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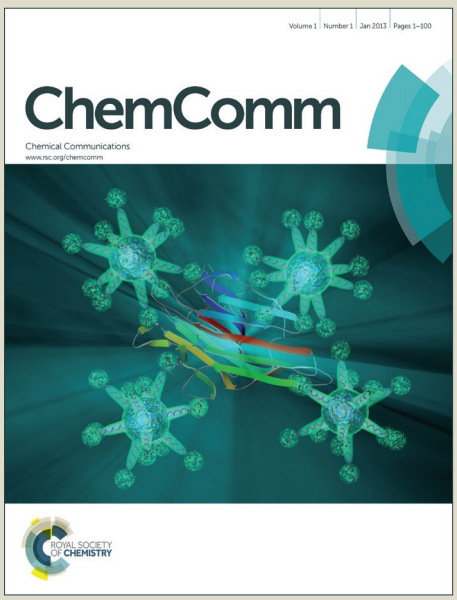

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Enantioselective sulfoxidation reaction catalyzed by G-quadruplex DNA metalloenzyme

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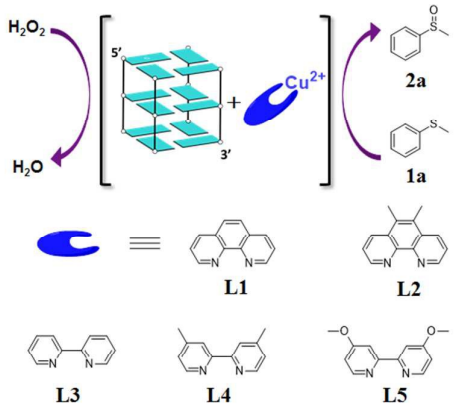
Enantioselective sulfoxidation reaction is achieved for the first time by DNA metalloenzyme assembled with human telomeric G-quadruplex DNA and Cu(II)-4,4′-bimethyl-2,2′-bipyridine complex, and the mixed G-quadruplex architectures are responsible for the catalytic enantioselectivity and activity.

The potential catalysis function of DNA has been explored in the past decade. Two distinct strategies are developed for constructing DNA catalysts --- *i*) *in vitro* selection from synthetic DNA libraries[1,2] and *ii*) anchoring transition metal complexes as cofactors to naturally occurring double stranded (ds) DNAs.[3,4] The former focuses on the transformation of nucleic acids and peptides, meanwhile asymmetric catalysis of small molecules is frequently applied in exploring the catalytic function of the latter.

DNA is usually a double helix *in vivo*, but single stranded guanine-rich DNAs can fold into alternative structural forms known as G-quadruplex.[5,6] These four helix DNA architectures possess advantages of abundant and plastic conformations.[7] Therefore, G-quadruplex DNAs have been introduced as chiral scaffolds for asymmetric Diels-Alder reaction with Cu(II)-bipyridine,[8,9] terpyridine[10] and porphyrin[11] complexes as cofactors. Another new type of ligand-free DNA metalloenzyme constructed by directly coordinating metal ion, such as Cu(II), with G-quadruplex DNA has been found to be active for asymmetric C-C bond formation reactions.[12-14] However, it is not clear whether the G-quadruplex DNA based chiral catalysis function can be extended to the other type reactions.

Biological oxidation reaction is one of the most important processes in nature. Most of these reactions are performed by highly evolved assemblies of proteins and cofactors, such as metalloporphyrin-containing enzymes.[15] Early tempts to catalyze enantioselective oxygen transfer reaction by G-quadruplex•heme metalloenzymes resulted in racemic products.[16-18] The reason was that the big planar aromatic porphyrin ligand was stacked to outer G-tetrad of G-quadruplex, namely, the active sites were not surrounded by G-quadruplex scaffold.[19]



Scheme 1. Enantioselective oxidation of thioanisole (**1a**) catalyzed by G-quadruplex DNA•Cu(II) complex (**L1**-**5**).

The copper ion is frequently involved in fundamental redox process,[20] and its coordinating ligands such as bipyridines and phenanthrolines[21] can interact with dsDNA *via* groove binding and intercalation, respectively. We anticipate that these small sized Cu(II)-bidentate ligand complexes (Scheme **1**, Cu**L1**-**5**) would be the appropriate cofactors to the G-quadruplex DNA for asymmetric oxidation reactions. Herein we report the enantioselective sulfoxidation reaction catalyzed by human telomeric G-quadruplex DNA and Cu(II) complexes with H_2O_2 as the oxidant and up to 77% *ee* was achieved. To the best of our knowledge, this is the first report on DNA based enantioselective sulfoxidation reaction.

