Published in final edited form as:

Biotechnol Prog. 2016 July 08; 32(4): 891–898. doi:10.1002/btpr.2281.

G-Quadruplex DNA-Based Asymmetric Catalysis of Michael Addition: Effects of Sonication, Ligands, and Co-Solvents

Hua Zhao*, Kai Shen

Department of Chemistry and Forensic Science, Savannah State University, Savannah, GA 31404, USA

Abstract

There is an escalating interest of using double stranded DNA molecules as a chiral scaffold to construct metal-biomacromolecule hybrid catalysts for asymmetric synthesis. Several recent studies also evaluated the use of G-quadruplex DNA-based catalysts for asymmetric Diels-Alder and Friedel-Crafts reactions. However, there is still a lack of understanding of how different oligonucleotides, salts (such as NaCl and KCl), metal ligands and co-solvents affect the catalytic performance of quadruplex DNA-based hybrid catalysts. In this study, we aim to systematically evaluate these key factors in asymmetric Michael addition reactions, and to examine the conformational and molecular changes of DNA by circular dichroism (CD) spectroscopy and gel electrophoresis. We achieved up to 95% yield and 50% enantiomeric excess (ee) when the reaction of 2-acylimidazole **1a** and dimethylmalonate was catalyzed by 5'-G₃(TTAG₃)₃-3' (G4DNA1) in 20 mM MOPS (pH 6.5) containing 50 mM KCl and 40 μ M [Cu(dmbipy)(NO₃)₂], and G4DNA1 was pre-sonicated in ice bath for 10 min prior to the reaction. G-quadruplex-based hybrid catalysts provide a new tool for asymmetric catalysis, but future mechanistic studies should be sought to further improve the catalytic efficiency. The current work presents a systematic study of asymmetric Michael addition catalyzed by G-quadruplex catalysts constructed via non-covalent complexing, and an intriguing finding of the effect of pre-sonication on catalytic efficiency.

Keywords

quadruplex DNA; DNA-based hybrid catalyst; asymmetric catalysis; Michael addition; ionic liquid

Introduction

Recently, duplex DNA molecules have been explored as a chiral scaffold for binding metal complexes to design so called 'DNA-based hybrid catalysts'. The new type of hybrid catalysts is being actively evaluated in asymmetric reactions,^{1–3} and has resulted in high activities and enantioselectivities in the Michael addition,^{4, 5} Diels-Alder reaction,^{6–8} Friedel-Crafts alkylation,⁹ intramolecular cyclopropanation,¹⁰ electrophilic fluorination of β -keto esters,¹¹ and asymmetric hydration,⁷ etc.

*Corresponding author: Dr. Hua Zhao, Department of Chemistry and Forensic Science, Box 40154, Savannah State University, Savannah, GA 31404, USA, zhaoh@savannahstate.edu (or huazhao98@gmail.com), Phone: +1-912-358-4448.

Meanwhile, G-quadruplex DNA (G4DNA) molecules have shown potential applications in catalysts, biosensors, and DNA-based architectures. G-quadruplex DNA has unique four-stranded helices, and is folded from guanine-rich DNA sequences by the stacking of planar quartets composed of four guanines that interact by Hoogsteen hydrogen bonding.^{12, 13} G-quadruplex structures are highly polymorphic; for example, human telomeric sequences (containing a repeating string of TTAGGG units) can adopt at least five different intramolecular G-quadruplexes.¹⁴ Several groups reported the use of quadruplex DNA in asymmetric catalysis.¹⁵ The Moses group¹⁶ constructed G-quadruplex chiral catalysts by using human telomeric (*h*-Tel) DNA [5'-AG₃(TTAG₃)₃-3'] or *c*-kit promoter quadruplex 'c-kit 87up'(*c*-kit) [5'-(AGGGAGGGCGCTGGGAGGAGGG)-3'] in 90 mM KCl as chiral scaffold of Cu²⁺-ligand complex. They found that the hybrid catalyst in a Diels–Alder reaction gave >85% conversion, 96:4 *endo:exo* ratio, and moderate ees (–24% *endo* ee and –51% *exo* ee). This finding suggests the transfer of chirality from quadruplex structures to reacting species, although duplex DNA-based catalysts have been reported to produce much high ee values (up to 99%).^{6, 17} The Li group¹⁸ measured circular dichroism (CD) spectra of human telomeric 5'-G₃(TTAG₃)₃-3' complexing with Cu²⁺ ions in Na⁺ or K⁺ salt solutions, and observed the antiparallel G-quadruplex conformation in Na⁺ solutions¹⁹ and the hybrid conformations in K⁺ solutions;²⁰ the G4DNA–Cu²⁺ metalloenzyme in 50 mM NaCl catalyzed the enantioselective Friedel–Crafts reactions, resulting in up to 99% conversion and 74% ee.¹⁸ The same catalytic system in 50 mM NaCl was employed by this group²¹ in Diels–Alder reactions, achieving up to 99% conversion, 98:2 (*endo:exo* ratio) and 74% ee; they also found that upon the addition of 50 wt% PEG 200, the G4DNA's conformation was switched from antiparallel to parallel, which produced a reversed absolute configuration of the products. Wilking and Hennecke²² evaluated various oligonucleotides in 50 mM KCl as G-quadruplex scaffold for cationic porphyrin complex with Cu²⁺ to catalyze the Diels–Alder reaction of aza-chalcone and cyclopentadiene, and obtained up to 97% conversion, 98:2 (*endo:exo* ratio) and 67% ee. Dey and Jäschke²³ constructed DNA quadruplexes with a covalent linkage to Cu²⁺ complexes at a specific location of the biomolecules, and found that quadruplexes with a covalent modification at position 10 led to the (–)-enantiomer with up to 92 % ee in Michael additions, but the DNA modified at position 12 resulted in the (+)-enantiomer with up to 75 % ee.

