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Catalysis of Michael additions by covalently modified G-quadruplex DNA

Surjendu Dey, Carmen L. Rühl and Andres Jäschke*

Institute of Pharmacy and Molecular Biotechnology

Heidelberg University, 69120 Heidelberg, Germany.

E-mail: jaeschke@uni-hd.de

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Abstract:

Enantioselective catalysis utilizing G-quadruplex DNA-based artificial metalloenzymes has emerged as a new approach in the field of aqueous-phase homogeneous catalysis. Recently, a catalytic asymmetric Michael addition employing a covalently modified G-quadruplex in combination with Cu(II) ions has been reported. Here we assess by systematic chemical variation and using various spectrometric techniques a variety of parameters that govern rate acceleration and stereoselectivity of the reaction such as the position of modification, the topology of the quadruplex, the nature of the ligand, the length of the linker between ligand and DNA, the chemical identity of monovalent ions and transition metal complexes. The DNA quadruplex modified at position 10 (dU10) with hexynyl-linked bpy ligand showed twice the initial rate as the the DNA strand derivatized at position 12 (dU12). The strikingly different dependence of the stereoselectivity on the linker length, and their different spectroscopic properties indicate large differences in the architecture of the catalytic centers between the dU10-derivatized and the dU12-modified quadruplexes. Upon addition of Cu(II), both types of bpy-derivatized DNA strands form defined 1:1 Cu-DNA complexes stable enough for mass-spectrometric analysis, while the underivatized strands exhibit weak and unspecific binding, correlated with much lower catalytic rate acceleration. Both dU10 and dU12-derivatized quadruplexes could be re-used 10 times without reduction of stereoselectivity.

Introduction

Asymmetric Michael addition reactions are important carbon-carbon or carbon-heteroatom bond forming reactions, which have been widely used to construct valuable building blocks in organic synthesis.[1] During the last decades, elegant approaches using various Lewis acid catalysts and organocatalysts have been developed to perform the asymmetric Michael addition reactions in aqueous media aimed at green & sustainable chemistry.[2]

The development and application of DNA-based artificial metalloenzymes has become an attractive new approach in aqueous-phase homogeneous asymmetric catalysis, where a (typically double-stranded - ds) DNA has been commonly used to control the stereochemical outcome of different transition metal-catalyzed reactions.[3] This approach has already been successfully applied to perform different

asymmetric reactions.[4] In this context, the Roelfes group reported the first catalytic asymmetric Michael addition in water using a DNA-based artificial metalloenzyme, which was (non-covalently) composed of double stranded (ds) DNA and different Cu(II)-ligand complexes.[4g] Later, the Li group applied a similar dsDNA-based artificial metalloenzyme for the Michael addition varying a wide range of substrates.[4i] Besides the application of dsDNA, recently DNA hairpins were also utilized as scaffold for enantioselective catalysts.[5]

Among the various DNA structures[6], the G-quadruplex is particularily interesting due to its high structural diversity, which is based on various combinations of parallel and antiparallel strand pairing and the associated different loop shapes of the connector sequences. These feature make G-quadruplexes a favorable candidate to create artificial metalloenzymes.[7] Applying these artificia metalloenzymes, asymmetric Diels–Alder, Friedel–Crafts and sulfoxidation reactions were performed i which two different well-known G-quadruplex-forming sequences, namely the human telomeric G quadruplex (h-Tel) and a region of the c-kit promoter (c-kit) were utilized, whereby h-Tel showed highe catalytic activity.[8] Most of these studies utilized Cu(II) as a Lewis acid catalyst, however, the binding o Cu(II) to the G-quadruplex sequences occurs in an unspecific, most likely electrostatic, manner, whicl makes it impossible to pinpoint the exact location of the metal-binding catalytic site as well as th substrate binding pocket. This limitation prevents prediction and detailed understanding of the factor that determine activity and selectivity. To overcome these problems, a specific covalent attachmen strategy is an alternative in which the transition-metal ligand complex is covalently attached to DNA allowing to more precisely localize the metal binding center and to study the structure-functio relationship.[4l, 9]

In a recent communication, we reported a new class of DNA-based metalloenzymes, namely G quadruplex DNA with covalently attached bipyridyl (bpy) ligands in combination with Cu(II) ions whicl efficiently and selectively catalyzed asymmetric Michael additions in water.[10] The catalytic activity an stereoselectivity of the G-quadruplex based artificial metalloenzymes was found to depend on severa factors, such as the position of modification, the topology of the quadruplex, the nature of the ligand and – importantly - the length of the linker that connects ligand and DNA. Surprisingly, this latte parameter turned out to be of prime importance for stereoselective bond formation within th investigated structure space (from propargyl to hexynyl linkers). Herein, we present a comprehensiv study in which we extend the linker length further, and investigate a variety of other parameters tha influence the catalytic activity and selectivity.