A natural 21mer human telomeric sequence (5′-$(G_3T_2A)_3G_3$-3′, HT21) was utilized as the chiral scaffold, and thioanisole (**1a**) was chosen as the model substrate. Firstly, five achiral

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Table 1. Enantioselective oxidation of thioanisole (**1a**).

Entry [a]	Catalyst	Conv. (%) [b]	Ee. (%) [c]
1	none	3	0
2	HT21	3	0
3	HT21-Cu**L1**	15	<5
4	HT21-Cu**L2**	7	<5
5	HT21-Cu**L3**	26	<5
6	Cu**L4**	95	0
7	HT21-Cu**L4**	99	56
8	HT21-Cu**L5**	83	39
9 [d]	HT21-Cu**L4**	9	<5
10 [e]	HT21-Cu**L4**	63	<5
11 [f]	cgmHT21-Cu**L4**	51	<5
12 [g]	rHT21-Cu**L4**	70	0

[a] Reaction conditions: **1a** (5 mM), H_2O_2 (7.5 mM, 1.5 eq.), HT21 (10 μM, 0.2 mol%), Cu**L4** (50 μM, 1 mol%), KCl (150 mM), MOPS buffer (1 mL, 20 mM, pH 7.0), 15 °C, 5 h. All data are repeated at least two separated experiments. [b] Determined for the crude product by chiral-phase HPLC within reproducibility of ±5%. The overoxidized product sulfone is untraceable. [c] Determined by chiral-phase HPLC within reproducibility of ±5%.[d] NaCl (150 mM) is added instead of KCl. [e] KCl is removed and no metal ion is added. [f] Central guanine mutation HT21 (cgmHT21, 5′-(GGG TTA)$_2$ G**T**G TTA GGG-3′) is added instead of HT21. [g] RNA G-quadruplex (rHT21. 5′-(ggg uua)$_3$ ggg-3′) is added instead of DNA one.

bidentate ligands, which can be categorized into phenanthroline type (1,10-phenanthroline, **L1**; 5,6-dimethyl-1,10-phenantiroline, **L2**) and bipyridine type (2,2′-bipyridine, **L3**; 4,4′-bimethyl-2,2′-bipyridine, **L4**; 4,4′-bimethoxy-2,2′-bipyridine, **L5**) were investigated and the results were depicted in Table **1**. Cu**L1** and Cu**L2** complexes combined with HT21 catalyze the reaction with extremely low conversions (15% and 7%, respectively) and nearly zero enantioselectivities. In contrast, the bipyridine type Cu**L3**-**5** complexes combined with HT21 lead to higher conversions, and more importantly, the products exhibit chiral selectivity. Notably, Cu**L4** complex with methyl group at 4,4′-position of bipyridine gives the product with the full conversion and 56% *ee*. The methyl group of Cu**L4** replaced by H or CH_3O forming Cu**L3** and Cu**L5** complexes give the product with lower enantioselectivities (22% and 39% *ee*, respectively) and conversions.

HT21 sequence adopts mixed conformations (including antiparallel, parallel and hybrid forms) in K^+ solution and a three layered antiparallel conformation in the presence of Na^+ (Fig. S2).[22-25] Hence control experiments were carried out in MOPS buffer containing K^+, Na^+ or no ion. HT21 alone shows no catalytic activity in the sulfoxidation reaction (Table 1, Entries 1, 2). In the presence of Cu**L4** alone, **1a** is almost completely converted and racemic phenyl methyl sulfoxides (**2a**) are produced (Table 1, Entry 6). Interestingly, K^+-stabilized HT21 assembled with Cu**L4** gives full conversion and 56% *ee* (Table 1, Entry 7). When K^+ is replaced by Na^+ or removed, conversions are decreased dramatically and racemic products are obtained (Table 1, Entries 9, 10). These results reveal that the K^+ plays a vital role in inducing the G-quadruplex architecture for chiral control.

To test whether the quadruplex helix backbone is indispensable, a single base mutation experiment was performed (Table 1, Entry 11). A central guanine mutation sequence (cgmHT21) was added instead of HT21. The only difference between cgmHT21 and HT21 is that a single central guanine (G14) base from the third G-tract is replaced by thymine (T14). This mutation leads to the collapse of G-quadruplex backbone evidenced by CD spectra (Fig. S3), which is in accordance with previous result.[26] Along with this unwinding of the ordered G-quadruplex to disordered state, the conversion is restored to about 50% of the wild-type level. More importantly, the enantioselectivity of sulfoxides drops to near-zero (Table 1, Entry 11). These results emphasize that maintaining the integrity of quadruplex helix backbone is essential to chiral control in sulfoxidation reaction.