G-quadruplex DNA structures are typically maintained in aqueous solutions, and thus their relevant applications are usually carried out in aqueous phases. Several recent studies suggest that G-quadruplex DNA could be stable in some organic ionic solutions. Fujita and Ohno²⁴ observed that dG₃(T₂AG₃)₃ DNA maintains the G-quadruplex structure in aqueous [choline][H₂PO₄] of various concentration, but not in many other salt solutions. They indicated that the G-quadruplex structure is preserved in kosmotropic salt solutions because the DNA hydration state is strongly affected by the ion kosmotropicity. Lannan et al.²⁵ reported that human telomere sequence (HTS) DNA exhibits a G-quadruplex with the parallel-stranded fold in choline chloride/urea (1:2, molar ratio). This is consistent with the observation that a reduced water activity results in the parallel fold while a high water activity leads to alternative folds. More interestingly, after HTS DNA was thermally denatured and then quickly cooled back to room temperature, the refolding of DNA back to the parallel structure in the deep eutectic solvent (DES) requires several months (vs < 2 min

in an aqueous solution). Zhao et al.²⁶ studied the structures of ten G-quadruplexes in neat DES (choline chloride/urea, 1:2 molar ratio) using UV melting, CD and fluorescence spectroscopy, and found different intramolecular, intermolecular, and higher-order G-quadruplex structures in DES, especially the parallel structure. G-quadruplexes in neat DES seem to be more stable than in aqueous media; some G-quadruplex DNA molecules could be thermally stable at over 110 °C.

In this study, we further extend our earlier study on the effects of sonication, ligands, and co-solvents on duplex DNA-based catalysts to quadruplex oligonucleotide-based asymmetric catalysis. We systematically evaluate the enantioselective Michael addition catalyzed by human telomeric quadruplex DNA-based hybrid catalysts (with non-covalent modification) in terms of different oligonucleotide sequences, salts, ligands, pre-sonication times, co-solvents and substrates.

Materials and methods

Materials

Three oligonucleotides, i.e. 5'-G₃(TTAG₃)₃-3' (**G4DNA1**), 5'-AG₃(TTAG₃)₃-3' (**G4DNA2**), and 5'-TG₃(TTAG₃)₃-3' (**G4DNA3**) were synthesized by Sigma-Aldrich (St. Louis, MO, USA). 4,4'-Dimethyl-2,2'-bipyridine (dmbipy), 2,2'-bipyridine (bipy), and 1,10-phenanthroline (phen) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2'-Bipyrimidine (bipym) was produced by OxChem (Irwindale, CA, USA), and berberine was obtained from TCI America (Philadelphia, PA, USA). Potassium tetrachloroplatinate(II) was produced by Acros Organics (Geel, Belgium). The synthesis of 2-acyl imidazole substrates (**1a-f**) was a modification of literature methods,²⁷⁻²⁹ and is described in detail in our recent study.⁵ (*E*)-4,4-dimethyl-1-(1-methyl-1*H*-imidazol-2-yl)-2-penten-1-one (**1g**) was prepared following a literature method.³⁰

Ligand preparations

The preparations of [Cu(dmbipy)(NO₃)₂], [Cu(bipy)(NO₃)₂], [Cu(phen)(NO₃)₂], [Cu(bipym)(NO₃)₂], and [Cu(berberine)(NO₃)₂] are based on a literature method (in its *Supporting Information*).⁶ Using [Cu(dmbipy)(NO₃)₂] as an example, a solution of Cu(NO₃)₂·3H₂O (1.0 g or 4.14 mmol dissolved in 50 mL ethanol) was mixed with a solution of 0.3825 g (2.08 mmol) 4,4'-dimethyl-2,2'-bipyridine in 50 mL ethanol at room temperature. The mixture was incubated in an ethyl acetate bath for 2 days. The blue solid was collected by filtration, washed with ethanol and dried in air.

[Pt(bipym)Cl₂] was prepared following these two steps. **(1)** Based on the literature method,³¹ 0.1 g (0.24 mmol) potassium tetrachloroplatinate(II) K₂PtCl₄ suspended in 1.0 mL distilled water was mixed with 0.72 mmol (51 μL) DMSO. The mixture was stirred at room temperature until yellow crystals precipitated. The Pt(dmsO)₂Cl₂ crystals were collected by filtration, followed by washing with water, ethanol and diethyl ether. The product was dried in vacuum for 4 h. **(2)** A solution of 50 mg (0.316 mmol) 2,2'-bipyrimidine in 5 mL methanol was added dropwise to a suspension of 115 mg (0.275 mmol) Pt(dmsO)₂Cl₂ in 10 mL methanol under gentle agitation. The mixture was stirred at room temperature for 12 h.

The red precipitates were sonicated for 3 h and then filtered under vacuum, followed by washing with ethanol and diethyl ether. The product Pt(bipym)Cl₂ was dried in vacuum for 12 h.

G-Quadruplex-based catalytic enantioselective Michael reaction

A stock solution of 100 μM oligonucleotide (e.g. G4DNA1, 5'-G₃(TTAG₃)₃-3') was prepared in 20 mM MOPS containing 50 mM KCl (or NaCl). The DNA stock solution (500 μL) was mixed with 89 μL 0.45 mM [Cu(dmbipy)(NO₃)₂] in 20 mM MOPS pH 6.5. Additional 410 μL MOPS (20 mM MOPS pH 6.5 with 50 mM KCl or NaCl) containing co-solvent was added to make a total volume of ~1.0 mL. The final oligonucleotide concentration was 50 μM, and the final copper complex concentration was 40 μM. To this mixture, 1 μmol of substrate in 3 μL acetonitrile/2-propanol (3/2, v/v) was added and cooled to below 5 °C. The reaction was initiated by adding 100 eq. dimethylmalonate (19 μL) and stirred at 5 °C for 72 h. The product was isolated by extraction with diethyl ether followed by drying with Na₂SO₄ and removal of the solvent. The crude product was dissolved in 1.0 mL methanol-d₄ and examined by ¹H-NMR (300 MHz, JEOL at Tokyo, Japan), and LC-20AT Shimadzu HPLC (Kyoto, Japan) equipped with a SPD-20A UV-Vis dual-wavelength detector (Chiralpak-AD 2.1 × 150 mm, 10 μm; 0.2 mL/min *n*-heptane/*i*-PrOH 90/10 (v/v), 210 nm).

