Electronic Supplementary Information (ESI†) for

G-quadruplex Facilitated Fluorescent Turn-Off Chemosensor for Selective Detection of Cupric Ion

Haixia Qin, Jiangtao Ren, Jiahai Wang* and Erkang Wang*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin,130022, China, and Graduate School of the Chinese Academy of Sciences, Beijing, 100039, China.

*To whom correspondence should be addressed. E-mail: ekwang@ciac.jl.cn or jhwang@ciac.jl.cn

Materials and Methods

Materials: NMM was purchased from Porphyrin Products (Logan, UT). Oligonucleotides (24GT: GGGTTTTgggTTTTGGGTTTTGGG, sDNA: CTGGCTATCGCTGGATGTG) were synthesized by Sangon Biotechnology Co., Ltd (Shanghai, China). All reagents were used as received without further purification. The stock solution of NMM (1 mM) was prepared in dimethyl sulfoxide (DMSO), stored in darkness at -20 □. The stock solution of oligonucleotides (3 μM) were prepared in 20 mM HEPES buffer (pH 7.0) containing 140 mM NaCl and 5 mM KCl and accurately quantified using UV-vis absorption spectroscopy. All water used to prepare buffer solutions was obtained by using a Milli-Q water system. Before use, the NMM and oligonucleotide solutions were diluted to required concentrations with the working buffer (20 mM HEPES, 140 mM NaCl and 5 mM KCl, pH 7.0). The stock solution of $CuCl_2$ (10 mM) and other metal ion stock solutions (10 mM) were prepared in MilliQ water, and MilliQ water was also used for further dilution.

Apparatus: Cary 500 Scan UV/Vis Spectrophotometer (Varian, USA) was used to quantify the oligonucleotides. Fluorescence intensities were recorded on a LS-55 Luminescence Spectrophotometer (Perkin-Elmer, USA). The emission spectra were recorded in the wavelength of 550-750 nm upon excitation at 399 nm. Slit widths for the excitation and emission were set at 5 and 15 nm, respectively.

Fluorescence measurement for Cu^{2+} without 24GT: Different concentrations of Cu^{2+} were added to the NMM solutions (10 μM), allowing the Cu^{2+} react with NMM for 30 min (Figure S6A). Then the fluorescence assays were carried out in working buffer which contained 2 μM NMM and different concentrations of Cu^{2+}. The assay procedures for other metal ions (50 μM) were the same as those for Cu^{2+} ions, except that other metal ions were used instead of Cu^{2+}.

Fluorescence measurement with 24GT: First, the 24GT solutions were heated at 96□ for 5 min to dissociate any intermolecular interaction, and gradually cooled to room temperature for 30 min to form a stable G-quadruplex. Subsequently, required concentration of 24GT was added to the mixture solution of NMM (2 μM) and different concentrations of Cu^{2+}. Finally, fluorescence assays were carried out after the mixture solutions incubated for 30 min (Figure S6B). Also, other metal ions (50 μM) took place of Cu^{2+} for the selective assays.

Application: Lake water and tap water were used to confirm the feasibility of this sensor for analysis of real-world sample. Water samples were filtered through 0.22 μM membranes. 2 μM $CuCl_2$ was added into water samples to test the recovery. Then the samples were diluted 2-fold with HEPES buffer and analyzed in HEPES buffer (20 mM, pH=7.0) which contained 500 nM 24GT and 2 μM NMM.

Safety considerations: Because most of the tested heavy metals are highly toxic and harmful to human health, all the experiments involving in heavy-metal ions should be performed with protective gloves. The waste solutions that contain heavy-metal ions should be collectively reclaimed to avoid the environment pollution.

Discussions

As shown in Figure S5, both G-quadruplex and copper ion will bind NMM, which elicits the change of UV-vis spectra of NMM. Figure S1 shows that copper ion damages the antiparallel conformation of G-quadruplex, therefore, the specific interaction between NMM and G-quadruplex is prevented. The stoichiometry of copper ion and G-quadruplex is roughly 20:1 (Figure S1B).

