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COMMUNICATION

Metal-mediated DNA assembly using the ethynyl linked terpyridine ligand†

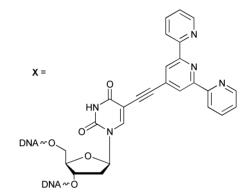Thomas Ehrenschwender,[a] Anna Barth,[b] Holger Puchta[b] and Hans-Achim Wagenknecht*[a]

The terpyridine ligand directly attached to the 5-position of a uridine allows metal-mediated DNA assembly towards potentially electronically coupled DNA conjugates.

DNA represents an increasingly important tool for the construction of nanoarchitectures or nanoscaled devices due to the predictable Watson–Crick base pairing.[1–6] In order to enhance complexity of DNA-based nanostructuring it would be highly desirable to develop additional binding motifs that behave chemically orthogonal to conventional hydrogen bonding of Watson–Crick base pairing that is applied typically as so-called sticky ends. The first alternative, hydrophobic π–π interactions have been used mainly between perylene bisimides as DNA caps to aggregate DNA[7] and Y-shaped DNA constructs[8] in a reversible fashion. Metal ion–ligand interactions represent the second alternative motif. The latter idea is not new; conjugates of nucleic acids and metal-chelating moieties have been investigated intensively.[9–15] Especially the 2,2':6',2''-terpyridine (terpy) ligand is known to efficiently form stable complexes with a broad variety of metal ions[16–21] and has been used to assemble oligonucleotides.[22–24] However, it is important to point out that all of these studies have been carried out with oligonucleotides that were terminally modified with terpy using a flexible alkyl linker. In supramolecular electronics a strong electronic coupling is provided mainly by acetylene bridges.[25] In order to go one step further towards DNA-based nanoelectronics, we present the DNA building block terpy-dU in which the terpy ligand is linked to the 5-position of 2'-deoxyuridine *via* the ethynyl bridge. This building block allows internal and terminal terpy-dU modification and thereby provides the basis for metal-mediated DNA assembly.

The synthesis of 2'-deoxyuridine carrying the terpy-acetylene moiety in the 5-position was recently reported.[26] Accordingly, the preparation of the corresponding building block was carried out *via* Sonogashira coupling between DMT-protected 5-iodo-2'-deoxyuridine and 4'-ethynyl-2,2':6',2''-terpyridine followed by standard phosphoramidite formation (see Supporting Information†). The single strands **DNA1–DNA4** (Scheme 1) were prepared with one terpy-dU modification in the middle of the

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DNA1	3´–A–G–T–C–A–C–T–T–**X**–T–T–C–T–G–A–C–G–5´
DNA2	5´–T–C–A–G–T–G–A–A–**X**–A–A–G–A–C–T–G–C–3'
DNA3	3´–A–G–T–C–A–C–T–G–**X**–G–T–C–T–G–A–C–G–5´
DNA4	5´–T–C–A–G–T–G–A–C–**X**–C–A–G–A–C–T–G–C 3'
DNA5	3´–A–G–T–C–A–C–T–T–G–T–T–C–T–G–A–T–**X**–5´
DNA6	5´–**X**–C–A–G–T–G–A–A–C–A–A–G–A–C–T–A–A–3'

Scheme 1 Structure of terpy-dU in oligonucleotides and sequences of single strands **DNA1–DNA6**. Duplexes between two modified oligonucleotides are called **DNA1-2**, **DNA3-4** and **DNA5-6**. Duplexes of only one of the modified oligonucleotides with corresponding unmodified counterstrands are called **DNA1Y** *etc.* (with Y = base opposite to terpy-dU, *e.g.* A in **DNA1A**).

sequence and surrounded by T, A, G or C; strands **DNA5** and **DNA6** carry a terminal terpy-dU label. **DNA1** is complementary to **DNA2**, **DNA3** to **DNA4**, and **DNA5** to **DNA6** (Fig. 1).
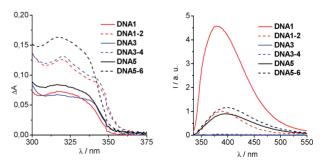

Fig. 1 Absorption (left) and fluorescence spectra (right) of terpy-dU–modified single strands **DNA1**, **DNA3** and **DNA5** and double strands **DNA1-2**, **DNA3-4**, **DNA5-6**; 2.5 μM in Na–P$_i$ buffer at pH 7, 250 mM NaCl, 100 μM EDTA, 20 °C, excitation at 325 nm.