Circular dichroism (CD) spectra of oligonucleotide in aqueous solutions

Oligonucleotide was dissolved in 20 mM MOPS (pH 6.5) containing 50 mM KCl (or NaCl) to make a 100 μM stock solution. The stock solution (300 μL) was diluted with co-solvent and MOPS (20 mM, pH 6.5) containing 50 mM KCl (or NaCl) to make a 3.0 mL solution (final DNA concentration 10 μM). Co-solvents were weighed to meet the desired final concentration. The background CD spectrum of each sample was scanned with the corresponding buffer containing the co-solvent. An aliquot of the mixture was scanned in the range of 216–340 nm by a JASCO J-825 CD Spectrometer (Tokyo, Japan). The instrument parameters were set as the following: data pitch 0.1 nm, scanning speed 100 nm/min, band width 1.00 nm, slit width 100 μm, DIT 1 sec, standard sensitivity, 3 accumulations, and the cell temperature of 25 °C.

DNA binding with Cu²⁺ ligand in aqueous solutions of organic solvents or ionic liquids

Oligonucleotide was dissolved in 20 mM MOPS (pH 6.5) containing 50 mM KCl (or NaCl) to make a 100 μM stock solution. An aliquot of stock DNA solution (0, 50, 10 and 150 μL) was mixed with 90 μL of 0.45 mM Cu(dmbipy)(NO₃)₂ in MOPS (20 mM, pH 6.5), followed by the addition of co-solvent and MOPS buffer containing 50 mM KCl (or NaCl) to make the total volume of 1.5 mL. The copper complex was maintained at 27 μM. The mixture was then analyzed by a Thermo Scientific NanoDrop 2000 (Waltham, MA, USA) for its absorbance at 260 nm. The binding constant (*K_b*) can be determined by the following equation,^{17, 32}

$$\frac{D}{\Delta\epsilon_{ap}} = \frac{1}{\Delta\epsilon} D + \frac{1}{\Delta\epsilon K_b}$$

where $\varepsilon_{\text{ap}} = |\varepsilon_{\text{a}} - \varepsilon_{\text{f}}|$, $\varepsilon = |\varepsilon_{\text{b}} - \varepsilon_{\text{f}}|$, and ε_{a} , ε_{f} and ε_{b} are the apparent, free and bound extinction coefficients for the complex respectively, and D is the DNA concentration in base pairs. $D/\varepsilon_{\text{ap}}$ was plotted against D , and the K_{b} value was calculated from the ratio of the slope to the y-intercept.

Gel electrophoresis of DNA

The compositions of DNA samples were characterized using agarose gel electrophoresis. A brief description of the procedures is as the following: 10 μL Sybr Safe DNA stain (10,000 $\times$) was added into 100 mL 2% agarose gel during gel casting. DNA samples were prepared by mixing 10 μL DNA solution (10 μM) with 2 μL Bio-Rad nucleic acid sample loading buffer. For each well, 6 μL of DNA sample or DNA standard (EZ Load 1 kb DNA ladder, Bio-Rad) was loaded. Gel cell was immersed in ice-water bath to keep the buffer temperature low and to minimize small oligonucleotides diffusing out of the gel. Gel electrophoresis was conducted at 110 V for 30–90 min. Gel images were acquired on a Bio-Rad Gel Doc EZ system (Hercules, CA, USA). The concentration of DNA samples were quantitated by measuring their UV absorption at 260 nm on a Thermo Scientific Nanodrop 2000 system.

Results and discussion

The quadruplex DNA-based hybrid catalysts were constructed by mixing G4DNA in MOPS buffer (20 mM, pH 6.5) containing 50 mM KCl or NaCl with a metal-ligand such as $\text{Cu}(\text{dmbipy})(\text{NO}_3)_2$ (dmbipy = 4,4'-dimethyl-2,2'-dipyridyl). To evaluate the catalytic activity of the catalysts, a Michael addition reaction was selected as our primary model system: the conversion of (*E*)-(1-methyl-1*H*-imidazole-2-yl)-3-phenylprop-2-en-1-one (**1a**) to (*R*)-dimethyl 2-(3-(1-methyl-1*H*-imidazol-2-yl)-3-oxo-1-phenylpropyl)malonate (**2a**) (see Scheme 1).

Effect of different quadruplex DNA and salts

Through custom synthesis, three human telomeric oligonucleotides, i.e. 5'-G₃(TTAG₃)₃-3' (**G4DNA1**), 5'-AG₃(TTAG₃)₃-3' (**G4DNA2**) and 5'-TG₃(TTAG₃)₃-3' (**G4DNA3**) were chosen for this study. In earlier studies, these G4DNA/Cu²⁺ hybrid catalysts have exhibited relatively high activities and enantioselectivities in Diels-Alder reactions in KCl^{16, 22} or NaCl solutions,²¹ and Friedel-Crafts reactions in NaCl solutions.¹⁸ Based on circular dichroism (CD) spectra (Figure 1), human telomeric oligonucleotides tend to fold intramolecularly into an antiparallel G-quadruplex conformation in 50 mM NaCl (with a strong minimum band at 265 nm and a maximum at 295 nm). However, in 50 mM KCl (Figure 2), the oligonucleotide forms an equilibrium mixture of species including a hybrid (3+1) species and the (2+2) anti-parallel basket structure (see Scheme 2), with a substantial proportion of the anti-parallel structure (as suggested by strong band at 290 nm in Figure 2).^{16, 20, 33} Without the addition of any salt in MOPS buffer, the characteristic bands seem weaker especially after 10 min of sonication (Figure 1), implying the instability of quadruplex structures without NaCl or KCl.