Results and discussion:

Synthesis

For the design of G-quadruplex based artificial metalloenzymes, the c-kit wild-type (wt) sequence (5'-AGGGAGGGCGCTGGGAGGAGGG-3'), which is known to form a unique all-parallel G-quadruplex structure (as shown in Figure 1a), was our primary choice. As in our previous study[10], positions 12 and 10 were chosen as modification sites based on the high-resolution 3D structure obtained from X-ray crystallography[11] and NMR spectroscopy[12]. Position 12 is placed in an apical loop region, while position 10 is crucial for forming the unique all-parallel folding topology. In case of position 12,

10.1002/chem.201700632

crystallographic investigations suggested that attachement of a bulky group may have little impact on the quadruplex folding. On the other hand, mutating G10 with T, namely c-kit-T10 induces an alternative folding. The exact structure of the c-kit-T10 mutant is unknown, but several lines of evidence suggest a mixed parallel-antiparallel topology.[12] A hypothetic structure of this quadruplex is drawn in Figure 1d. Bulky groups attached to T10 are expected to have a stronger influence on folding. In order to systematically investigate catalysis by these new metalloenzymes, we replaced T12 and T10, respectively, by various deoxyuridine derivatives systematically modified at position 5 of the nucleobase. The deoxyuridine derivatives were transformed to phosphoramidites, and the target deoxyoligonucleotides assembled by solid–phase synthesis and purified by HPLC (Figure S1, see the Supporting Information for details).

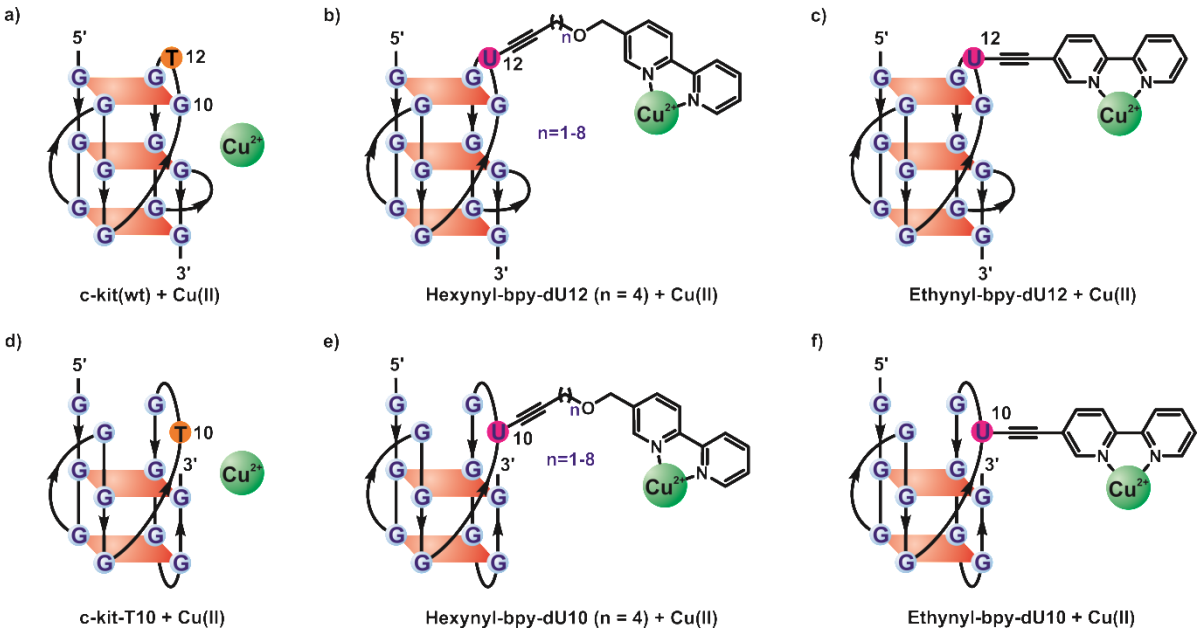


Figure 1. Folding of different G-quadruplex DNA sequences in the presence of Cu(II).
a) Folding of c-kit(wt) DNA as observed.[11-12] b) Schematic representation of hypothetical folding and metal binding of dU12-modified DNA. b) Schematic representation of hypothetical folding and metal binding of ethynyl-bpy-dU12 DNA. d) Schematic representation of hypothetical folding of c-kit-T10 DNA. e) Schematic representation of hypothetical folding and metal binding of dU10-modified DNA. f) Schematic representation of hypothetical folding and metal binding of ethynyl-bpy-dU10 DNA.

Catalytic properties

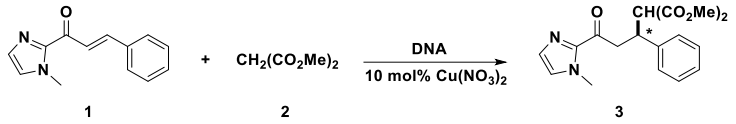
The catalytic activity of the modified G-quadruplexes with covalently linked bpy-moieties in combinatio with Cu(II) was evaluated in an asymmetric Michael addition of 1-(1-methyl-1*H*-imidazol-2-yl)-3 phenylprop-2-en-1-one (**1**) as Michael acceptor and dimethyl malonate (DMM, **2**) as nucleophile. Fo dU12-modified G-quadruplexes it was noticed previously that the increase in stereoselectivity wa almost linear from the propargyl linker (n=1) to the hexynyl linker (n=4), from 31% to 52% *ee* (Table 1 entries 3-6) in favor of the (+)-enantiomer.[10] This counter-intuitive trend led us to investigate longe. linkers (n>4, Table 1, Figures S2-6). Surprisingly, reversal of stereoselectivity was observed for the heptynyl to nonynyl linkers, ranging from -34 to -45% *ee* (Table 1, entries 7-9). For the decynyl linker, the (+)-enantiomer was again (slightly) preferred with 15% *ee* (Table 1, entry 10).

