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Sensors

Label-free Electrochemiluminescent Enantioselective Sensor for Distinguishing between Chiral Metallosupramolecular Complexes

Li Wu,[a, b] Chuanqi Zhao,[a] Jinsong Ren,[a] and Xiaogang Qu*[a]

Abstract: Chiral molecular recognition of DNA is important for rational drug design and for developing structural probes of DNA conformation. Developing a convenient and inexpensive assay for sensitive and selective identification of DNA-specific binding compounds with rapid, easy manipulation is in ever-increasing demand. Here, we present a "turn-on" and label-free electrochemiluminescent (ECL) biosensor for distinguishing chiral metallosupramolecular complexes based on DNA three-way junction formation selectively induced by the analyte. The fabricated ECL sensor shows excellent performance in the chiral discrimination of two enantiomers with an enantioselective recognition ratio of up to 4.4. More importantly, as a "turn-on" detection system, the ECL chiral sensor does not suffer from false positives and limited signal range of "signal-off" systems. Therefore, this concept may provide a new insight into the design of efficient sensors for distinguishing chiral molecules and for investigating the interactions between DNA and small molecules.

DNA structure is dynamic and exists in a variety of distinct conformations, which play important functional roles in gene expression.[1] Recently, there has been increased attention focused on small molecules binding to specific DNA structures to inhibit the biological functions in which these particular structures participate. Among these studies, chiral molecular recognition of DNA is important for rational drug design and for developing structural probes of DNA conformation.[2] Over the past few decades, considerable effort has been devoted to designing DNA-binding chiral agents for conversions of the chirality and diverse conformations of DNA, such as the DNA B-Z transition, G-quadruplex, i-motif, and also the DNA junction structure.[3]

[a] L. Wu, C. Zhao, Prof. J. Ren, Prof. X. Qu
Laboratory of Chemical Biology
Division of Biological Inorganic Chemistry
State Key Laboratory of Rare Earth Resource Utilization
Changchun Institute of Applied Chemistry
Chinese Academy of Science
Changchun, Jilin 130022 (R.P. China)
E-mail: xqu@ciac.ac.cn

[b] L. Wu
University of the Chinese Academy of Science
Beijing, 100039 (R.P. China)

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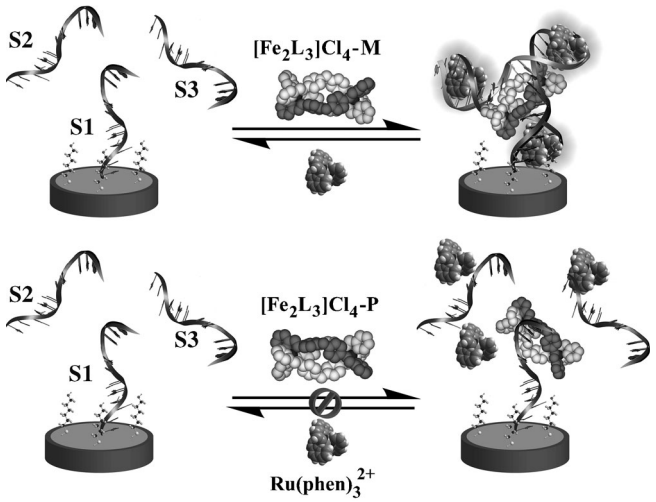
DNA junctions, unique branched structures that consist of several double strands converging at one point, are involved in a series of important biological events. For example, three-way junctions (TWJs) occur both in RNA and DNA and are associated with several human genetic disease.[4] Thus, DNA TWJs have aroused much interest as a mode for DNA recognition and could serve as well-defined potential structural targets for novel, highly specific drugs. Therefore, developing a convenient and inexpensive assay for the sensitive and selective identification of TWJ-specific binding compounds with rapid, easy manipulation is in ever-increasing demand.[5] Hannon and co-workers reported that chiral metallosupramolecular helicate $[Fe_2L_3]Cl_4$ could selectively bind and stabilize DNA three-way junctions.[3e,6] Here, choosing a $[Fe_2L_3]Cl_4$ stabilized TWJ as a model system, we developed a turn-on, label-free ultrasensitive ECL sensor for distinguishing chiral metallosupramolecular complexes.