In addition to their influence on quadruplex structures, different salts (KCl or NaCl) also change the catalytic behavior of hybrid catalysts. As shown in Table 1 (Entries 2–3), without any salt (KCl or NaCl), G4DNA1-based catalyst gave low yields (25–29%) and low ees (19–21%) regardless of pre-sonication or not. Entries 4–10 illustrate that the use of 50 mM KCl with three G4DNA always led to higher yields than that of 50 mM NaCl, more significantly in the case of G4DNA1 where the yield was improved from 40% to 95%. However, the effect on enantiomeric excess (ee) of product **2a** appears to be a different scenario: the addition of 50 mM KCl improved ee to 50% from 3% (when using 50 mM NaCl) for G4DNA1, whereas the enantioselectivity was higher in NaCl than in KCl for G4DNA2 and G4DNA3. Wilking and Hennecke²² also observed a lower activity and enantioselectivity of G4DNA1/cationic porphyrin-Cu²⁺ catalyst in 50 mM NaCl than in 50 mM KCl, although the Li group reported a relatively high activity and enantioselectivity of G4DNA in 50 mM NaCl for Diels–Alder²¹ and Friedel–Crafts reactions.¹⁸ The use of 100 mM KCl (Entry 5) showed no improvement in either yield or ee for G4DNA1 when comparing with 50 mM KCl (Entry 4); Table 2 shows an unusually high binding constant of $49.2 \times 10^5 \text{ M}^{-1}$ in 100 mM KCl (vs $8.26 \times 10^5 \text{ M}^{-1}$ in 50 mM KCl), which implies that an excessively strong binding between oligonucleotide and Cu²⁺-ligand might hinder the catalytic ability of copper ions. The addition of adenosine or thymidine at the 5' end of oligonucleotide led to lower activity and enantioselectivity of the hybrid catalyst (Entries 7–10). Overall, G4DNA1 in 50 mM KCl gave the highest yield (95%) and ee (50%) although they are still inferior to the results of the duplex DNA catalyst (Entry 1). Other G-quadruplex-based asymmetric catalysis suggested similar results. G4DNA-catalyzed Diels–Alder reactions gave moderate ees (–24% *endo* ee and –51% *exo* ee) in 90 mM KCl,¹⁶ 67% ee in 50 mM KCl,²² and 74% ee in 50 mM NaCl;²¹ however, duplex DNA-based catalysts have been reported to produce much high ee values (up to 99%).^{6, 17} G4DNA–Cu²⁺-catalyzed Friedel–Crafts reactions in 50 mM NaCl resulted in up to 74% ee,¹⁸ while double-stranded DNA hybrid catalyst produced up to 93% ee.⁹

Effect of pre-sonication and ligands

Our earlier study⁵ suggested that pre-sonication of double stranded DNA in buffer allowed a better dispersion and less aggregation of DNA in aqueous media, which led to improved catalytic performance of the DNA-based hybrid catalyst. Therefore, we also pre-sonicated G4DNA1 in ice bath for 0, 5, 10 or 15 min (Entries 11–15 in Table 1), and found that pre-sonication for 10 min prior to the reaction gave the highest yield and ee (Entry 14). CD spectra in Figures 1 and 2 show that pre-sonication does not have an appreciable impact or destruction on the quadruplex structures. Gel electrophoresis of G4DNA1 in Figure 3 indicates that pre-sonication does not cause any molecular fragmentation of the oligonucleotide in the presence or absence of [Cu(dmbipy)(NO₃)₂]. However, pre-sonication of G4DNA1 for 10 min led to the highest binding constant ($K_b = 8.26 \times 10^5 \text{ M}^{-1}$) toward Cu²⁺ ligand among entries 1–4 in Table 2; the moderately strong (but not excessively strong) binding is likely responsible for the high catalytic performance of hybrid catalyst.

We further screened a number of ligands for binding Cu²⁺ ions (Entries 16–22 in Table 1). Several ligands (dmbipy, bipy and phen) have been used in constructing duplex DNA-based hybrid catalysts;⁶ bipym could act as a doubly bidentate bridging ligand to form DNA-ligand

complexes;³⁴ Pt(bipym)Cl₂ was considered because platinum complexes are known to bind with DNA molecules;^{34–36} berberine is a plant alkaloid that is known to bind with DNA molecules.³⁷ Among all ligands studied, only [Cu(dmbipy)(NO₃)₂] (Entry 16), [Cu(phen)(NO₃)₂] (Entry 18) and [Cu(bipym)(NO₃)₂] (Entry 21) afforded reasonable product yields (74–95%); both [Cu(dmbipy)(NO₃)₂] and [Cu(berberine)(NO₃)₂] led to about 50% ee, but the latter ligand only gave 7% yield. The addition of [Pt(bipym)Cl₂+Cu(NO₃)₂] (Entry 19) only produced 12% yield and 9% ee; although platinum complexes bind with double helical DNA molecules (Scheme 3),^{35, 36} they may not bind efficiently to those quadruplex structures examined by this study. The use of Cu(NO₃)₂ alone without ligand resulted in a product of an opposite optical rotation (+) with only 4–6% ee (Entries 23 and 24), which is likely due to a poor Cu²⁺ complexing with the chiral DNA without the ligand. The concentration of the best metal-ligand complex [Cu(dmbipy)(NO₃)₂] was further optimized (Entries 25–30), and 40 μM of Cu²⁺ complex was identified to enable the highest catalytic capability (Entry 29, 95% yield and 50% ee).