For the dU10-modified G-quadruplexes, the previous data had revealed a similar linear increase in stereoselectivity from n=1-4, however, in favor of the other isomer, the (-) enantiomer (Table 1, entries 3-6).[10] The best system (hexynyl) yieled remarkable 95% conversion and 92% *ee*. For the linkers with

10.1002/chem.201700632

n>4, this systematic trend is broken. Enantioselectivity varies unsystematically between 52 and 83%, but unlike the dU12-quadruplexes, no reversal of enantioselectivity is observed (Table 1, entries 7-10). Thus, the influence of the linker length on the stereoselectivity of the reaction is quite different for the two attachment sites. In contrast, all catalysts yield quite similar conversions under these conditions.

Table 1: Results of the Michael addition reaction catalyzed by dU12/dU10-modified c-kit DNA and Cu(II).[a]



1 2 3

Entry	DNA/linker	n	dU12-modified DNA		dU10-modified DNA	
			conv (%)[b]	*ee* (%)[b], [c]	conv (%)[b]	*ee* (%)[b], [c]
1[d]	c-kit(wt)	-	6	-9	-	-
2[d]	c-kit-T10	-	-	-	8	-11
3[d]	Propargyl-bpy	1	94	+31	92	-32
4[d]	Butynyl-bpy	2	92	+40	92	-57
5[d]	Pentynyl-bpy	3	94	+43	91	-69
6[d]	Hexynyl-bpy	4	95	+52	95	-92
7	Heptynyl-bpy	5	92	-34	94	-46
8	Octynyl-bpy	6	95	-31	96	-83
9	Nonynyl-bpy	7	97	-45	96	-52
10	Decynyl-bpy	8	96	+15	97	-59
11	Ethynyl-bpy	-	94	+51	96	<-5

[a] See the Experimental Section for detailed reaction condition. All experiments were performed in triplicate. [b] Both conversion and *ee* were calculated by using chiral HPLC results are reproducible within ±5%. [c] (+) and (-) symbols refer to isomer with low and high retention time respectively from chiral HPLC column. [d] see reference.[10]

In all previous experiments the bpy ligand was attached to the deoxyuridine base via long flexible linker with up to 10 rotatable atoms (sp^3-hybridized C, ether-O), offering extensive translational and rotationa freedom and limiting the positioning of the ligand relative to the DNA only by the length of the linker We next compared these flexible systems with a rigid one, in which the bpy ligand was attached to C5 o the deoxyuridine via an ethynyl bridge with no translational and very little rotational freedom. For botl attachment sites, we observe high catalytic activity, however only in case of the dU12 quadruplex w observe significant (51% *ee*) enantioselectivity (Figure S7). The favored enantiomer (+) is the same tha is preferred by other dU12 quadruplexes with short linkers, and remarkably, the enantioselectivity fo the rigid ethynyl bridge is higher than for the more flexible propargyl derivative (compare entries 3 an 11 in table 1). Thus, for the dU12 quadruplex the chiral induction appears optimal when the distanc between DNA and bpy ligand is short, but it never reaches the high *ee* values (92 and 83%) seen for the dU10 quadruplexes, which depend on optimized long linkers. These differences in stereoinduction suggest quite different architectures of the respective catalytic centers.

10.1002/chem.201700632

CD spectroscopy

To better understand these differences and the relationships between structure and catalytic performance (shown in Table 1), circular dichroism (CD) spectra of different modified quadruplexes along with Cu(II) were recorded (Figure 2, see also Figure S8-9). For all dU12 quadruplexes, these spectra are nearly identical and show a minimum around 240 nm and a maximum around 260 nm, which are typical features of parallel quadruplexes. Neither the attachment of the bpy-linker, nor the addition of Cu(II) ions induce significant changes to the spectrum (Fig. S8). Overall, the attachment of flexible linker-bpy conjugates to the dU12 position has – according to the CD spectra – very little influence on the quadruplex folding. The bpy ligand may interact, e.g. by stacking, with an intact and autonomously folded DNA quadruplex. The spectra of the dU10 quadruplexes reveal three differences: they show an additional shoulder at 290 nm, which is a feature of antiparallel quadruplexes, they exhibit a much stronger influence of the attached linker on the CD spectrum, and thus, presumably on the folding, and the spectra are also much more sensitive to copper ions. Interestingly, for longer linkers the 290 nm band decreases and the 240 and 260 nm bands increase. (Figure 2b, see also Figure S9). These spectra showed the features of either hybrid structures with mixed parallel/anti-parallel strands, or of a mixed population of parallel quadruplexes and antiparallel quadruplexes.[13]

These observations and considerations also hold true for the rigid ethynyl linkers (Figure S10).

