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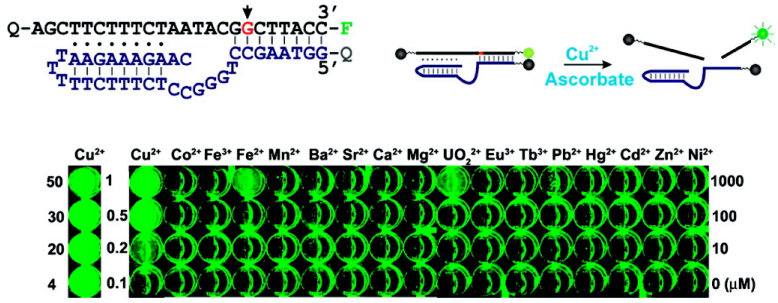


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# A DNAzyme Catalytic Beacon Sensor for Paramagnetic $Cu^{2+}$ Ions in Aqueous Solution with High Sensitivity and Selectivity

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Design of fluorescent metal sensors has recently become one of the most active research areas because the sensors can provide in situ and real-time information for a number of applications including environment monitoring, industrial process control, metalloneurochemistry, and biomedical diagnostics.[1] A widely used strategy is to link the metal recognition portion closely with a signal generation moiety such as a fluorophore. While quite successful in designing sensors for diamagnetic metal ions such as $Pb^{2+}$, $Hg^{2+}$, $Zn^{2+}$, and $Cu^{+}$,[2] this method has been applied to paramagnetic metal ions such as $Cu^{2+}$ with only limited success, due to their intrinsic fluorescence quenching properties.[3,4] Most $Cu^{2+}$ sensors showed decreased emission upon $Cu^{2+}$ binding,[3] which was undesirable for analytical purposes. First, the room for signal change was at most 1-fold. Second, such "turn-off" sensors may give false positive results by quenchers in real samples. Among the reported "turn-on" $Cu^{2+}$ sensors,[4] few have nanomolar sensitivity,[4a,d,f,g] with high selectivity,[4a,d] and free of organic solvents.[4a] One way to circumvent this quenching problem is to spatially separate the metal recognition part from the fluorescent signaling moiety so that they are independent of each other. A significant challenge then is to transduce metal binding to signal enhancement when the two parts are well-separated. We have previously reported a novel metal sensing platform with DNAzyme catalytic beacons that spatially separated the two parts by rigid double-stranded DNA,[5,6] and sensors for diamagnetic metal ions such as $Pb^{2+}$ and $UO_2^{2+}$ have been demonstrated.[7,8] Herein, we apply this method to turn-on sensing of paramagnetic $Cu^{2+}$ with high sensitivity and selectivity.

Copper is a widely used metal that can leak into the environment through various routes. Low concentration of copper is an essential nutrient. However, exposure to high level of copper even for a short period of time can cause gastrointestinal disturbance, while long-term exposure causes liver or kidney damage.[9] The U.S. Environmental Protection Agency (EPA) has set the limit of copper in drinking water to be 1.3 ppm (~20 $\mu$M).

We chose a $Cu^{2+}$-dependent DNA-cleaving DNAzyme reported by Breaker et al. as a basis for the sensor design.[10−12] On the basis of the original DNAzyme sequences, we rationally designed a $Cu^{2+}$ sensor as shown in Figure 1A. The sensor contained two DNA strands that formed a complex. The substrate (in black) was labeled with a FAM fluorophore (6-carboxyfluorescein) at the 3′-end and a quencher (Iowa Black FQ) at the 5′-end, while the enzyme (in blue) contained a 5′-quencher. Such a dual-quencher approach was employed to suppress background signals.[13] The substrate and enzyme associate through two base-pairing regions. The 5′-portion of the enzyme binds the substrate via Watson−Crick base pairs and the 3′-region through formation of a DNA triplex. Initially, the FAM emission was quenched by the nearby quenchers. In the presence of $Cu^{2+}$, the substrate was irreversibly cleaved at the cleavage site (the guanine in red). Following cleavage, we hypothesized that the cleaved pieces were released due to decreased affinities to the enzyme, leading to increased fluorescence (Figure 1B). This hypothesis was supported by the observation that the FAM emission increased by ~13-fold after addition of $Cu^{2+}$ (Figure 1C). Such a signal generation method was termed catalytic beacon because the involvement of catalytic reactions.[7,8] The sensor system also contained 50 $\mu$M ascorbate because it can significantly enhance the reaction rate (Figure S9, Supporting Information).[10−12] Ascorbate was also useful for suppressing quenching. For example, FAM quenching was <15% with 50 $\mu$M $Cu^{2+}$ (Figure S8).

