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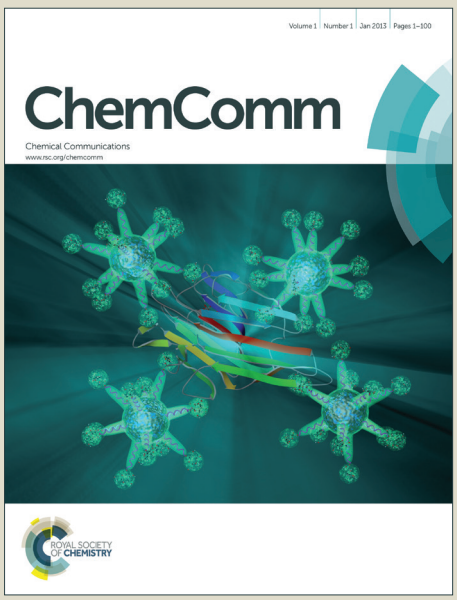

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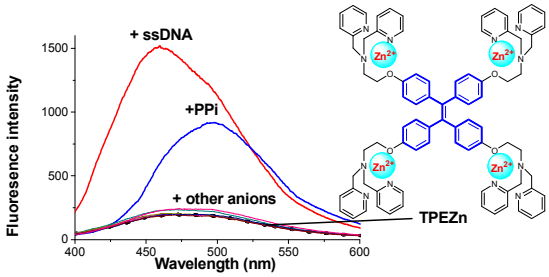
Table of contents



A new DNA probe based on coordination interaction exhibited higher sensitivity than the corresponding probe based on electrostatic interaction.

Tetraphenylethene-based zinc complex as sensitive DNA probe by coordination interaction

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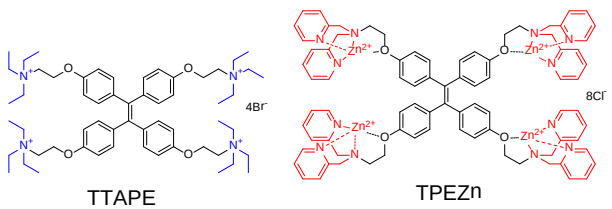
We developed a new DNA probe by utilizing the coordination interaction of Zn^{2+} with DNA and consequent emission. Because the coordination interactions do not depend on the length of DNA, the new probe exhibited much higher sensitivity in detecting short ssDNA than the corresponding probe based on electrostatic interactions.

It is of important significance to develop rapid, sensitive and cost-effective methods for the detection of nucleic acid in the fundamental biological/biomedical research.[1] Fluorescence as a ultrasensitive, relatively rapid and easy operation approach has attracted great attention in nucleic acid detection. These merits open up opportunities for developing promising reagents in diagnosing genetic diseases and monitoring biological processes in cells.[2] Most classic fluorescent DNA dyes (e.g., ethidium bromide,[3] TOTO,[4] Sybr Green,[5] $[Ru(phen)_2dppz]^{2+}$,[6] DAPI and Hoechst dyes[7]) mainly bind DNA with secondary structures by intercalation or groove binding, and thus many of them do not show significant fluorescence response for ssDNA without secondary structures.[3-7]

To sense ssDNA effectively and conveniently, some new fluorescent dyes based on tetraphenylethene (TPE) have been developed by Tang et al.[8] The sensitivity of these sensors results from the fact that, tetraphenylethene (TPE)-based dyes are barely fluorescent in their separated monomer forms, while highly emissive upon aggregation. This aggregation-induced emission (AIE) is a result of non-radiative pathway blocked by restriction of intramolecular rotations in the aggregated state.[9] By utilizing the AIE and electrostatic interaction simultaneously, Tang et al. felicitously designed the DNA probes of TTAPE[8b] etc. (Scheme 1), in which the electrostatic interaction between tetraalkylammonium cations and anionic moieties of the DNA chains leads to dye aggregation and the consequent AIE.

The ssDNA probes by way of electrostatic interactions have been also used in other fluorescent molecules such as pyrene,[2b] polythiophene, polyfluorene etc.[10] However, the practical applications of these ionic probes are limited, because electrostatic interactions are easily affected by other electrolytes. Moreover, this type of non-covalent interaction is easily suppressed in aqueous medium.[11,12] It would be preferable to incorporate another stronger binding force for DNA than electrostatic interaction. This may not only decrease the interference of other electrolytes, but also enhance the sensitivity. Metal-ligand coordination interaction have been widely used in anions recognition and sensing.[11-13] Among many anions receptors based on coordination interaction, Zn^{2+}-DPA (dipicolylamine) units are capable of binding a variety of analytes containing anionic phosphate groups, such as phosphate, pyrophosphate, ATP and phosphorylated peptides, etc.[12,13] As DNA is a phosphodiester polymer, Zn^{2+}-DPA functionalized PNA[14] and naphthalene diimide[15] show improved affinity for DNA due to the coordination interaction between Zn^{2+} and phosphorus-oxygen bond of DNA. Besides phosphodiester backbone, DNA bases may bind Zn^{2+} complexes.[16] This binding mode is intrinsically different with intercalation or groove binding, and provides anther way to sense ssDNA.