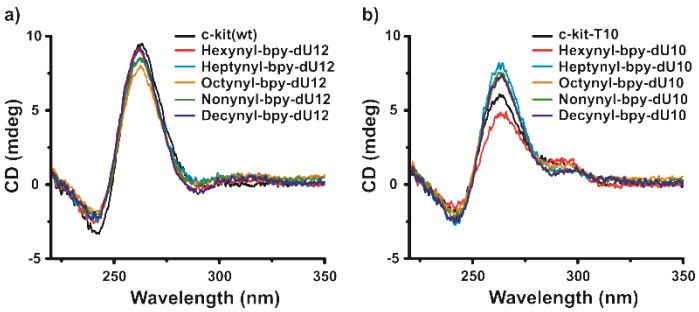


Figure 2. Comparison of different 12 or 10 position modified quadruplexes folding in the presence of Cu(II).
a) CD spectra of c-kit(wt) and different dU12 position modified quadruplexes in the presence of Cu(II). b) CD spectra of c-kit-T10 and different dU10 position modified quadruplexes in the presence of Cu(II).

One possible structural interpretation is that in the dU12 quadruplexes, the catalyst is the bpy-bound copper ion which is only weakly associated with the DNA. The DNA influences mainly the stereochemical outcome by restricting the approach of substrates from certain directions, and the linker length modulates which orientations the weakly bound bpy can assume. In the case of dU10, on the other hand, copper, bpy, and tightly bound DNA may all become integral parts of the catalytic center, which may even resemble a catalytic pocket. The linker length only slightly modulates the stereoselectivity, and reversal of enantioselectivity is never observed, again supporting tight binding and a pocket-like architecture. Key difference between the dU12 and dU10 quadruplexes might be the architecture of the copper binding site. In terms of catalytic activity, the dU10 derivatives are the better catalysts, which gave much higher conversions than the dU12 derivatives at reduced catalyst loading[10].

To further investigate the role of the individual components for the formation of catalytically competent structures, we next investigated the system by UV-Vis absorption spectroscopy, thermal denaturation studies, and mass spectrometry.

UV-Vis absorption spectroscopy

UV-Vis absorption data were collected for the hexynyl-bpy modified dU12 and dU10 quadruplexes as well as for the unmodified c-kit(wt) and c-kit-T10 quadruplexes and are shown in the Figure 3 (see also Figure S11a-d). The absorption spectra for both the unmodified quadruplexes (wt and c-kit-T10) are virtually identical with or without Cu(II), indicating that the unspecific binding of Cu(II) to the unmodified quadruplexes has no effect on the absorption characteristics. Modification with the hexynyl-bpy linker for the dU12 and dU10 quadruplexes gives a small increase in absorption at around 310 nm. The addition of copper ions to both the hexynyl-bpy-dU12 and hexynyl-bpy-dU10 quadruplexes displays a small shoulder at around 317 nm. Similar effects are observed with the respective nucleoside, hexynyl-bpy-deoxyuridine (Figure S11e, f).[14]

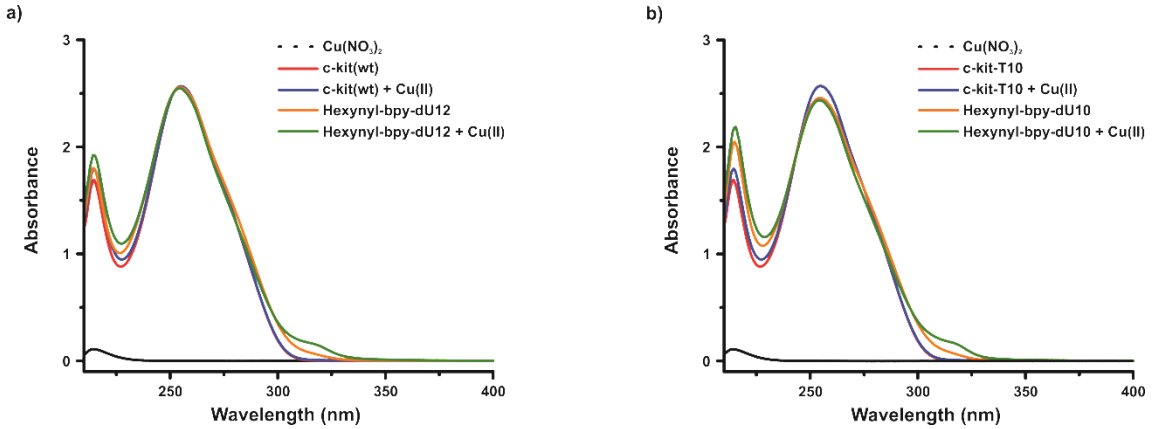


Figure 3. UV-Vis absorption spectra.
a) UV-Vis spectra of c-kit(wt) and hexynyl-bpy-dU12 in the absence and presence of Cu(II). b) UV-Vis spectra of c-kit-T10 and hexynyl-bpy-dU10 in the absence and presence of Cu(II).

