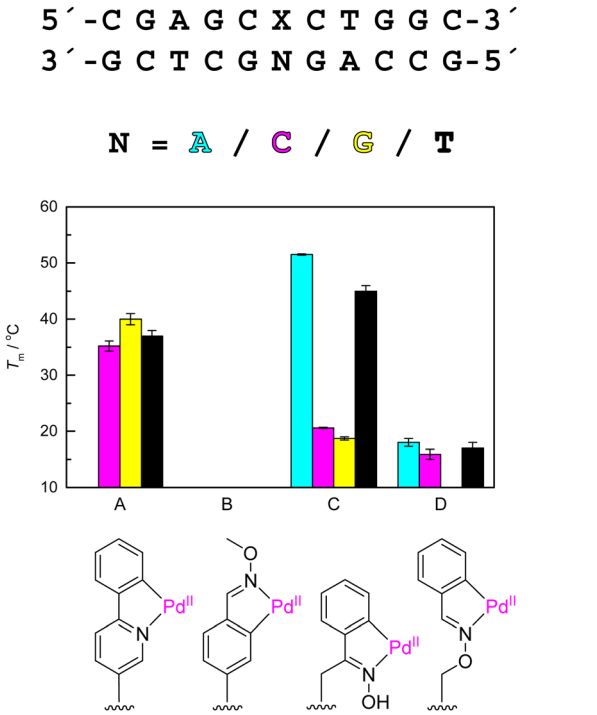


Fig. 4 UV melting temperatures of 11-mer oligodeoxyribonucleotide duplexes pairing either (A) phenylpyridine palladacycle[108] or (B)–(D) benzaldoxime palladacycles of varying flexibility[167,168] with adenine (cyan), cytosine (magenta), guanine (yellow) or thymine (black). Missing columns indicate cases where a sigmoidal melting curve was not observed. In case of multiphasic melting curves, the highest melting temperature is given. For experimental conditions, see the original publications.

processes involve changes in Pd(II)-mediated base pairing, they are too slow to be studied by conventional UV melting temperature measurements. We have explored FRET-based competition assays[151] and ^{15}N NMR spectroscopy[167] as alternative methods, with moderate success.

Our earlier studies on coordinative Pd(II)-mediated base pairing had revealed that the same base pair could be stabilizing at the end of a double helix but destabilizing in the middle, suggesting suboptimal geometry that is better tolerated outside of the base stack than within.[169,170] This turned out to be the case also with the palladacyclic phenylpyridine – 11-mer duplexes incorporating this modification in the middle melted at a lower temperature than their unmodified counterparts[108] while the opposite was true for duplexes bearing the same modification at either end.[151] Interestingly, base-pairing preferences of the palladacyclic C-nucleoside analogue were also somewhat different depending on whether it was incorporated at the 3′- or 5′-terminus.

Besides placing it at the end of the double helix, the strain caused by a geometrically incompatible metal-mediated base pair could also be alleviated by making the metalated nucleoside analogue more flexible. We tested this approach with three isomeric oligonucleotides bearing oxime palladacyles of varying rigidity as the base moiety of their central nucleoside analogue (Fig. 4(B)–(D)).[167,168] For duplexes incorporating the most rigid version (Fig. 4(B)), sigmoidal melting curves were not observed so their stabilities remain obscure. With the most flexible version (Fig. 4(D)), melting temperatures could be determined and were found to be very low. The acetophenone oxime palladacycle (Fig. 4(C)), representing an isomer of intermediate flexibility, was tested as both α and β anomers, with markedly different results. The α anomer favored pairing with thymine and guanine but the resulting duplexes were still less stable than unmodified counterparts featuring a single mismatch in the middle. The β anomer, in turn, was stabilizing when placed opposite to an adenine or thymine residue, the melting temperatures of the corresponding duplexes approaching those of fully matched unmodified duplexes.

3. Oligonucleotides bearing organometallic labels

The properties of some organometallic complexes make them useful as labels to facilitate imaging of oligonucleotides or elucidation of their secondary structures. The field is dominated by studies on ferrocene-labeled electrochemical probes and sensors[73–77] but recently luminescent organometallic labels[119,171] have also received attention.

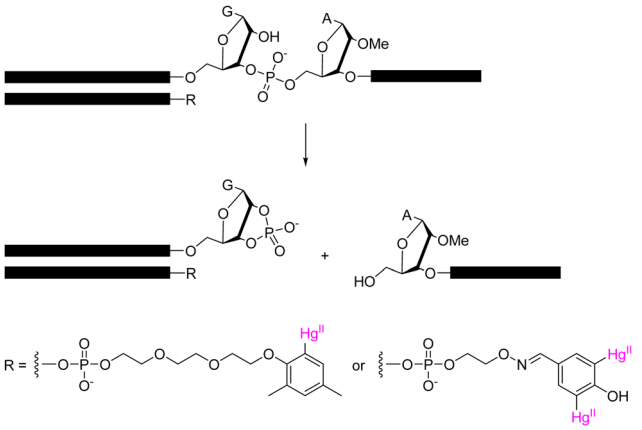
Ferrocene-labeled oligonucleotides can be detected in femtomolar quantities.[111,172] Depending on the intended application, the signal can be rendered either insensitive or sensitive to secondary structure by either separating the label and the oligonucleotide by a long and flexible linker[73] or by incorporating the label as part of the backbone.[173–175] Changes in secondary structure, in turn, can be induced by hybridization with another nucleic acid,[176,177] coordination of metal ions[140,178] or aptamer-type interaction with a variety of analytes.[179,180] The strong correlation between the oxidation current of a ferrocene label and its proximity to the electrode can be easily harnessed to create electrochemical sensors by immobilizing the labeled oligonucleotide on the surface of the electrode.[181] Finally, the oxidation potential of ferrocene can be tweaked by changing the substituents of the cyclopentadiene rings, allowing simultaneous detection on multiple channels.[182]