Effect of co-solvents and different substrates

Our earlier study⁵ suggested that the addition of co-solvents (such as 0.4 M glycerol and several ionic liquids) could improve the performance of duplex DNA-based hybrid catalyst by dissolving more substrates in aqueous solutions and allowing the Michael addition to be carried out at room temperature with a high enantioselectivity. After adding 0.2–0.5 M co-solvents [including methanol, glycerol, ionic liquids and choline/glycerol (1:2 molar ratio)] in the G4DNA1-based Michael reaction (Entries 32–39 in Table 1), lower yields and ees were observed. CD spectra in Figure 2 indicate that low concentrations of co-solvents caused no significant changes of the quadruplex structures [note: ionic liquids caused some interferences at the far-UV wavelength range (200–250 nm)³⁸ that are not necessarily associated with the DNA structural changes]. Gel electrophoresis of G4DNA1 in Figure 3 confirms that these co-solvents impose no significant molecular fragmentation of the oligonucleotide with or without [Cu(dmbipy)(NO₃)₂]. Table 2 suggests that the binding constants in the presence of ionic co-solvents (Entries 9–11 in Table 2) are markedly higher ($31\text{--}43 \times 10^5 \text{ M}^{-1}$), which might explain the poor catalytic performance of hybrid catalyst in these co-solvents. The impact of methanol and glycerol on the binding constants (Entries 7–8 in Table 2) is less substantial, so does on the product yields and ees (Entries 32–35 in Table 1).

Furthermore, we expanded the catalytic reaction to other substrates (**1b–1g**, Entries 41–46 in Table 1), and obtained high yields (>90%) for **1d** and **1e** and relatively low ees (23–29%) for most substrates; these ee values are substantially lower than similar reactions catalyzed by duplex DNA-based catalyst.⁵ More in-depth molecular-level understanding is needed to understand the differences and to further improve the catalytic ability of quadruplex DNA hybrid catalysts. The Li group^{18, 21} indicated that the loop sequence is essential to the stereoselectivity of quadruplex catalysts. As pointed out by Dey and Jäschke,²³ the activity and selectivity of G-quadruplex catalysts are controlled by several key factors including the topology of the quadruplex, the binding site of the metal ligand, and the type of ligand, etc.

In general, comparing with the high specificity and selectivity of duplex DNA-based hybrid catalysts³⁹ and conventional biocatalysts (such as enzymes),^{40, 41} current G-quadruplex-based catalytic systems exhibit a high catalytic activity but are lack of a high specificity and selectivity. However, multiple G-quadruplex structures (Scheme 2) have more complexed topology and more structural variety than duplex DNA, which could potentially provide versatile binding sites for metal ligands enabling some unique asymmetric catalysis.¹⁵ Currently, the G-quadruplex-catalytic system has not been well explored and optimized (for example, customized metal-ligand complexes may be needed for efficient binding), and thus the true potential of quadruplex-hybrid catalysts is yet to be fully exploited.

Conclusions

We have identified several key factors that influence the quadruplex DNA-based hybrid catalyst, including oligonucleotide base sequence, pre-sonication, and the type and concentration of Cu²⁺ ligands. Pre-sonication could improve the binding between oligonucleotide and Cu²⁺ ligand, leading to higher yields and ees, while pre-sonication does not seem to cause appreciable conformational and molecular changes of the quadruplex structures based on CD spectra and gel electrophoresis images. The use of organic and ionic solvents as additives induced lower catalytic activities of the hybrid catalyst, mostly likely due to the excessively strong binding between oligonucleotide and Cu²⁺ ligand. Future mechanistic studies are needed to understand the differences between quadruplex and duplex DNA in the hybrid catalyst, and to provide a rational tool to improve the catalytic performance of quadruplex DNA-based catalysts.

Acknowledgements

HZ acknowledges the supports by the Henry Dreyfus Teacher-Scholar Award (2012–2017), NIH MBRS-RISE grant (1R25GM096956), NIH NIBIB contract award (HHSN268201200011C), and the National Natural Science Foundation of China (21328601). The authors declare no conflict of interest.

References

1. Boersma AJ, Megens RP, Feringa BL, and Roelfes G, DNA-based asymmetric catalysis. *Chemical Society Reviews* 2010; 39: 2083–2092. [PubMed: 20411188]
2. Park S, and Sugiyama H, DNA as a chiral scaffold for asymmetric synthesis. *Molecules* 2012; 17: 12792–12803. [PubMed: 23114615]
3. Park S, and Sugiyama H, DNA-based hybrid catalysts for asymmetric organic synthesis. *Angewandte Chemie, International Edition in English* 2010; 49: 3870–3878.
4. Coquière D, Feringa BL, and Roelfes G, DNA-based catalytic enantioselective Michael reactions in water. *Angewandte Chemie, International Edition in English* 2007; 46: 9308–9311.
5. Zhao H, and Shen K, DNA-based asymmetric catalysis: Role of ionic solvents and glymes. *RSC Adv* 2014; 4: 54051–54059 [PubMed: 25386337]
6. Roelfes G, Boersma AJ, and Feringa BL, Highly enantioselective DNA-based catalysis. *Chemical Communications* 2006; 635–637. [PubMed: 16446834]
7. Rosati F, and Roelfes G, A ligand structure–activity study of DNA-based catalytic asymmetric hydration and Diels–Alder reactions. *ChemCatChem* 2011; 3: 973–977.
8. Boersma AJ, Feringa BL, and Roelfes G, α,β -Unsaturated 2-acyl imidazoles as a practical class of dienophiles for the DNA-based catalytic asymmetric Diels–Alder reaction in water. *Organic Letters* 2007; 9: 3647–3650. [PubMed: 17685624]