Thermal denaturation analysis

To investigate the influence of the covalent modification and the addition of Cu(II) on the thermodynamic stability of the quadruplexes, we carried out temperature-dependent CD measurements for the two unmodified quadruplexes (c-kit(wt) and c-kit-T10) and their two hexynyl-bpy derivatives at the characteristic maximum at 260 nm (Figure 2), as is common for the investigation of quadruplexes.[15] The obtained melting temperatures (T_m) are shown in Table 2.

Quadruplex c-kit(wt) is more stable than the T10 mutant, and the same applies to their bpy derivatives. For none of the four oligonucleotides a significant effect of copper ions is observed. Derivatization with the hexynyl-bpy moiety has a strongly stabilizing effect in case of position 12 (ΔT_m = 10.6 °C), while the effect is much smaller for position 10 (ΔT_m = 0.8 °C). Polyacrylamide gel electrophoresis (Figure S12) and HPLC analysis (Figure S13-16) of the quadruplex solutions before and after the denaturation analysis confirmed that heating the samples in the presence of copper ions did not cause significant degradation.

10.1002/chem.201700632

Table 2: Melting temperatures (T_m) of different quadruplexes in the absence (-) and presence (+) of Cu(II).

Entry	DNA	$Cu(NO_3)_2$	T_m[a] [°C]
1	c-kit(wt)	-	64.2
2	c-kit(wt)	+	64
3	c-kit-T10	-	57.4
4	c-kit-T10	+	57.2
5	Hexynyl-bpy-dU12	-	74.8
6	Hexynyl-bpy-dU12	+	74.8
7	Hexynyl-bpy-dU10	-	58.2
8	Hexynyl-bpy-dU10	+	58.2

[a] T_m values are averaged over three separate measurements and are reproducible within ±1.0 °C.

Mass spectrometry

To corroborate the binding of Cu(II) to the bpy-moiety of the hexynyl-bpy modified dU12 and dU1(quadruplexes, the oligonucleotides were analyzed by ESI-mass spectrometry. The mass spectra o hexynyl-bpy modified dU12 and dU10 quadruplexes in the absence and presence of Cu(II) are shown i the Figure 4. For hexynyl-bpy-dU12, the deconvoluted spectrum contained the signal [M] = 7261.313 Da, and after incubation with Cu(II) the signal was shifted to 7323.2180 Da, which is the indication o formation of [M+Cu(II)-2H$^+$] (Figure 4a). Similarly, for hexynyl-bpy-dU10 the main signal is shifted fron [M] = 7236.2802 Da to 7298.2380 Da (Figure 4b). These are clear indications for the formation of a stable Cu(II) adduct with both the hexynyl-bpy-dU12 and the hexynyl-bpy-dU10 quadruplexes. Accordin to the integrated signal areas, less than 5% of the oligonucleotides remained in a copper-free form.

In contrast, the mass spectra of the unmodified c-kit(wt) and c-kit-T10 quadruplexes displayed the mas of different unspecifically bound Cu(II) species (Figure S17c, d). In these cases, only a fraction of th quaduplexes are attach to Cu(II), and also show a single to multiple Cu(II) binding. These results shov that Cu(II) can bind to the quadruplexes weak and unspecifically, whereas the attachment of a bpy moiety drives the system towards DNA derivative tightly bound to a single Cu(II) ion. The reason for th tight binding is the covalent bpy ligand, as corroborated using the nucleoside derivative hexynyl-bpy dUrd, which yielded a near-quantitative mass shift upon Cu(II) addition, while no changes could be see in the mass spectrum of unmodified deoxyuridine (Figure S17e,f).

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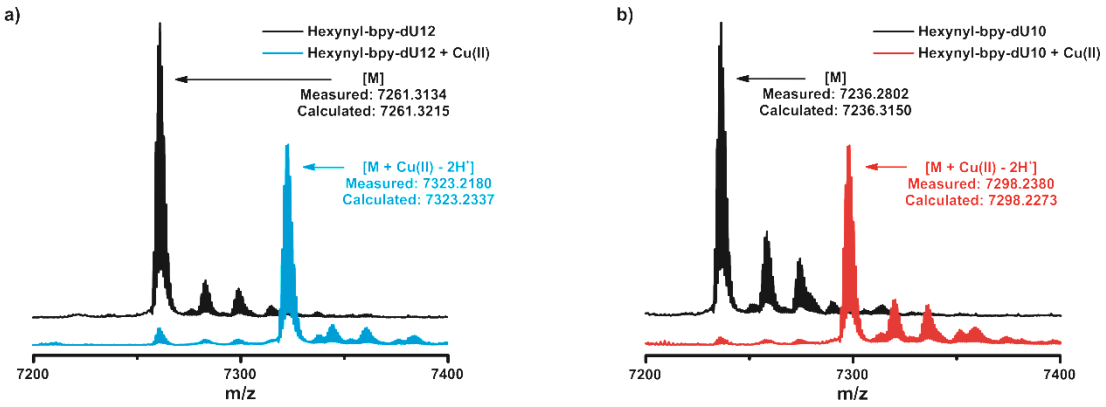

Figure 4. ESI-mass spectra.
a) Mass spectra of hexynyl-bpy-dU12 quadruplex in the absence and presence of Cu(II). b) Mass spectra of hexynyl-bpy-dU10 quadruplex in the absence and presence of Cu(II).