Tridentate platinacyclic chelates of 1-(1,2,3-triazol-4-yl)-6-phenylpyridine represent an interesting alternative to organic fluorophores and lanthanide chelates as luminescent labels, combining some of the desirable characteristics of both.[183,184] Their luminescence lifetimes are relatively long (although not quite on par with lanthanide chelates), allowing time-resolved experiments. In contrast to the bulky lanthanide chelates, on the other hand, planar platinacycles readily intercalate within the base stack of a double helix, a useful feature for many applications involving nucleic acids. As a step towards such applications, the platinacyclic chelate was recently incorporated into short double-helical oligodeoxynucleotides through either DNA- or GNA-type scaffolds pointing it towards the center of the double helix or through uridine-C5, placing it in the major groove.[119] Oligonucleotides placing the platinacycle within the base stack had longer fluorescence lifetimes than those placing it in the major groove and this difference was attributed to shielding from water and triplet oxygen. This interpretation was further borne out by the fact that the shortest lifetimes were observed with corresponding single-stranded oligonucleotides.

4. Oligonucleotides bearing organometallic catalytic moieties

Given the programmable sequence recognition properties, the most obvious and also the most extensively studied use for oligonucleotide catalysts is the cleavage of other nucleic acids.[185–191] Efficient artificial nucleases are accessible through the simple and modular approach of covalently tethering a cleaving agent to a guiding oligonucleotide strand. Alternatively, catalytic oligonucleotides can be found through an iterative process known as systematic evolution of ligands by exponential enrichment (SELEX).[192,193] In the latter case, the scope of the catalyzed reactions is not limited to cleavage (or formation) of RNA or DNA phosphodiester linkages. Regardless of the design and application, most oligonucleotide catalysts require metal ion cofactors.

As discussed above in Section 1.2.3., dissociation under dilute conditions makes most coordinative metal complexes unsuitable for applications in biological media. Organometallic complexes could offer a solution to this problem and we recently set out to test this hypothesis on simple RNA cleavage model systems. The requirement for a hydrolytically stable organometallic complex ruled out the metal ions most extensively studied in the context of RNA cleavage and led us to



Scheme 2 Cleavage of the single RNA phosphodiester linkage within a target oligonucleotide by oligonucleotide conjugates of two different arylmercury complexes.

choose Hg(II) instead. The potential of this overlooked metal ion was first tested in the free form and found to be sufficient to warrant further experiments.[194] Replacing free Hg(II) by an arylmercury compound led to lower apparent catalytic activity but the loss was mostly attributable to lower affinity for the scissile phosphodiester, an issue that could be addressed by the inclusion of an appropriate guiding element, such as an oligonucleotide.[195]

Two oligonucleotide conjugates, differing in the arylmercury group as well as the linker between this moiety and the guiding sequence (Scheme 2), were tested for their ability to catalyze the cleavage of the sole RNA phosphodiester linkage within a complementary 2′-*O*-methyl-RNA strand.[196] Both conjugates were active and the observed rates indicated a significant proximity effect on tethering the cleaving agent to a guiding oligonucleotide. The linker length and flexibility also played a major role, with the conjugate incorporating a triethylene glycol linker being approximately 6 times as active as the one incorporating the shorter and more rigid ethylene glycol oxime linker. While the cleavage efficiency fell short of what has been achieved with the best metal-dependent artificial ribonucleases, we were nevertheless able to demonstrate the feasibility of furnishing oligonucleotides with organometallic catalytic functions.

5. Conclusion and outlook on future progression

Redox labeling of oligonucleotides by ferrocene is an established method that has already been exploited in myriad sensor applications. Apart from these, the most successful use of oligonucleotides bearing organometallic modifications has been as hybridization probes for SNP genotyping. Replacing the base moiety at the recognition site by an arylmercury moiety appears particularly promising, allowing robust discrimination of all canonical nucleobases with a single probe. Inexpensive point-of-care SNP genotyping tools based on Hg(II)-mediated base pairing by arylmercury nucleobase surrogates could become reality in near future. Owing to their more complicated coordination chemistry and slower ligand-exchange kinetics, palladacycles are less suitable for this role but have instead shown some promise as modifications of therapeutic oligonucleotides.

Interesting properties of organometallic modifications as labels are not limited to redox chemistry, as exemplified by the recent report on oligonucleotides bearing phosphorescent platinacyclic chelates. Many more studies have been published on related nucleoside, nucleotide and dinucleotide analogues,[171,197–199] predicting this to be an active field in the coming years. The atomic weights and isotope distributions of many metals forming stable organometallic complexes, notably mercury and the platinum group metals, are very distinct from those of the biogenic elements, possibly allowing laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) or nanoscale secondary ion mass spectrometry (NanoSIMS) imaging of correspondingly modified oligonucleotides.

The field of oligonucleotides featuring organometallic catalytic groups is still in its infancy but the concept has been proven in the case of artificial ribonucleases. The catalytic activity was modest but can likely be improved considerably by optimizing the linker and the overall architecture of the oligonucleotide conjugate. Organometallic modifications could expand the catalytic repertoire of DNAzymes and ribozymes to reactions that have so far remained elusive. Stereoselective C–C cross-couplings appear a particularly attractive application given the ubiquity of palladacycles as pre-catalysts for such reactions.

Conflicts of interest

There are no conflicts to declare.

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