G-quadruplex RNA based catalysts were capable of catalyzing the oxidation of thioanisole[17] and the G-quadruplex RNA•Cu**L4** complex was also investigated here. The RNA used in this work shares the sequence of HT21 and is named as rHT21. CD spectra reveal that rHT21 folds into a characteristic parallel conformation (positive peak at 265 nm and negative peak at 240 nm, Fig. S3) in K^+ solution.[27] Though thioanisole can be converted by RNA based catalyst, no preference for generating any enantiomer of sulfoxides is observed (Table 1, Entry 12).

Then, reaction conditions including (a) K^+ concentration, (b) molar ratio between Cu**L4** and HT21, (c) reaction temperature and (d) buffer pH were optimized (Fig. S4). CD titration spectra show that the conformation of HT21 transforms from a random coil into an antiparallel type with the addition of K^+, further, into a stable mixed type[23] by increasing the K^+ concentration (Fig. S5). Correspondingly, both conversions and enantioselectivities are increasing continuously and going up to the highest values in the presence of 150 mM K^+ (Fig. S4a). The optimal molar ratio between HT21 and Cu**L4** is five (Fig. S4b), albeit the binding stoichiometry is unknown (*vide infra*). The HT21•Cu**L4** catalyst

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shows the best results at 15 °C and pH of 7.0 (Figs. S4c and S4d). The following experiments were conducted under the optimal conditions ([K^+] = 150 mM, [Cu**L4**]/[HT21] = 5, Temp. = 15 °C, pH = 7.0).

Small molecular ligands usually bind to G-quadruplex architectures at terminal G-tetrads or grooves which can be distinguished by biochemical characterization methods.[28-33] Very recently, the Cu**L4** combined with dsDNA *via* groove binding was identified.[21] So far there is no identification of the binding mode between G-quadruplex DNA and Cu(II)-bipyridine. Compared to the CD profiles of HT21 alone, tiny peak intensity changes between 250-275 nm and no ICD signal of Cu**L4** are induced beyond 310 nm after the addition of Cu**L4** (Fig. S2a). Moreover, by adding HT21 into Cu**L4** solution, no wavelength shifts, and even no absorbance changes at 307 nm are observed in UV/vis absorption spectra (Fig. S6). What's more, the melting temperatures of HT21 in the absence or presence of Cu**L4** are almost identical (Fig. S7). Taking the above spectral characterization together, the binding affinity between Cu**L4** and HT21 is too weak to identify their interaction mode.

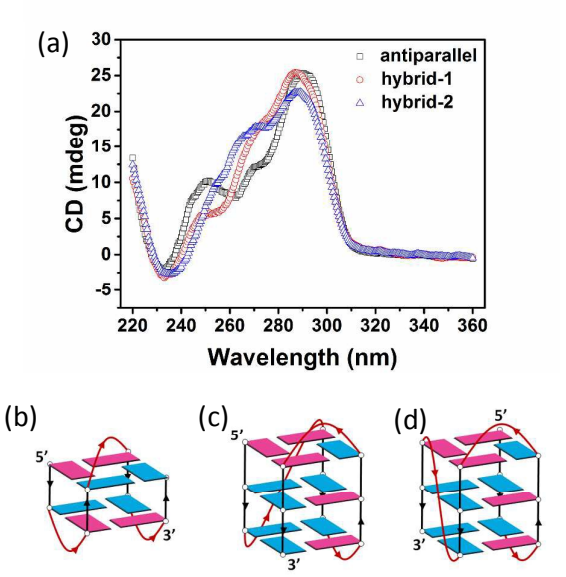

Fig. 1 (a) CD spectra of three determined human telomeric G-quadruplexes in K^+ (150 mM) solution. (b-d) The effect of different G-quadruplex conformations on enantioselective oxidation of thioanisole in K^+ (150 mM) conditions: (b) antiparallel[39] (HT21+T, PDB ID: 2KF8); (c) hybrid-1[35] (TA+HT21, PDB ID: 2JSM); (d) hybrid-2[37] (TA+HT21+TT, PDB ID: 2JSL).

Table 2 Dependence of conversion and enantioselectivity on DNA sequences.