Since among all the bpy-linkers tested, from the propargyl-bpy to the decynyl-bpy, the best results were achieved with the hexynyl-bpy modified dU12 and dU10 quadruplexes, we chose these two catalysts to explore the Michael addition further under different catalytic conditions.

Initial rate and enantioselectivity

The conversion and enantioselectivity of Michael additions were monitored over 1-6 h time range (Figure 5). The initial rate of the reaction using the hexynyl-bpy-dU10 was about twice the rate of hexynyl-bpy-dU12, while in all cases the enantioselectivity was unaltered during the course of the reaction. In agreement with our earlier data on the dependence of conversion and *ee* on the catalyst loading[10], these results demonstrate that the hexynyl-bpy modified dU10 quadruplex is catalytically more active than the dU12-quadruplex.

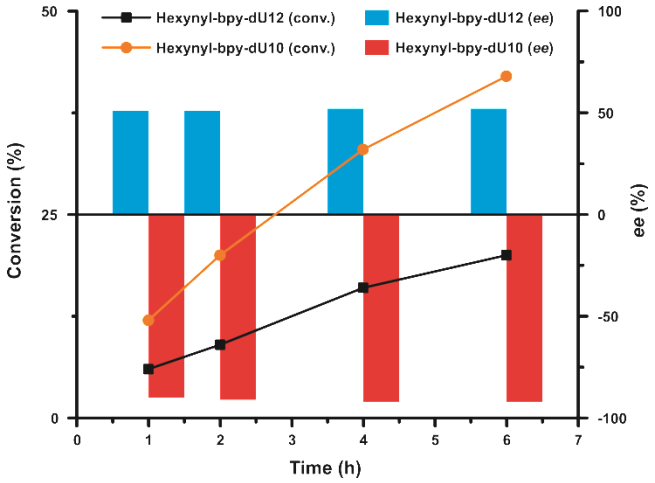

Figure 5. Conversion and *ee* over time using the hexynyl-bpy modified dU10- and dU12-quadruplexes.
Line represents the conversion and bar shows the enantioselectivity.

Role of monovalent ions

Monovalent ions are known to stabilize the structure and control the formation of G-quadruplexes.[16] The ability of alkali metal ion to stabilize G-quadruplexes follows the order: $K^+ >> Na^+ > Rb^+ > Cs^+ >> Li^+$ [16c] or $K^+ > Rb^+ > Na^+ > Li^+$ or Cs^+ [16b]. Among the alkali metal ions, K^+ which can coordinate optimally with eight carbonyl oxygen atoms present in two adjacent stacked G quartets, is preferred due to the

combined effect of relatively high hydration energy and coordination factor.[17] A G-quadruplex folding not only depends on the type of monovalent ions but also on its concentration.[18] The folding topology of a G-quadruplex can be switched from one form to another form just by changing the monovalent ions.[19]

We used different monovalent ions (in combination with 100 µM Cu(II)) to tune the G-quadruplex folding and applied those in the Michael addition of **1** with **2** to analyse their effects on the product formation and stereoselectivity (Table 3, Figure S18). CD spectra were recorded to detect different G-quadruplex foldings (Figure 6).

Table 3: Dependence of the DNA-catalyzed Michael addition of **1** with **2** on the monovalent ions.[a]

Entry	MCl	Hexynyl-bpy-dU12 conv (%)[b]	Hexynyl-bpy-dU12 *ee* (%)[b], [c]	Hexynyl-bpy-dU10 conv (%)[b]	Hexynyl-bpy-dU10 *ee* (%)[b], [c]
1[d]	-	3	n.d.[e]	19	-26
2	LiCl	34	+49	33	-31
3	NaCl	47	+66	36	-15
4[f]	KCl	95	+52	95	-92
5	RbCl	83	+45	74	-52
6	CsCl	81	+50	37	-45
7	NH_4Cl	63	+46	49	-39

[a] All the experiments were performed in MOPS buffer (20 mm, pH 7) containing 100 mM MCl, where M = Li^+, Na^+, K^+, Rb^+, Cs^+, NH_4^+. [b] Both conversion and *ee* wer calculated by using chiral HPLC; results are reproducible within ±5%. [c] (+) and (-) symbols refer to isomer with low and high retention time respectively from chiral HPL column. [d] Performed in pure water (pH 7). [e] n.d. = not determined. [f] see reference.[10]

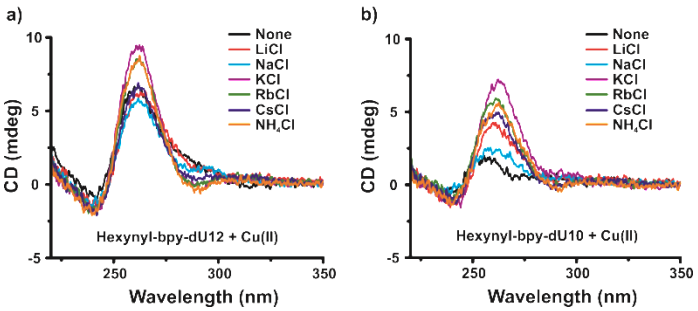

Figure 6. Dependence of the quadruplex folding on different monovalent ions.
a) CD spectra of the hexynyl-bpy-dU12 quadruplex with Cu(II) in the presence of different monovalent ions. a) CD spectra of the hexynyl-bpy-dU10 quadruplex with Cu(II) in th presence of different monovalent ions.