Table 1 Melting temperatures (T_m) and spectroscopic data of double strands without metal ions

DNA	λ_{abs} [nm]	λ_{em} [nm]	T_m [°C]	ΔT_m [°C][a]
DNA1A	316	378	56.8	−5.7
DNA1-2	321	390	65.9	+2.4
DNA3A	316	—	63.9	−4.1
DNA3-4	316	—	71.6	+3.6
DNA5A	316	400	61.4	—
DNA5-6	321	400	62.7	—

[a] Compared to the unmodified references: T_m = 62.5 °C for **DNA1A** and T_m = 68.0 °C for **DNA5A**, each with T instead of terpy-dU.

First we studied the influence of a single terpy-dU modification on the melting temperatures (T_m) of double strands (Table 1). If **DNA1** and **DNA3** are hybridized with completely unmodified counterstrands including A opposite to terpy-dU (yielding double strands **DNA1A** and **DNA3A**) the T_m values reveal a strong destabilization (−5.7 °C and −4.1 °C) compared to completely unmodified duplexes. The destabilization is slightly stronger with other bases opposite to terpy-dU (double strands **DNA3T**, **DNA3G** and **DNA3C**) (see Supporting Information†). Obviously, the terpy-dU unit exhibits a small preference for adenine as the counterbase although the metal ligand has been attached via the short ethynyl bridge to the 5-position. In contrast, the duplexes **DNA1-2** and **DNA3-4** bearing two terpy-dU moieties opposite to each other are stabilized quite significantly (+2.4 °C and +3.6 °C, respectively) compared to completely unmodified duplexes. This is a remarkable result and shows that the hydrophobic interaction of the two terpy unit regains more hybridization energy than the destabilization introduced by the terpy units. Similar results have been obtained with bipyridine pairs[27] and binaphthyl pairs[28] inside DNA.

The terpy chromophore can be excited selectively at 325 nm yielding a characteristic fluorescence (Fig. 1). Compared to duplex **DNA1A** the fluorescence of double strand **DNA1-2** is quenched. This is the typical result of chromophore aggregation and thereby supports the idea of a hydrophobically interacting terpy "base pair" inside **DNA1-2**. The fluorescence of duplexes **DNA3A** and **DNA3-4** does not allow this interpretation since it is almost completely quenched, probably due to photoinduced charge transfer processes to adjacent guanines. The fluorescence intensity of **DNA5** and **DNA5-6** is approximately equal since hydrophobic terpy pairing enforced by the surrounding DNA architecture (as in **DNA1-2**) is unlikely with terminal modifications.

More importantly, the terpy fluorescence and its quenching can be used to follow and quantify metal ion coordination. We chose Cu^{2+}, Ni^{2+}, Zn^{2+} and Fe^{2+} as typical representatives, known to form stable complexes with the terpy ligands. First, we examined double strands **DNA1A** and **DNA5A** bearing only one terpy-dU in the middle or at the terminus. It is expected that addition of metal ions induces dimerization. From the titration experiments (see Supporting Information†) we calculated the quenched fraction of fluorescence intensity (Fq) at characteristic emission maxima. The results show that fluorescence quenching is complete after addition of 0.5–0.75 equiv. of metal ions (Fig. 2, top). This observation together with the absorption changes (see Supporting Information†) indicate approximately the expected stoichiometry. To further evidence the dimer formation we performed non-
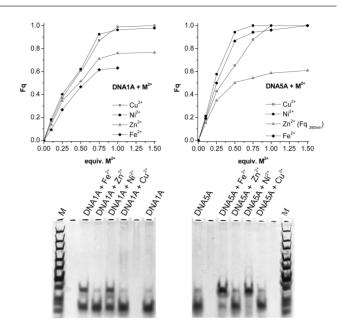

Fig. 2 Top: Fluorescence quenching (Fq) for **DNA1A** (left) and **DNA5A** (right) upon addition of metal ions; bottom: non-denaturing gel electrophoresis (8% TBM-PAGE) of **DNA1A** and **DNA5A** in absence and presence of metal ions after silver staining.

denaturing polyacrylamide gel electrophoresis (Fig. 2, bottom). The gels show dimerization of **DNA5A** and **DNA1A** in the presence of Ni^{2+} and Fe^{2+} by a band of slower mobility. This is a remarkable result by keeping in mind how short the acetylene linkers are between the metal chelators and the nucleic acids on both sides of the complex. On the other hand, dimers of **DNA5A** and **DNA1A** in the presence Cu^{2+} and Zn^{2+} which are indicated by the fluorescence measurements seem to be not stable enough for non-denaturing gel analysis.