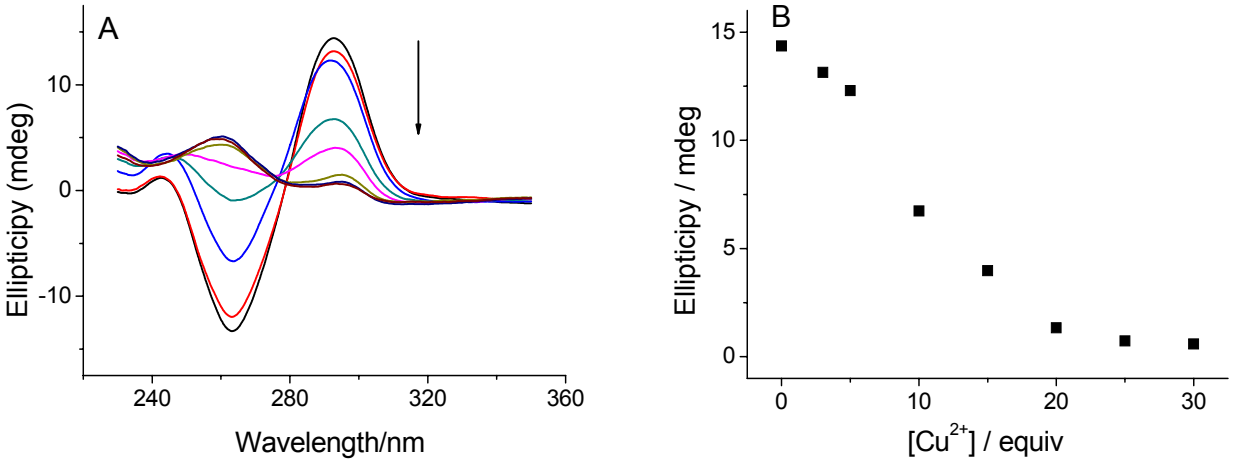

Figure S1. 24GT quadruplex denaturation as a function of Cu^{2+} ion concentration, followed by CD spectroscopy. (A) Cu^{2+} (3equiv, 5equiv, 10equiv, 15equiv, 20equiv, 25equiv, 30equiv) is added into 24GT (4 μM) buffered in 20mM HEPES buffer (pH 7.0) containing 140 mM NaCl and 5 mM KCl. (B) Dependence of ellipticipy at 292 nm on the Cu^{2+} concentration (equiv of 24GT).

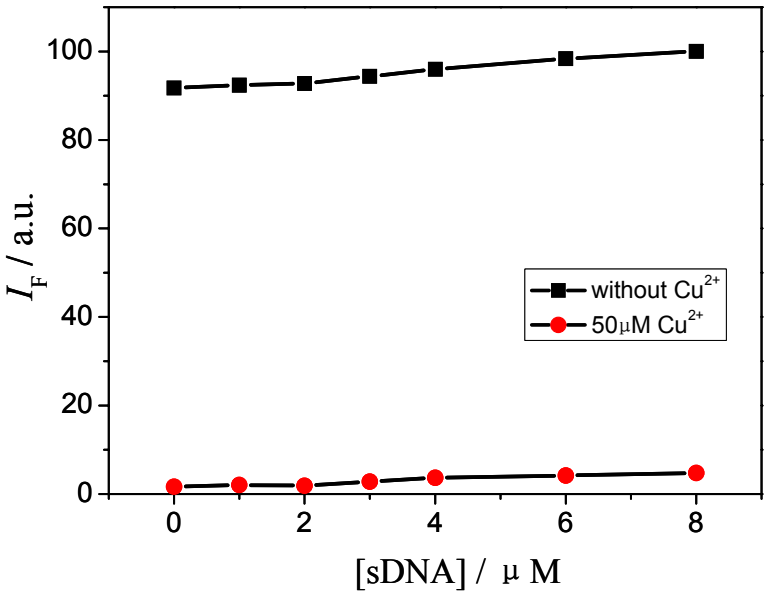

Figure S2. Impact of various concentrations of sDNA on the fluorescence responses of NMM + 24GT in absence (black square) and presence (red circle) of 50 μM Cu^{2+}. Experimental conditions: 2 μM NMM, 0.5 μM 24GT, 20 mM HEPES buffer (pH 7.0) containing 140 mM NaCl and 5 mM KCl, excitation of NMM at 399 nm.

Table S1 Sensitivity of NMM (or NMM + 24GT) towards various metal ions

	NMM		G-Quadruplex / NMM	
	F[a]	QF[b]	F	QF
blank (F_0)	10.01	1.00	85.71	1.00
Hg^{2+}	10.16	0.99	87.97	0.97
Mg^{2+}	10.95	0.91	87.79	0.98
Ca^{2+}	10.57	0.95	86.00	1.00
Cd^{2+}	10.54	0.95	84.76	1.01
Ni^{2+}	10.22	0.98	85.45	1.00
Co^{2+}	10.13	0.99	86.42	0.99
Mn^{2+}	10.33	0.97	86.11	1.00
Cr^{3+}	6.37	1.57	—	—
Cr^{3+} + sDNA	9.90	1.01	82.75	1.04
Fe^{3+}	2.86	3.50	—	—
Fe^{3+} + sDNA	10.05	1.00	86.06	1.00
Cu^{2+}	0.63	15.84	—	—
Cu^{2+} + sDNA	0.50	19.87	1.07	80.06

[a] F stands for fluorescence intensity (a.u.).
[b] QF stands for quenching factor, QF= F_0/F.