Electrochemiluminescence, the combination of chemiluminescence and electrochemistry, has become a powerful analytical tool in the detection of biomolecules, clinical diagnostics, environmental and food monitoring, and biowarfare agent detection owing to its simplified set-up, the fact that it is label-free, and has low background signal and high sensitivity.[7] Label-free biosensors have the unique advantage of providing real-time quantitative information on the progress of the biochemical reaction under study and its rate without any risk of undesired effects.[8] Most label-free ECL sensors are based on the principle of formation of a duplex DNA structure during target recognition as many small molecules have been reported to bind double-stranded DNA (ds-DNA) with high affinity.[9] However, there has been no report of the use of label-free ECL biosensors for chiral discrimination and detection of chiral compounds based on target selectively induced DNA TWJ formation.

In this study, a commonly used ECL probe, $[Ru(phen)_3]^{2+}$ (phen = phenanthroline), which can bind to ds-DNA, was employed for the discrimination of chiral metallosupramolecular complexes. The DNA sequences were designed according to the previous report; in the presence of $[Fe_2L_3]Cl_4$ the DNA could form a TWJ structure that had arms six base-pairs (bps) long.[6] As shown in Scheme 1, one of the three DNA strands, S1, was first immobilized onto a gold electrode through a gold–thiol bond. As indicated previously, $[Fe_2L_3]Cl_4$ can induce DNA TWJ structure formation and bind to the junction region. The ECL probe, $[Ru(phen)_3]^{2+}$, then bound to the duplex sections and generated measurable ECL emission. The intensity of the ECL signal from $[Ru(phen)_3]^{2+}$ with tripropyl-



Scheme 1. Schematic illustration of the three-way DNA junction based label-free electrochemiluminescent sensor for distinguishing between chiral metallosupramolecular complexes.

amine (TPrA) as a coreactant is related to the quantity of the target $[Fe_2L_3]Cl_4$ in the testing buffer and serves as the quantitative signal for the target determination. Moreover, based on the fact that one of the enantiomers is several times more efficient in promoting and stabilizing the TWJ than its counterpart, the fabricated ECL sensor shows excellent performance in the chiral discrimination of the two enantiomers. Given the same concentration of the two enantiomers, the enantioselective recognition ratio,[10] defined as $\Delta ECL_M/\Delta ECL_P$, reaches 4.4. Such a large difference in ECL signal change makes the sensor practically useful for enantioselective recognition, and it is comparable to most enantioselective sensors reported previously.[11] As the ECL probe, $[Ru(phen)_3]^{2+}$, can bind tightly to DNA, the background signal is decreased and the result is a highly sensitive detector for the target enantiomer. Furthermore, as a "turn-on" detection system, the ECL chiral sensor does not suffer from false positives and the limited signal range of "signal-off" systems.[11d]

As an effective method to measure electrode surface changes, electrochemical impedance spectroscopy (EIS) is highly suitable for evaluating the interfacial electron transfer efficiency at different stages of biosensor preparation.[12] The EIS curves and corresponding equivalent circuit are shown in Figure 1a. The bare AuE exhibits small impedance (line A), whereas the self-assembly of S1 onto the electrode surface results in a substantial increase in the electrochemical impedance (line B), which indicates the anchoring of thiolated DNA on the electrode surface. The blocking of the AuE surface by 6-mercapto-1-hexanol (MCH) further increased the value of electron-transfer resistance (R_{et}). The presence of $[Fe_2L_3]Cl_4$ enantiomers can induce the formation of a three-way DNA junction by bringing the other two single strands of DNA close to the electrode surface, a process that results in a clear increase in the impedance value owing to the electrostatic repulsion between the net negative charges of the DNA phosphate backbone and the negatively charged ferricyanide (Figure 1a, lines D and F).