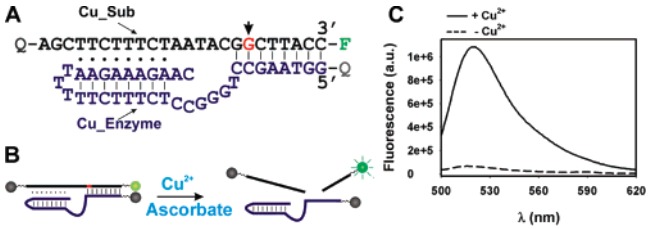


***Figure 1.*** (A) The secondary structure of the $Cu^{2+}$ sensor DNAzyme. F and Q denote fluorophore and quencher, respectively. The cleavage site is indicated by an arrow. (B) Signal generation scheme of the $Cu^{2+}$ catalytic beacon. (C) Fluorescence spectra of the sensor before and 10 min after addition of 20 $\mu$M $Cu^{2+}$.

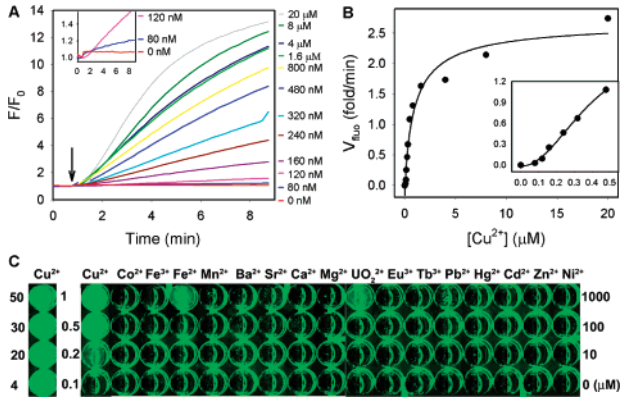


***Figure 2.*** (A) Kinetics of fluorescence increase over background at varying $Cu^{2+}$ levels. The arrow indicates the point of $Cu^{2+}$ addition. Inset: responses at low $Cu^{2+}$ levels. (B) The rate of fluorescence enhancement plotted against $Cu^{2+}$ concentration. Inset: rates at the low $Cu^{2+}$ region. (C) Sensor selectivity. The buffer contained 1.5 M NaCl, 50 mM HEPES, pH 7.0, and 50 $\mu$M ascorbate. $Cu^{2+}$ concentrations were labeled on the left side of each well, while others were on the right end (in $\mu$M).

To test sensitivity, the kinetics of fluorescence increase at 520 nm in the presence of varying concentrations of $Cu^{2+}$ were monitored. As shown in Figure 2A, fluorescence enhancement rates were higher with increasing levels of $Cu^{2+}$. The rates in the time window of 2−4 min were plotted in Figure 2B. A detection limit of 35 nM (2.3 ppb) was determined, which represents one of the most sensitive turn-on $Cu^{2+}$ sensors.[4a,d,f,g] The sensor has a dynamic range up to 20 $\mu$M, which is useful for detecting $Cu^{2+}$ in drinking water because the U.S. EPA has defined a maximum contamination level of 20 $\mu$M. In addition to being highly sensitive and possessing



10.1021/ja0717358 CCC: $37.00 

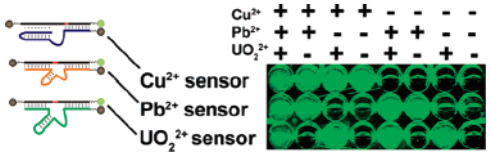


***Figure 3.*** Responses of a DNAzyme sensor array to metal mixtures.

turn-on signals, the sensor response was also fast, and quantitative results can be obtained within several minutes.