In the absence of the monovalent ions, the hexynyl-bpy-dU12 shows a negligible product formation, an low conversion and stereoselectivity were also noticed for hexynyl-bpy-dU10 (Table 3, entry 1). Th corresponding CD spectra show a labile parallel quadruplex folding for the latter case (Figure 6). In cas of Li^+, both the conversion and stereoselectivity were improved (Table 3, entry 1 vs entry 2), CD spectra indicate a similar folding pattern for the hexynyl-bpy-dU12 (Figure 6a), and on the other hand little more stability was observed in case of the hexynyl-bpy-dU10 (Figure 6b). Among all alkali metal ions, K^+ was found to be the most efficient in terms of both the product formation and stereoselectivity (Table 3, entry 4), and also yielded the most intense CD signal, which may indicate the highest proportion of folded G-quadruplex relative to other conformations (Figure 6a, b). The catalytic efficiency of NH_4^+

(Table 3, entry 7) was lower in comparison to K^+, although NH_4^+ (ionic radius 1.45 Å) has a similar size as K^+ (ionic radius 1.33 Å).

To trace the source for these variations in rate and stereoselectivity, we also measured catalysis of the Michael reaction by the modified hexynyl-bpy-dUrd in the presence of Cu(II) and different monovalent cations, applying the same catalytic conditions (Table S1). Low conversions (18 – 26%) and negligible *ee* (always in favour of the – enantiomer) were observed. In combination with the CD data (Fig. 6), these findings demonstrate that the monovalent ions control the stability and the structure of the quadruplexes, rather than modulating the Cu(II)-bpy interaction.

Properties of different transition metal complexes

To investigate the effect of various anions and transition metal cations on the Michael addition, the reaction of **1** and **2** was explored using different transition metal complexes (MX_2, 10 mol%) with hexynyl-bpy-dU12 and hexynyl-bpy-dU10 quadruplexes. The influence of anions (X) was analyzed by employing different complexes of the same transition metal (M). For this purpose, a series of Cu(II) complexes were taken, having a range of anions, and a minor effect on the stereoselectivity was observed without affecting the conversion (Table 4, entries 1-6). These results indicate that the anion of Cu(II) exert a negligible influence on the Michael addition, which is similar to the results previously reported for the Diels-Alder reaction.[8c] This missing effect of the anion is not surprising, as in all case the $[Cu(H_2O)_6]^{2+}$ complex should form in aqueous solution. On the other hand, applying various transition metal complexes of the same anion (NO_3^- in this case) we found that the transition metal itself exerts a huge effect on both the conversion and stereoselectivity (Table 4, entries 1, 7-9). We observed that Cu(II) complexes are the best choice to accomplish the Michael addition.

Table 4: Influence of the transition metal complexes on the Michael addition reaction.[a]

Entry	MX_2	Hexynyl-bpy-dU12 conv (%)[b]	Hexynyl-bpy-dU12 *ee* (%)[b], [c]	Hexynyl-bpy-dU10 conv (%)[b]	Hexynyl-bpy-dU10 *ee* (%)[b], [c]
1[d]	$Cu(NO_3)_2$	95	+52	95	-92
2	$CuCl_2$	96	+51	94	-92
3	$CuBr_2$	95	+51	97	-88
4	CuI_2	94	+47	96	-84
5	$Cu(OAc)_2$	93	+48	96	-86
6	$Cu(OTf)_2$	94	+44	97	-85
7	$Co(NO_3)_2$	7	<+5	12	<-5
8	$Ni(NO_3)_2$	15	+16	23	-10
9	$Zn(NO_3)_2$	<5	n.d.[e]	5	<-5

[a] See the Experimental Section for detailed reaction condition.. All experiments were performed in triplicate. [b] Both conversion and *ee* were calculated by using chiral HPLC; results are reproducible within ±5%. [c] (+) and (-) symbols refer to isomer with low and high retention time respectively from chiral HPLC column. [d] see reference.[10] [e] n.d. = not determined.

According to the Irving-Williams studies, the stability order for the first transition series bivalent ions is Mn < Fe < Co < Ni < Cu > Zn irrespective of the ligand nature.[20] This can explain why the Cu(II)-

complexes were more effective in terms of catalytic activity than the Ni(II)-complexes. Moreover, the exchange rate of $[Cu(H_2O)_6]^{2+}$ is also greater than those of other first row divalent transition metal ions, which is due to the axial ligands labilization via Jahn-Teller distortion. Cu(II) shows a tendency to form square planar or elongated tetragonal complexes.[21] Furthermore, Lewis-acidity may contribute to rate enhancement, and is highest for copper and nickel among the investigated transition metals. These physical properties make the Cu(II) an effective ion in both binding substrates and activating them for a nucleophilic addition.