9. Boersma AJ, Feringa BL, and Roelfes G, Enantioselective Friedel–Crafts reactions in water using a DNA-based catalyst. *Angewandte Chemie, International Edition in English* 2009; 48: 3346–3348.
10. Oelerich J, and Roelfes G, DNA-based asymmetric organometallic catalysis in water. *Chem. Sci* 2013; 4: 2013–2017.
11. Shibata N, Yasui H, Nakamura S, and Toru T, DNA-Mediated enantioselective carbon–fluorine bond formation. *Synlett* 2007; 7: 1153–1157.
12. Parkinson GN, Lee MPH, and Neidle S, Crystal structure of parallel quadruplexes from human telomeric DNA. *Nature* 2002; 417: 876–880. [PubMed: 12050675]
13. Huppert JL, Four-stranded nucleic acids: Structure, function and targeting of G-quadruplexes. *Chemical Society Reviews* 2008; 37: 1375–1384. [PubMed: 18568163]
14. Mashimo T, Yagi H, Sannohe Y, Rajendran A, and Sugiyama H, Folding pathways of human telomeric type-1 and type-2 G-quadruplex structures. *Journal of the American Chemical Society* 2010; 132: 14910–14918. [PubMed: 20882978]
15. Wang L-X, Xiang J-F, and Tang Y-L, Novel DNA catalysts based on G-quadruplex for organic synthesis. *Advanced Synthesis & Catalysis* 2015; 357: 13–20.
16. Roe S, Ritson DJ, Garner T, Searle M, and Moses JE, Tuneable DNA-based asymmetric catalysis using a G-quadruplex supramolecular assembly. *Chemical Communications* 2010; 46: 4309–4311. [PubMed: 20458390]
17. Megens RP, and Roelfes G, Organic co-solvents in aqueous DNA-based asymmetric catalysis. *Org. Biomol. Chem* 2010; 8: 1387–1393. [PubMed: 20204212]
18. Wang C, Li Y, Jia G, Liu Y, Lu S, and Li C, Enantioselective Friedel–Crafts reactions in water catalyzed by a human telomeric G-quadruplex DNA metalloenzyme. *Chemical Communications* 2012; 48: 6232–6234. [PubMed: 22595813]
19. Wang Y, and Patel DJ, Solution structure of the human telomeric repeat d[AG₃(T₂AG₃)₃] G-tetraplex. *Structure* 1993; 1: 263–282. [PubMed: 8081740]
20. Lim KW, Amrane S, Bouaziz S, Xu W, Mu Y, Patel DJ, Luu KN, and Phan AT, Structure of the human telomere in K⁺ solution: A stable basket-type G-quadruplex with only two G-tetrad layers. *Journal of the American Chemical Society* 2009; 131: 4301–4309. [PubMed: 19271707]
21. Wang C, Jia G, Zhou J, Li Y, Liu Y, Lu S, and Li C, Enantioselective Diels–Alder reactions with G-quadruplex DNA-based catalysts. *Angewandte Chemie, International Edition in English* 2012; 51: 9352–9355.
22. Wilking M, and Hennecke U, The influence of G-quadruplex structure on DNA-based asymmetric catalysis using the G-quadruplex-bound cationic porphyrin TMPyP4-Cu. *Org. Biomol. Chem* 2013; 11: 6940–6945. [PubMed: 23959382]
23. Dey S, and Jäschke A, Tuning the stereoselectivity of a DNA-catalyzed Michael addition through covalent modification. *Angewandte Chemie, International Edition in English* 2015; 54: 11279–11282.
24. Fujita K, and Ohno H, Stable G-quadruplex structure in a hydrated ion pair: cholinium cation and dihydrogen phosphate anion. *Chemical Communications* 2012; 48: 5751–5753. [PubMed: 22552502]
25. Lannan FM, Mamajanov I, and Hud NV, Human telomere sequence DNA in water-free and high-viscosity solvents: G-Quadruplex folding governed by Kramers rate theory. *Journal of the American Chemical Society* 2012; 134: 15324–15330. [PubMed: 22651378]
26. Zhao C, Ren J, and Qu X, G-quadruplexes form ultrastable parallel structures in deep eutectic solvent. *Langmuir* 2013; 29: 1183–1191. [PubMed: 23282194]
27. Evans DA, Fandrick KR, and Song H-J, Enantioselective Friedel–Crafts alkylations of α,β -unsaturated 2-acyl imidazoles catalyzed by bis(oxazolonyl)pyridine–scandium(III) triflate complexes. *Journal of the American Chemical Society* 2005; 127: 8942–8943. [PubMed: 15969558]
28. Hayakawa S, Michiue T, Okamoto M, Hatakeyama S, and Ohta S, Syntheses of α,β -unsaturated ketones starting from vinylic and allylic Grignard reagents via 2-imidazolylmethanol intermediates. *Heterocycles* 1988; 27: 457–473.