In this communication, by taking advantage of the coordination interaction of Zn^{2+} and the emission of restricted TPE, we designed and synthesized a new TPE derivative, named TPEZn, by using Zn^{2+}-DPA units to replace the tetraalkylammonium moieties of TTAPE (Scheme 1). The synthetic route of TPEZn is shown in Scheme S1. The DPA functionalized ligand **2** was synthesized by substitution of 1,1,2,2-tetrakis(4-(2-bromoethoxy)phenyl)ethane (**1**) with 2,2'-dipicolylamine. The reaction of ligand **2** with $ZnCl_2$ solutions afforded the complex of TPEZn as a white precipitate (see Electronic Supplementary Information).



Scheme 1 Structure of TPE probes based on electrostatic interaction (TTAPE) and coordination interaction (TPEZn).

The fluorescence spectra of TPEZn upon the addition of DNA were tested in H_2O/DMSO (99:1, v/v) solutions. The sequences of synthetic DNA used in this study are shown in Table S1. The fluorescence spectra of titration experiments with a 10 nt ssDNA (X10) are displayed in Fig. 1. TPEZn showed significant fluorescence enhancement upon the addition of X10. Because the excess Zn^{2+} was added to the solution, the free ligand **2** could not exist in solution. Therefore the fluorescence enhancement could not result from the emission of the ligand **2** (Fig.S1), instead it

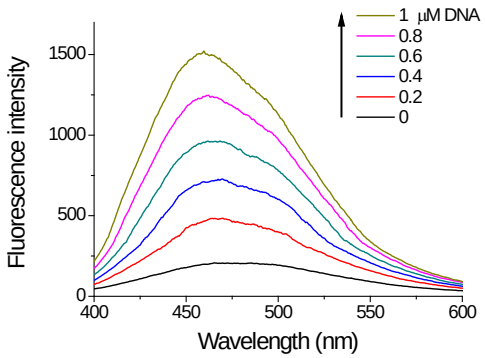


Fig. 1 Fluorescence spectra of 5 μM TPEZn upon the addition of DNA in H_2O/DMSO (99:1, v/v) solutions. [$Zn(NO_3)_2$] = 0.1 mM. λ_{ex} = 330 nm. ssDNA was X10 (DNA sequences are shown in Table S1).

should attribute to the restricted intramolecular rotation of TPE upon Zn^{2+}-DPA units binding to DNA through coordination interaction. Y10 (the complementary DNA of X10) and the hybridized dsDNA of X10 with Y10 were also tested under the identical conditions. TPEZn showed significant fluorescence enhancement and the similar emission maximum at about 465 nm to these DNAs (Fig. S2 and S3). To figure out the deference between ssDNA and dsDNA, the fluorescence intensity *vs.* concentration of total phosphodiester units ([P]) was shown in Fig. 2. The addition of X10 and Y10 caused comparable fluorescence enhancement, while their hybridized dsDNA exhibited much weaker fluorescence enhancement at the same concentration of phosphodiester units. This suggests that Zn^{2+}-DPA units many not only coordinate to the phosphodiester backbone but also the bases of DNA. As Watson-Crick hydrogen bonds formed between base pairs in the hybridized dsDNA, the available coordination sites to Zn^{2+} decreased, which leaded to weaker binding with TPEZn, and consequently lower fluorescence enhancement.

For comparison, the fluorescence response of the reported TTAPE with the tetraalkylammonium cations to these DNAs was also tested under the identical conditions (Fig. 2). As expected, TTAPE showed much weaker fluorescence enhancement upon

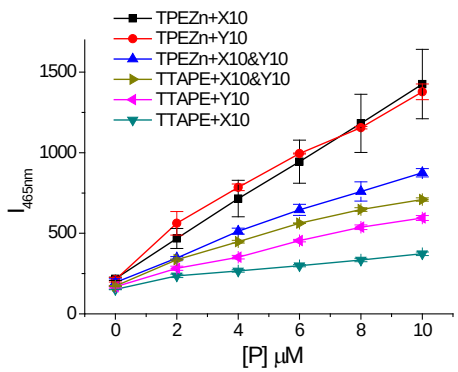


Fig. 2 Fluorescence intensity of 5 μM TPEZn or TTAPE vs. concentration of phosphodiester units in H_2O/DMSO (99:1, v/v) solutions. [P] = concentration of total phosphodiester units. [$Zn(NO_3)_2$] = 0.1 mM. λ_{ex} = 330 nm. λ_{em} = 465 nm. X10&Y10 was hybridized dsDNA of X10 and Y10 (DNA sequences are shown in Table S1).