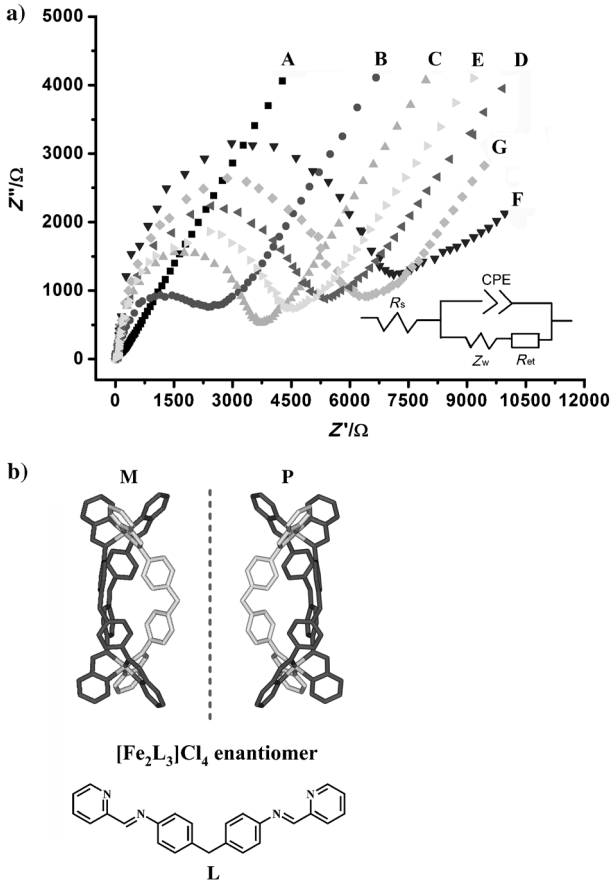


Figure 1. a) Typical Nyquist diagrams obtained for the working gold electrode (AuE) prepared at different stages: bare AuE (A); S1-modified AuE (S1/AuE) (B); the MCH-modified as-prepared S1/AuE (C); TWJ formed on electrode surface stabilized by $[Fe_2L_3]Cl_4$-P (D) or $[Fe_2L_3]Cl_4$-M (F); the intercalation of $[Ru(phen)_3]^{2+}$ in TWJ DNA stabilized by $[Fe_2L_3]Cl_4$-P (E) or $[Fe_2L_3]Cl_4$-M (G). b) Structures of the M- and P-enantiomers of the $[Fe_2L_3]^{4+}$ cation and the bis(pyridylimine) ligand, L.

The supramolecular cylinder $[Fe_2L_3]Cl_4$ is helical and exists as two enantiomers, M and P (Figure 1b). It has been reported that both enantiomers are capable of stabilizing the TWJ structure formed by S1, S2, and S3, and that the M enantiomer is more effective than the P enantiomer for stabilizing the TWJ structure.[6] DNA UV melting studies indicate the formation of TWJ structure (Figure S1, see the Supporting Information). The R_{et} value obtained from one of the enantiomers, $[Fe_2L_3]Cl_4$-M, is much larger than that from $[Fe_2L_3]Cl_4$-P, demonstrating that $[Fe_2L_3]Cl_4$-M indeed exhibits a higher affinity in TWJ formation on the electrode surface. The subsequent binding of positively charged $[Ru(phen)_3]^{2+}$ within the formed duplex section slightly attenuates R_{et} (Figure 1a, lines E and G), as it can help to neutralize partial negative charges on DNA molecules.

Fluorescence experiments were performed to characterize the interaction between $[Ru(phen)_3]^{2+}$ and DNA. Under our experimental conditions, $[Ru(phen)_3]^{2+}$ has fluorescence (Figure S2, see the Supporting Information), whereas the enantiomers are not fluorescent. Moreover, the fluorescence intensity of $[Ru(phen)_3]^{2+}$ is weakened in the presence of both $[Fe_2L_3]Cl_4$-M and $[Fe_2L_3]Cl_4$-P enantiomers to the same degree