In the second part of this study we performed similar experiments with double strands bearing two terpy-dU units either opposite to each other in the middle (**DNA1-2**) or at the termini (**DNA5-6**). It is expected that these DNA probes potentially are forming larger DNA assemblies. The Fq analysis of **DNA1-2** (Fig. 3, top) reveals a complete fluorescence quenching after addition of 1.5 equiv. metal ions which is 0.5 equiv. more than expected. The gel analysis (Fig. 3, bottom) shows dimers of **DNA1-2** only in the presence of Ni^{2+} but no larger aggregates. Due to the fact that optical changes clearly indicate metal coordination, it looks reasonable to assume that the two terpy-dU moieties of **DNA1-2** are forming a metal-mediated base pair inside the duplex instead of networking between duplexes.

It is important to note that it is problematic to compare T_m values of the metal-ion coordinated samples of **DNA1-2** directly with the metal free **DNA1-2** since the T_m of the latter duplex revealed an astonishingly stabilized, hydrophobically interacting terpy-dU pair (as discussed above). Compared to a completely unmodified reference double strand, however, **DNA1-2** shows significantly higher melting temperatures in the presence of 1 equiv. of Ni^{2+}, Fe^{2+} and Cu^{2+} (Table 2). With Ni^{2+} or Fe^{2+} two different T_m values are obtained, of which one is even higher than the metal free **DNA1-2** (ΔT_m positive). The latter observation strongly supports the idea of a metal ion-mediated, internal terpy-dU base pair that interferes with the formation of higher DNA
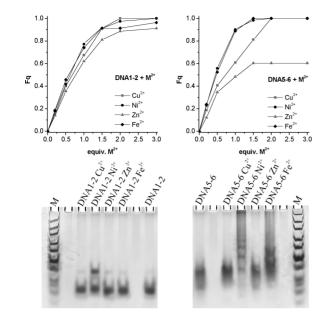
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Fig. 3 Top: Fluorescence quenching (Fq) for **DNA1-2** (left) and **DNA5-6** (right) upon addition of metal ions; bottom: non-denaturing gel electrophoresis (8% TBM-PAGE) of **DNA1-2** and **DNA5-6** in absence and in presence of metal ions after silver staining.

Table 2 Melting temperatures (T_m) of **DNA1-2** in presence of metal ions

Metal ions	T_m [°C]	ΔT_m [°C][a]
(With EDTA)	65.9	+2.4
NiCl$_2$	54.1/69.4	−8.4/+6.9
CuCl$_2$	61.7	−0.8
FeCl$_2$	53.3/70.0	−9.2/+7.5
ZnCl$_2$	64.4	+1.9

[a] Compared to the unmodified references: T_m = 62.5 for **DNA1-2** with an A-T base pair instead of the terpy-dU modifications.

assemblies. The second T_m value at lower temperatures (ΔT_m negative) corresponds to duplex assemblies that are conjugated by the terpy-dU complexes, and therefore both hydrophobic and metal-mediated stabilization inside the DNA duplex are lost.

The situation looks different and more straightforward in case of the terminally labeled **DNA5-6**. The gels show clearly several bands of slower mobility in the presence of Ni^{2+} and Fe^{2+} thereby supporting the existence of DNA assemblies larger than dimers. With these metal ions the band of isolated duplexes **DNA5-6** has nearly completely vanished and Fq analysis shows complete fluorescence quenching upon addition of slightly more than 1.0 equiv. metal ions, as expected.

In conclusion it became evident from both fluorescence measurements and gel analysis that metal-mediated DNA assemblies do not require long and flexible alkyl chain linkers between the metal chelator and the nucleic acids. Even a short linker, as the acetylene linker, allows dimerization and formation of stable and larger assemblies of terpy-dU-modified DNA in the presence of Ni^{2+} and Fe^{2+}. Internal metal-mediated base pairing between two terpy-dU modifications interfere with the formation of higher DNA assemblies. This problem can be solved by placing two terpy-dU modifications not exactly opposite to each other in two

complementary strands. Higher structures can be formed with doubly terminally labeled DNA. In principal, the short acetylene linkers should provide strong electronic coupling between the metal–ligand complex and the DNA. Hence it is expected that these kind of DNA materials[29] have a significant potential for DNA-based nanoelectronics.

Acknowledgements

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