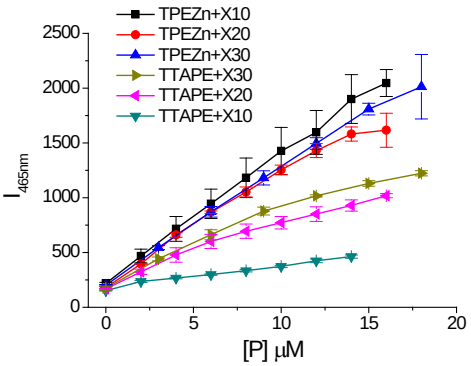


Fig. 3 Fluorescence intensity of 5 μM TPEZn or TTAPE vs. concentration of phosphodiester units in H_2O/DMSO (99:1, v/v) solutions. [P] = concentration of total phosphodiester units. [$Zn(NO_3)_2$] = 0.1 mM. λ_{ex} = 330 nm. λ_{em} = 465 nm. X10, X20 and X30 are ssDNA with length of 10, 20 and 30 nt respectively (DNA sequences are shown in Table S1).

the addition of all the DNAs, which proved that the coordination interaction is more effective than electrostatic interaction to improve the sensitivity.

The effect of DNA chain length on fluorescence change was also investigated by testing oligonucleotides with different length. Oligonucleotides (X10, X20 and X30) with repeated sequence of X10 from 10 nt to 30 nt were used for excluding the effect of sequence diversity. The fluorescence intensity of TPEZn and TTAPE *vs.* concentration of phosphodiester units ([P]) was shown in Fig. 3. As the length of oligonucleotides decrease from 30 nt to 10 nt, the fluorescence enhancement of TTAPE was significantly decreased, while that of TPEZn was less affected. Especially for X10 as short as 10 nt, the limited negative charge could not induce effective electrostatic interactions with tetraalkylammonium cations of TTAPE. In contrast, TPEZn based on coordination interactions exhibited significant fluorescence response to X10. This indicates that the fluorescence response of TPEZn do not depend on the length of DNA.

The selectivity of TPEZn was evaluated by testing the response to several usual anions, including SO_3^{2-}, SO_4^{2-}, AcO^-, HCO_3^-, oxalate, $H_2PO_4^-$ and PPi (pyrophosphate). No significant fluorescence change was obtained upon the addition of these anions except for PPi (Fig. 4). The titration experiments of

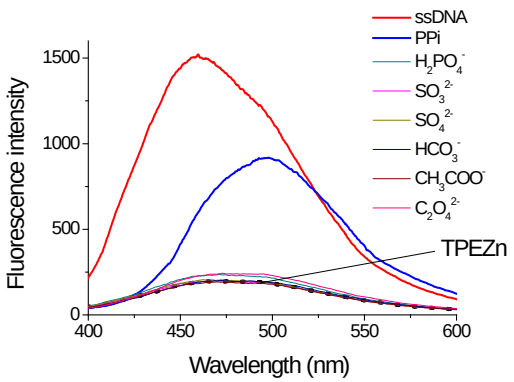


Fig. 4 Fluorescence spectra of 5 μM TPEZn upon the addition of different anions in H_2O/DMSO (99:1, v/v) solutions. [$Zn(NO_3)_2$] = 0.1 mM. λ_{ex} = 330 nm. ssDNA was 1 μM X10. [PPi] = 10 μM. Other anions are 30 μM respectively.

TPEZn with PPi showed that TPEZn could bind PPi to form 1:1 and 1:2 complexes with emission maximum at about 480 nm 490 nm, respectively (Fig. S4 and S5). The 1:1 complex exhibited about one fold higher fluorescence intensity than that of the 1:2 complex. This emission was quite different with the emission of TPEZn-DNA complexes, which indicated that TPEZn may bind various phosphate derivatives with different binding mode. The detailed mechanism is under investigation.

In conclusion, we developed a new kind of TPE-based DNA probe by utilizing coordination interaction of Zn^{2+} with DNA. TPEZn showed much higher sensitivity in detecting DNA than the corresponding probe based on electrostatic interaction. We believe that utilizing the coordination interaction provide a general principle for designing probes in the recognition of a broad range of biological molecules with improved sensitivity and selectivity.

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Notes and references

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† Electronic Supplementary Information (ESI) available: The syntheses and characterization of TPEZn and TTAPE, absorption spectra and fluorescence titration spectra and other experimental details. See DOI: 10.1039/b000000x/

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