(Figure S2, see the Supporting Information). This result is reasonable as a mirror-image enantiomeric pair of molecules share identical chemical properties.[2] The formation of three-way junction DNA in the presence of S1, S2, and S3 further decreases $[Ru(phen)_3]^{2+}$ fluorescence (FL). As the quenching effect of $[Fe_2L_3]Cl_4$-M and $[Fe_2L_3]Cl_4$-P toward $[Ru(phen)_3]^{2+}$ fluorescence is equal, the difference in the change of FL intensity in the presence of DNA (Figure S2, see the Supporting Information) can be attributed to more TWJ structures being formed under the stabilization of the M-enantiomer than that of the P-enantiomer. In addition, a redshift from 576.5 to 580.5 nm was observed for the maximum emission wavelength when $[Ru(phen)_3]^{2+}$ binds to DNA.[13] Figure S3 (see the Supporting Information) shows the fluorescence titration results. As the DNA concentration increased, the fluorescent emission of $[Ru(phen)_3]^{2+}$ decreased until a plateau was reached. Previous studies have shown that every four bps could embed one $[Ru(phen)_3]^{2+}$ molecule;[13–14] thus, one $[Ru(phen)_3]^{2+}$ molecule could bind to three double-stranded arms of the TWJ structure. As shown in Figure S3b, a breakpoint in the titration profile of the fluorescence of $[Ru(phen)_3]^{2+}$ versus the concentration of the TWJ was located at a $[Ru(phen)_3]^{2+}$/DNA ratio of 3:1 which may imply that one TWJ DNA molecule can bind three $[Ru(phen)_3]^{2+}$ molecules. Three ECL probe molecules bound to one target molecule can improve the sensitivity of the ECL chiral sensor.

Figure 2 shows the cyclic voltammograms (CVs) and the corresponding ECL of $[Ru(phen)_3]^{2+}$ on the modified electrode surface cultured with S2 and S3 in the absence or presence of $[Fe_2L_3]Cl_4$ enantiomers in Tris buffer (Tris = tris(hydroxymethyl)-aminomethane); ok 89 mM Tris salt, 89 mM borate, 10 mM NaCl, pH 7.2) containing 20 mM TPrA. A clear oxidation peak can be seen at 1.17 V (Figure 2a), a peak that results from the electrochemical oxidation of $[Ru(phen)_3]^{2+}$ and TPrA. Additionally, an ECL peak can be observed at this potential (Figure 2b). The CVs and ECL spectra show that both of the enantiomers have the ability to induce the formation of TWJ structures and the M-enantiomer is more effective than the P-enantiomer.

To illustrate the sensitivity of the proposed assay, we have investigated the changes between ECL and ECL_0 ($\Delta ECL = ECL - ECL_0$) by incubating the as-modified electrode with a series of different concentrations of $[Fe_2L_3]Cl_4$-M in the presence of an excess amount of single-strand DNA. In the absence of target $[Fe_2L_3]Cl_4$-M, the three-way junction formed by S1, S2, and S3 is unstable under the experimental conditions and can be easily removed from the electrode surface by rising with the buffered solution. The weak interaction between the ECL probe, $[Ru(phen)_3]^{2+}$, and the single-stranded capture DNA, S1, causes a considerably low ECL background signal, defined as ECL_0. The ECL count increased with increasing concentrations of $[Fe_2L_3]Cl_4$-M, as shown in Figure 3a. The relationship between responses of the ECL chiral sensor and different concentrations of chiral metallosupramolecular complexes ($[Fe_2L_3]^{4+}$-M/P) is shown in Figure 3b. The value of ΔECL for $[Fe_2L_3]Cl_4$-P is much smaller than that for $[Fe_2L_3]Cl_4$-M at same concentration. The linear range for $[Fe_2L_3]Cl_4$ is from 0.1 to 0.6 μM (Figure S4, see the Supporting Information) with the detection limit calculated to be 17.9 nM for $[Fe_2L_3]Cl_4$-M and 95.7 nM for $[Fe_2L_3]Cl_4$-P at 3σ. The above results demonstrate that the constructed ECL sensor shows higher chiroselectivity for one of the chiral metallosupramolecular enantiomers, $[Fe_2L_3]Cl_4$-M. The enantioselectivity ratio ($\Delta ECL_M/\Delta ECL_P$) for the ECL change is determined to be 4.4 for the fabricated ECL sensor at 0.6 μM analyte. The long-term stability of the proposed label-free ECL assay was also investigated by storing the fabricated electrode at a constant temperature of 4 °C in the refrigerator during a two-week period. The ECL signal remained at 96.25% of its original intensity after one week storage and at 93.5% after two weeks storage, indicating excellent stability of the developed ECL chiral sensor.