To test selectivity, 16 competing metal ions were assayed at three concentrations: 1 mM, 100 $\mu$M, and 10 $\mu$M. The assay was performed in a 96-well plate, and emission intensities at 12 min after addition of metal ions were compared. As shown in Figure 2C, besides $Cu^{2+}$, only the spots with 1 mM $Fe^{2+}$ and 1 mM $UO_2^{2+}$ lit up, and the intensities were lower than that with 0.5 $\mu$M of $Cu^{2+}$. Therefore, the sensor selectivity for $Cu^{2+}$ was at least 2000-fold higher than these two metals and >10 000-fold higher than any other tested metal ions. The relatively high selectivity of $Cu^{2+}$ over paramagnetic $Fe^{2+}$ may be due to either lack of DNAzyme recognition of $Fe^{2+}$ as the DNAzyme was in vitro selected for $Cu^{2+}$,[11,12] or lack of $H_2O_2$ in the sensor solution that is needed for $Fe^{2+}$ to go through the Fenton chemistry for DNA cleavage.[14] In gel-based assays, $UO_2^{2+}$ did not produce well-defined cleavage bands (Figure S4). Therefore, the increased emission by $UO_2^{2+}$ was attributed to DNA denaturation, although minor oxidative cleavage cannot be ruled out.[15] $Cu^+$ is unstable in water, and $Cu^+$ was tested using $[Cu(MeCN)_4](PF_6)$ in acetonitrile as a metal source. With ascorbate, the rate of fluorescence increase was similar to that with $Cu^{2+}$ (Figure S9). In the absence of ascorbate, both $Cu^{2+}$ and $Cu^+$ can induce fluorescence increase, with the rate with $Cu^+$ being much faster. Therefore, it is likely that $Cu^{2+}$ was reduced by ascorbate to $Cu^+$, which subsequently reacted with oxygen to oxidatively cleave DNA. $Ag^+$ was not tested because the reaction buffer contained 1.5 M NaCl, which can form insoluble AgCl. $Au^+$ was not tested because it is unstable in the open air aqueous solution. For testing environment samples, such as detection of $Cu^{2+}$ in drinking water, $Cu^+$ or $Fe^{2+}$ is unlikely to interfere due to the oxidative aqueous environment. $UO_2^{2+}$ is also unlikely to be present in millimolar concentration in drinking water. $Fe^{3+}$ with ascorbate can also cleave the DNAzyme. However, little fluorescence increase was observed due to the slow reaction rate and the quenching effect of $Fe^{3+}$ (Figures S6 and S7). It needs to be pointed out that the previously reported lead and uranium sensors were based on hydrolytic RNA cleavage.[7,8] In the current copper DNAzyme, the substrate was made completely of DNA, and the cleavage was oxidative.[10]

Finally, we constructed a sensor array as shown in Figure 3. The array contained three rows, and each row was loaded with a different DNAzyme-based sensor. Eight metal mixtures were prepared with all the possible combinations among $Cu^{2+}$, $Pb^{2+}$, and $UO_2^{2+}$ (1 $\mu$M each). As can be observed from Figure 3, the wells lit up only when the cognate metals were present and the metal compositions can be read directly from the array. We can imagine that larger metal sensor arrays can be built with the isolation of more metal-specific DNAzymes.[5]

In summary, we have demonstrated a highly effective fluorescent sensor that showed strong fluorescence enhancement in the presence of a paramagnetic metal ion: $Cu^{2+}$. This result further demonstrated that the DNAzyme-based metal sensing approach can be applied to a broad range of metal ions.

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**Supporting Information Available:** Experimental procedures, gel-based assays, controls and fluorescence quenching (PDF). This material is available free of charge via Internet at http://pubs.acs.org.

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