29. Myers MC, Bharadwaj AR, Milgram BC, and Scheidt KA, Catalytic conjugate additions of carbonyl anions under neutral aqueous conditions. *Journal of the American Chemical Society* 2005; 127: 14675–14680. [PubMed: 16231921]
30. Boersma AJ, Coquière D, Geerdink D, Rosati F, Feringa BL, and Roelfes G, Catalytic enantioselective syn hydration of enones in water using a DNA-based catalyst. *Nat. Chem* 2010; 2: 991–995. [PubMed: 20966958]
31. Price JH, Williamson AN, Schramm RF, and Wayland BB, Palladium(II) and platinum(II) alkyl sulfoxide complexes. Examples of sulfur-bonded, mixed sulfur- and oxygen-bonded, and totally oxygen-bonded complexes. *Inorganic Chemistry* 1972; 11: 1280–1284.
32. Wolfe A, Shimer GHJ, and Meehan T, Polycyclic aromatic hydrocarbons physically intercalate into duplex regions of denatured DNA. *Biochemistry* 1987; 26: 6392–6396. [PubMed: 3427013]
33. Phan AT, Luu KN, and Patel DJ, Different loop arrangements of intramolecular human telomeric (3+1) G-quadruplexes in K^+ solution. *Nucl. Acids Res.* 2006; 34: 5715–5719. [PubMed: 17040899]
34. Kiernan PM, and Ludi A, Complexes of platinum(II) with 2,2'-bipyrimidine: the effect of hydrogen bonding on intermetallic interactions. *Journal of the Chemical Society, Dalton Transactions* 1978; 1127–1130.
35. Mantri Y, Lippard SJ, and Baik M-H, Bifunctional binding of cisplatin to DNA: Why does cisplatin form 1,2-intrastrand cross-links with AG but not with GA? *Journal of the American Chemical Society* 2007; 129: 5023–5030. [PubMed: 17402732]
36. Mansuri-Torshizi H, Ghadimy S, and Akbarzadeh N, Synthesis, characterization, DNA binding and cytotoxic studies of platinum(II) and palladium(II) complexes of the 2,2'-bipyridine and an anion of 1,1-cyclobutanedicarboxylic acid. *Chemical and Pharmaceutical Bulletin* 2001; 49: 1517–1520. [PubMed: 11767068]
37. Li X, Hu Y, Wang H, Yu B, and Yue H, Molecular spectroscopy evidence of berberine binding to DNA: Comparative binding and thermodynamic profile of intercalation. *Biomacromolecules* 2012; 13: 873–880. [PubMed: 22316074]
38. Wehofsky N, Wespe C, Cerovsky V, Pech A, Hoess E, Rudolph R, and Bordusa F, Ionic liquids and proteases: A clean alliance for semisynthesis. *ChemBioChem* 2008; 9: 1493–1499. [PubMed: 18509837]
39. Rioz-Martínez A, and Roelfes G, DNA-based hybrid catalysis. *Current Opinion in Chemical Biology* 2015; 25: 80–87. [PubMed: 25579454]
40. Yang Z, and Russell AJ, Fundamentals of non-aqueous enzymology In: Koskinen AMP and Kilbanov AM *Enzymatic Reactions in Organic Media*, New York: Blackie Academic & Professional, 1996: 43–69.
41. Bornscheuer UT, and Kazlauskas RJ, *Hydrolases in Organic Synthesis* Weinheim: Wiley-VCH, 2006.
42. Luu KN, Phan AT, Kuryavyi V, Lacroix L, and Patel DJ, Structure of the human telomere in K^+ Solution: An intramolecular (3 + 1) G-quadruplex scaffold. *Journal of the American Chemical Society* 2006; 128: 9963–9970. [PubMed: 16866556]

Scheme 1.

G-quadruplex DNA-based asymmetric Michael addition.

Scheme 2.

Intramolecular G-quadruplexes formed by four-repeat human telomeric sequences: (a) basket-type anti-parallel (2+2) form for d[A(GGGTTA)₃GGG] in Na⁺ solution,¹⁹ (b) propeller-type form for d[A(GGGTTA)₃GGG] in a K⁺-containing crystal,¹² (c) (3 + 1) Form 1 for d[TA(GGGTTA)₃GGG] in K⁺ solution,⁴² and (d) (3 + 1) Form 2 for d[TA(GGGTTA)₃GGGTT] in K⁺ solution,³³ (e) Form 3 for d[GGGTTA(BrG)GGTTAGGGTTAGGGT] in K⁺ solution.²⁰ Loops are colored red; *anti* and *syn* guanines are colored cyan and magenta, respectively [Reprinted with permission from Ref²⁰ Copyright © 2009 American Chemical Society].

Scheme 3.

Complexing of Pt(bipym)Cl₂ with DNA oligonucleotide (based on refs^{35, 36}).

Figure 1.
CD spectra of G-quadruplex DNA in 20 mM MOPS (pH 6.5) containing 0 or 50 mM NaCl.

Figure 2.
CD spectra of G-quadruplex DNA in 20 mM MOPS (pH 6.5) containing 50 mM KCl and various co-solvents.

Lane legend

- a.** 80 $\mu\text{g/mL}$ Bio-Rad EZ load 1kb molecular ruler
- b.** MOPS, no sonication
- c.** MOPS, sonicated for 5 min
- d.** MOPS, sonicated for 10 min
- e.** MOPS, sonicated for 15 min
- f.** 0.2 M glycerol
- g.** 0.2 M Choline/glycerol (1:2)
- h.** 0.2 M methanol
- i.** 0.2 M [BMIM]Cl
- j.** 0.2 M [BMIM][BF₄]
- k.** 0.2 M [BMIM][CF₃COO]
- l.** MOPS, no sonication + 40 μM Cu²⁺ ligand
- m.** MOPS, sonicated for 5 min + 40 μM Cu²⁺ ligand
- n.** MOPS, sonicated for 10 min + 40 μM Cu²⁺ ligand
- o.** MOPS, sonicated for 15 min + 40 μM Cu²⁺ ligand
- p.** 0.2 M glycerol + 40 μM Cu²⁺ ligand
- q.** 0.2 M Choline/glycerol (1:2) + 40 μM Cu²⁺ ligand
- r.** 0.2 M methanol + 40 μM Cu²⁺ ligand
- s.** 0.2 M [BMIM]Cl + 40 μM Cu²⁺ ligand
- t.** 0.2 M [BMIM][BF₄] + 40 μM Cu²⁺ ligand

Figure 3.