Entry [a]	Name	Sequence (5′→3′)	Conv. (%)	Ee. (%)
Addition of flanking deoxynucleosides				
1	A+HT21	$AG_3(TTAG_3)_3$	74	14
2 [b]	TA+HT21	$TAG_3(TTAG_3)_3$	90	26
3	TTA+HT21	$TTAG_3(TTAG_3)_3$	85	21
4 [c]	HT21+T	$G_3(TTAG_3)_3T$	93	35
5	HT21+TT	$G_3(TTAG_3)_3TT$	96	26
6	HT21+TTA	$G_3(TTAG_3)_3TTA$	94	18
7 [d]	TA+HT21+TT	$TAG_3(TTAG_3)_3TT$	59	19
Modification of loop bases				
8	HT21-TTT	$G_3(TTTG_3)_3$	88	39
9	HT21-AAA	$G_3(AAAG_3)_3$	27	23
10	HT21-TAT	$G_3(TATG_3)_3$	44	10
11	HT21-ATT	$G_3(ATTG_3)_3$	61	29
12	HT21-TAA	$G_3(TAAG_3)_3$	34	37
13	HT21-ATA	$G_3(ATAG_3)_3$	50	15
14	HT21-AAT	$G_3(AATG_3)_3$	88	15

[a] Reaction conditions: 1a (5 mM), H_2O_2 (7.5 mM, 1.5 eq.), HT21 (10 μM, 0.2 mol%), Cu**L4** (50 μM, 1 mol%), KCl (150 mM), MOPS buffer (1 mL, 20 mM, pH 7.0), 15 °C, 5 h. All data are repeated at least two individual experiments. [b] TA+HT21, [c] HT21+T and [d] TA+HT21+TT adopt hybrid-1, antiparallel and hybrid-2 conformation in K^+ solution, respectively (see Fig. 1).

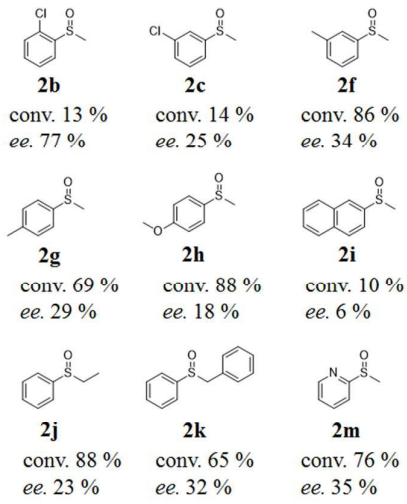

Fig. 2 Enantioselective sulfoxidation catalyzed with HT21•CuL4. All data are repeated at least two separated experiments.

Given the above results that the Cu**L4** is not able to disturb the structure of HT21 G-quadruplex, the G-quadruplex architectures induced by K^+ can represent the main structure of HT21•Cu**L4** catalyst. It is well known that human telomeric sequences are extremely polymorphic, especially for the truncated HT21 sequence, in K^+ solution.[34] Phan's and Yang's groups independently found that appropriate choices of extending the flanking nucleotides at HT21 G-tetrad core can overcome the conformational heterogeneity.[35-38] The effect of the G-quadruplex DNA sequence on the catalytic performance of the complex was investigated by addition of flanking deoxynucleosides. Both addition of 5′ and 3′ terminus flanking sequences decrease the enantioselectivities obviously, but 5′ addition has a lower conversion than 3′ (Table 2, Entries 1-6). Among these sequences, the HT21+T and TA+HT21 sequences can fold into a single basket-antiparallel-type G-quadruplex with two G-tetrad layers and hybrid-1 quadruplex in the K^+ solution, based on the CD spectra and literatures (Fig. 1a-c).[35,39] In addition, the TA+HT21+TT sequence adopts a hybdrid-2 conformation (Fig. 1d).[37] Reaction outcomes show that the distinct antiparallel quadruplex affords higher enantio-selectivity (35% *ee*) and conversion than the two hybrid conformations, and the hybrid-2 one shows the lowest catalytic activity (59% conv.) (Table 2, Entries 2, 4 and 7).

Finally, the loops modification of base types or sequence orders caused the notable decrease of both conversions and

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chiral selectivities (Table 2, Entries 8-14). These observations suggest that the enantioselective catalysis may occur both in the terminal G-tetrad and loop region.

To popularize the G-quadruplex DNA based enantioselective sulfoxidation, various prochiral sulfides including aryl alkyl sulfides, aryl benzyl sulfide and pyridine alkyl sulfide were converted to the corresponding sulfoxides by HT21•Cu**L4** catalyst (Fig. 2). It's interesting to note that the enantioselectivity is up to the highest of 77% *ee* with an electron-withdrawing chloro substituent at 2'-position of benzene ring.

Conclusions

In this communication, we present DNA based enantioselective sulfoxidation reaction and up to 77% *ee* was obtained, which is the highest enantioselectivity for DNA based oxidation reaction to date. The mixed G-quadruplex architectures of human telomeric sequence induced and stabilized by K^+ are responsible for the catalytic enantioselectivity and activity. The terminus and loops of G-quadruplex are rather strongly conserved for enantioselective induction. Furthermore, the antiparallel G-quadruplex affords higher enantioselectivity than the two hybrid forms in sulfoxidation reaction. This study expands the asymmetric catalytic repertoire of DNAzymes to oxidative process.

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