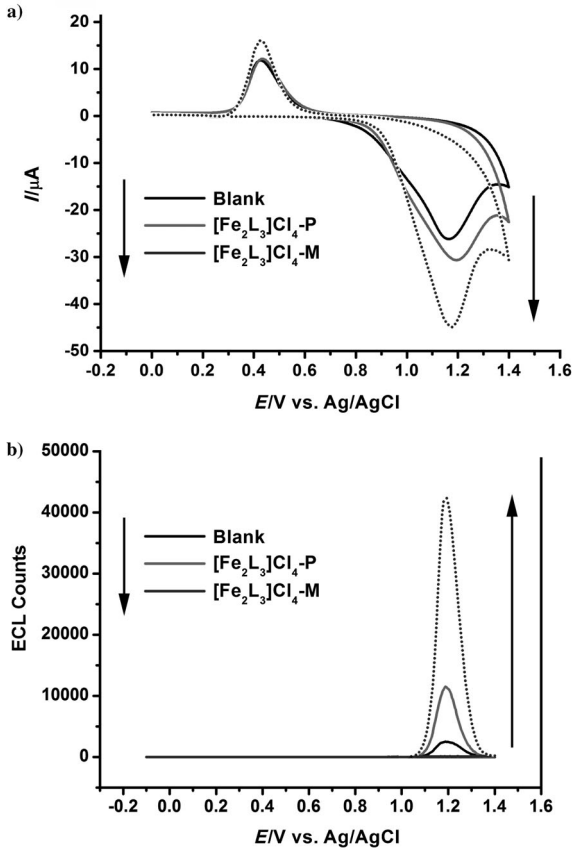


Figure 2. CVs (a) and ECL spectra (b) of $[Ru(phen)_3]^{2+}$ on different modified AuE surfaces: S1 modified surface (black line); TWJ DNA stabilized by the equal concentrations of $[Fe_2L_3]Cl_4$-P (grey line) and $[Fe_2L_3]Cl_4$-M (dashed line).

In summary, we have developed the first highly sensitive label-free ECL sensor for distinguishing between chiral metallosupramolecules based on the analyte-induced formation of DNA three-way junctions. The assay is sensitive and specific to one of the enantiomers, which permits selective discrimination of chiral complexes. The simple experimental procedure uses interactions of $[Ru(phen)_3]^{2+}$ with ds-DNA without requiring labeling of the DNA or the target and provides new insights into design of ECL sensors for distinguishing chiral molecules.