Catalyst recyclability

Organic chemists often consider nucleic acid-based catalysts as too fragile for practical applications. To investigate the long-term stability and re-usability of the catalyst, a series of succesive Michael addition of **1** and **2** were carried out using either hexynyl-bpy-dU12 or hexynyl-bpy-dU10 in the presence of Cu(II under standard Michael addition condition. After each cycle, the product was removed from the reaction medium by extraction with Et_2O, the aqueous solution containing the catalyst was recovered followed by the addition of the fresh substrates **1** and **2**. Conversion and *ee* were analyzed over 1(cycles of reaction and extraction, and the results are shown in Figure 7. An almost linear decrease i conversion, from 95% to 57% and practically no change in stereoselectivity was observed fo hexynyl-bpy-dU12 as catalyst (Figure 7a). In the case of hexynyl-bpy-dU10, conversion declined fron 95% to 74% without any noticeable loss of stereoselectivity (Figure 7b). Likely, the reason for the declin in conversion is the loss of part of the catalyst during the product extraction. These results indicate tha the hexynyl-bpy-dU10 is more effective than the hexynyl-bpy-dU12 in terms of catalyst recyclability.

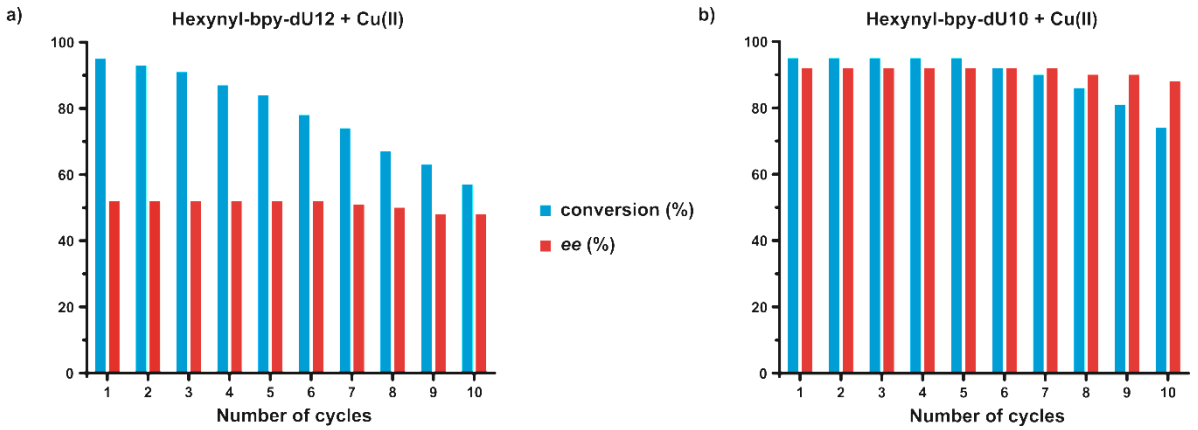

Figure 7. Catalyst recyclability.
a) Conversion and *ee* of the Michael addition product **3** in each catalytic cycle of hexynyl-bpy-dU12 catalyst. b) Conversion and *ee* of the Michael addition product **3** in each catalyt cycle of hexynyl-bpy-dU10 catalyst.

Conclusions

In conclusion, we have developed a new class of G-quadruplex DNA-based artificial metalloenzymes an applied these in an asymmetric Michael addition. We found that the position of modification, the topology of the quadruplex, the nature of the ligand, the linker length between ligand and DNA, the monovalent ion, and the chemical nature of transition metal complex are all crucial for the catalytic performance. Applying these newly synthesized metalloenzymes, up to 83% *ee* for the product was obtained. Moreover, the quadruplex modified at position 10 with a hexynyl-bpy linker showed twice the rate and a better recyclability compared to a DNA strand derivatized at position 12. In case of the dU12-

quadruplexes, comparison of catalytic assays with CD-spectrometric data indicates that the linker length has a very little influence on the quadruplex folding. The bpy-ligand may stack on the folded quadruplex which might influence the substrate accessibility and thus results in different enantiomeric preferences. For the dU10-quadruplexes, on the other hand, both linker length and Cu(II) addition impact on the folding and thus have an influence on the catalytic pocket formation. In addition to the (temperature-dependent) CD spectroscopy, other spectroscopic methods, such as UV-Vis absorption spectroscopy, and mass spectrometry were employed to investigate the interaction of Cu(II) with the bpy-linker modified G-quadruplexes. These findings are highly important to glean some guiding principles for new asymmetric DNA-based hybrid catalysts.

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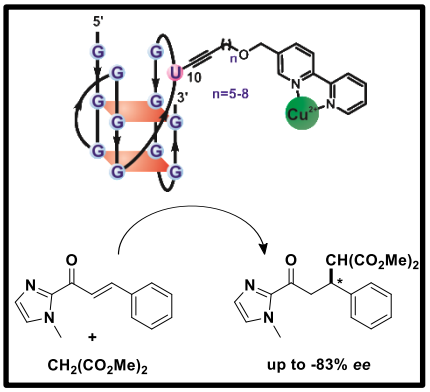
Figure for the Table of Contents



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