Gel electrophoresis of G4DNA1 (2% Agarose gel containing 10 μL Sybr® Safe DNA gel stain, TAE buffer, 6 μL sample for each lane, 110 V) at different electrophoresis running times: (a) 30 min, (b) 60 min, and (c) 90 min (Cu²⁺ ligand used was [Cu(dmbipy)(NO₃)₂]).

Table 1

G-Quadruplex-based catalytic Michael addition.

Entry	DNA	Ligand	Salt	Other Conditions	HPLC yield (%)	ee (%)	Optical rotation
<i>Duplex DNA</i>							
1	Salmon testes DNA (0.7 mg/mL)	158 μ M [Cu(dmbipy)(NO ₃) ₂]	No salt, 0.4 M glycerol	DNA sonicated for 5 min	96	>99	—
<i>Different quadruplex DNA and different salts</i>							
2	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	No salt	No sonication	29	19	—
3	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	No salt	DNA sonicated for 10 min	25	21	—
4	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	95	50	—
5	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	100 mM KCl	DNA sonicated for 10 min	64	23	—
6	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM NaCl	DNA sonicated for 10 min	40	3	—
7	G4DNA2	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	73	7	—
8	G4DNA2	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM NaCl	DNA sonicated for 10 min	17	14	—
9	G4DNA3	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	78	10	—
10	G4DNA3	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM NaCl	DNA sonicated for 10 min	17	33	—
<i>Pre-sonication time</i>							
11	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 0 min	90	28	—
12	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 5 min	89	44	—
13	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM NaCl	DNA sonicated for 5 min	33	22	—
14	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	95	50	—
15	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 15 min	83	25	—
<i>Different Ligands</i>							
16	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	95	50	—
17	G4DNA1	40 μ M [Cu(bipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	58	19	—
18	G4DNA1	40 μ M [Cu(phen)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	81	11	—
19	G4DNA1	40 μ M [Pt(bipy)Cl ₂ +Cu(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	12	9	—
20	G4DNA1	40 μ M [Cu(bipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	11	11	—
21	G4DNA1	40 μ M [Cu(bipy)(NO ₃) ₂]	100 mM KCl	DNA sonicated for 10 min	74	10	—
22	G4DNA1	40 μ M [Cu(berberine)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	7	53	—
23	G4DNA1	40 μ M Cu(NO ₃) ₂	50 mM KCl	DNA sonicated for 5 min	31	4	+

Entry	DNA	Ligand	Salt	Other Conditions	HPLC yield (%)	ee (%)	Optical rotation
24	G4DNA1	40 μ M Cu(NO ₃) ₂	50 mM KCl	DNA sonicated for 10 min	40	6	+
<i>Ligand concentration</i>							
25	G4DNA1	25 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 0 min	66	24	–
26	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 0 min	90	28	–
27	G4DNA1	68 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 0 min	98	22	–
28	G4DNA1	90 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 0 min	94	22	–
29	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	95	50	–
30	G4DNA1	55 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	DNA sonicated for 10 min	94	25	–
<i>Co-solvents/additives</i>							
31	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	MOPS, no co-solvent	95	50	–
32	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	0.2 M methanol	87	25	–
33	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	0.5 M methanol	58	37	–
34	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	0.2 M glycerol	53	36	–
35	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	0.4 M glycerol	55	28	–
36	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	0.2 M [BMIM]Cl	26	22	–
37	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	0.2 M [BMIM][CF ₃ COO]	40	18	–
38	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	0.2 M [BMIM][BF ₄]	26	30	–
39	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	0.2 M choline/glycerol (1:2)	18	15	–
<i>Different substrates</i>							
40	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	Substrate 1a	95	50	–
41	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	Substrate 1b	47	27	–
42	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	Substrate 1c	58	29	–
43	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	Substrate 1d	94	27	–
44	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	Substrate 1e	95	23	–
45	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	Substrate 1f	80	11	+
46	G4DNA1	40 μ M [Cu(dmbipy)(NO ₃) ₂]	50 mM KCl	Substrate 1g	16	3	+

Note: reaction conditions as the following (unless noted otherwise): 500 μ L of 100 μ M oligonucleotide (G4DNA) in 20 mM MOPS (pH 6.5) containing 50 mM KCl (or NaCl) sonicated in ice bath for 10 min, [Cu(dmbipy)(NO₃)₂] (final concentration 40 μ M), co-solvent, additional MOPS (20 mM MOPS pH 6.5 with 50 mM KCl or NaCl) to make the total volume of ~1.0 mL. 1 μ mol of substrate **1a** in 3 μ L acetonitrile/2-propanol (3/2, v/v), 100 μ mol dimethylmalonate, stirred at 5 °C for 72 h.

Table 2Binding constants (K_b) of G-quadruplex DNA with Cu^{2+} ligand

Entry	Salt	Pre-sonication (min)	Co-solvent	$K_b/10^5 \text{ (M}^{-1}\text{)}$
1	50 mM KCl	0	—	5.67
2	50 mM KCl	5	—	5.99
3	50 mM KCl	10	—	8.26
4	50 mM KCl	15	—	6.38
5	100 mM KCl	10	—	49.2
6	50 mM NaCl	10	—	1.56
7	50 mM KCl	10	0.2 M methanol	11.2
8	50 mM KCl	10	0.2 M glycerol	6.41
9	50 mM KCl	10	0.2 M Choline/glycerol (1:2)	43.0
10	50 mM KCl	10	0.2 M [BMIM]Cl	34.7
11	50 mM KCl	10	0.2 M [BMIM][BF ₄]	31.4

Note: binding between G4DNA1 and $[\text{Cu}(\text{dmbipy})(\text{NO}_3)_2]$ was studied in 20 mM MOPS (pH 6.5).