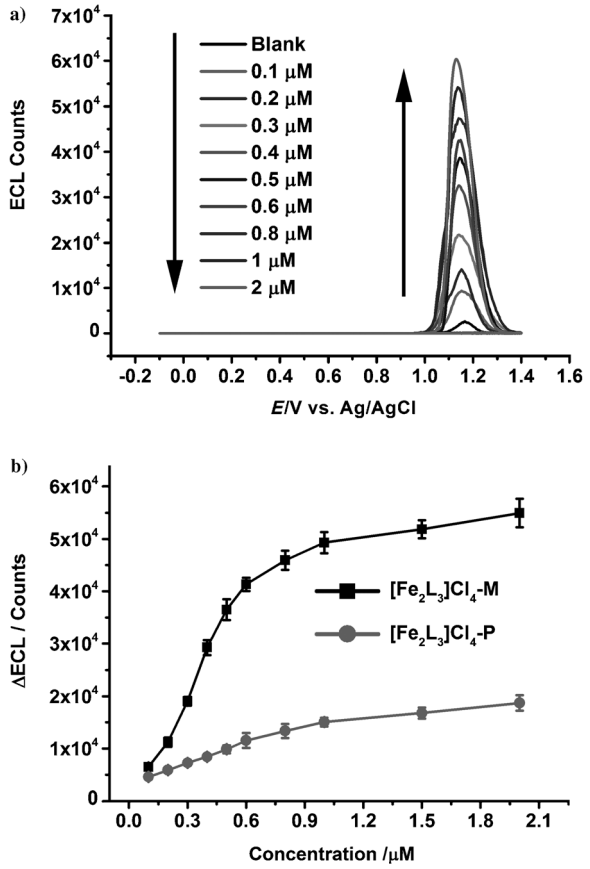


Figure 3. a) ECL counts for the fabricated sensor with $[Fe_2L_3]Cl_4$-M from 0 to 2 μM. b) Concentration dependence of ΔECL for $[Fe_2L_3]Cl_4$-M/P.

Experimental Section

Chemicals and materials

The oligonucleotides used in this paper were offered by Biotechnology Inc. (Shanghai, P.R. China). Their sequences were 5′-HS-$(CH_2)_6$-GGA ACG GCA CTC-3′ (S1), 5′-GAG TGC AGC GTG-3′ (S2), 5′-CAC GCT CGT TCC-3′ (S3).

The metallosupramolecular cylinder $[Fe_2L_3]Cl_4$ was synthesized and purified as previously reported.[3c] The enantiomerically pure $[Fe_2L_3]Cl_4$ was obtained by using a cellulose column (≈20 μm, Aldrich) and eluting with NaCl aqueous solution (20 mM). The purity was more than 95%, which was determined by ESI-MS and elemental analysis. CCDC 622770 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. UV/Vis spectroscopy was used to determine the concentration of the enantiomer. The samples of purified P- and M-enantiomer were collected and freeze-dried for further use.

Fabrication of sensors

The gold-disk electrodes ($\Phi = 2$ mm, CH Instruments, Austin, TX) were prepared by polishing with deagglomerated γ alumina (0.3 and 0.05 μm; BUEHLER, UAS) suspensions followed by sonication in water and multiple steps of electrochemical cleaning before modification with thiolated probe DNA. The cleaned gold surface was then immersed in buffered solution (10 mM Tris buffer, 1.5 M NaCl, 10 mM $MgCl_2$, pH 7.2) containing thiolated probes DNA S1 (0.1 μM) pretreated with TCEP (Tris = (2-carboxyethyl) phosphine hydrochloride); 10 mM) for 12 h at room temperature (ca. 25 °C). Subsequently, the modified surface was rinsed with buffer solution and then passivated with MCH in Tris buffer for 30 min. Then, the fabricated electrode was washed gently with distilled water and stored in air prior to use.

For the detection of the chiral complexes, $[Fe_2L_3]Cl_4$-M/P were first diluted to a known concentration in Tris buffer (89 mM Tris salt, 89 mM borate, 10 mM NaCl, pH 7.2) containing S2 and S3 DNA (each, 2 μM). Then, the modified electrodes were incubated with each sample containing different concentrations of the metallosupramolecules for 30 min at room temperature. After washing with Tris buffer, the modified electrode was soaked in Tris buffered solution (500 μL) containing $[Ru(phen)_3]^{2+}$ (2 mM) for 1 h. Next, the prepared electrodes were rinsed with buffer and dried with N_2. Finally, the ECL intensity of the resulting functionalized electrodes was recorded in Tris buffer (89 mM Tris salt, 89 mM borate, 10 mM NaCl, pH 7.2) containing TPrA (20 mM) from 0 to 1.4 V (versus Ag/AgCl) at the scan rate of 50 $mV\,s^{-1}$.

Electrochemical impedance spectroscopy (EIS) characterization of the fabricated sensor was performed on CHI 660B in 89 mM Tris buffer (89 mM borate, 10 mM NaCl, pH 7.2) containing $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$ (10 mM, 1:1) mixture with KCl (1 M) as the supporting electrolyte. The impedance spectra were recorded within the frequency range 10^{-2}–10^5 Hz. The amplitude of the applied sine wave potential in each case was 5 mV.

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Keywords: chirality · DNA · electrochemiluminescence · enantioselective recognition · metallosupramolecules · three-way junctions

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