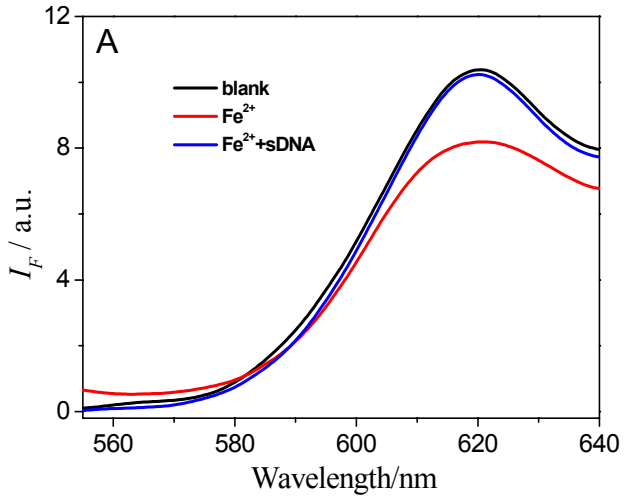

Figure S3. Fluorescence responses of NMM toward Fe^{2+}. Experimental conditions: 50 μM Fe^{2+}, 2 μM NMM, 20 mM HEPES buffer (pH 7.0) containing 140 mM NaCl and 5 mM KCl, excitation of NMM at 399 nm.

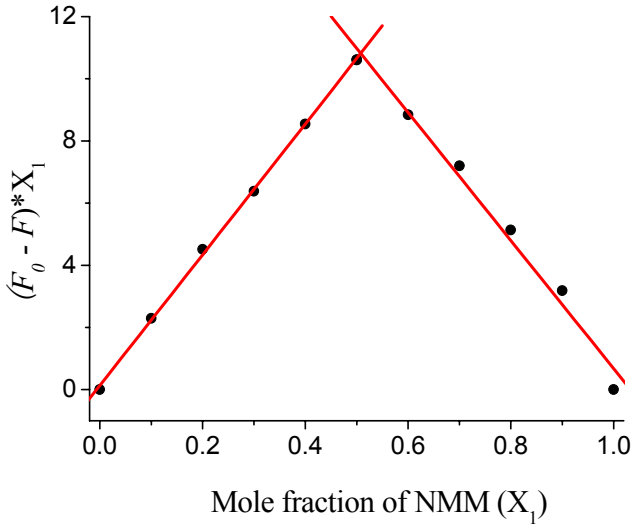

Figure S4. Job plot for determining the stoichiometry of NMM and Cu^{2+}. The total concentration of NMM and copper was 1.0×10^{-5} M. Molar fraction was given by [NMM] / ([NMM] + [Cu^{2+}]).

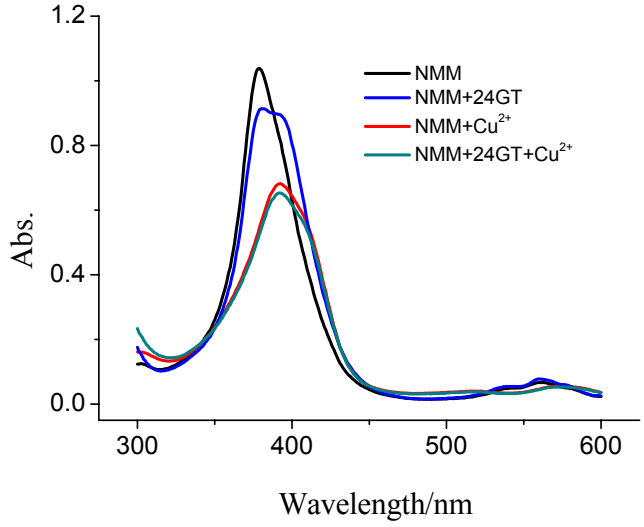

Figure S5. UV-vis spectra of NMM, NMM plus 24GT, NMM plus Cu^{2+}, NMM plus Cu^{2+} and 24GT. Experimental conditions: 50 μM Cu^{2+}, 2 μM NMM and 0.5 μM 24GT.

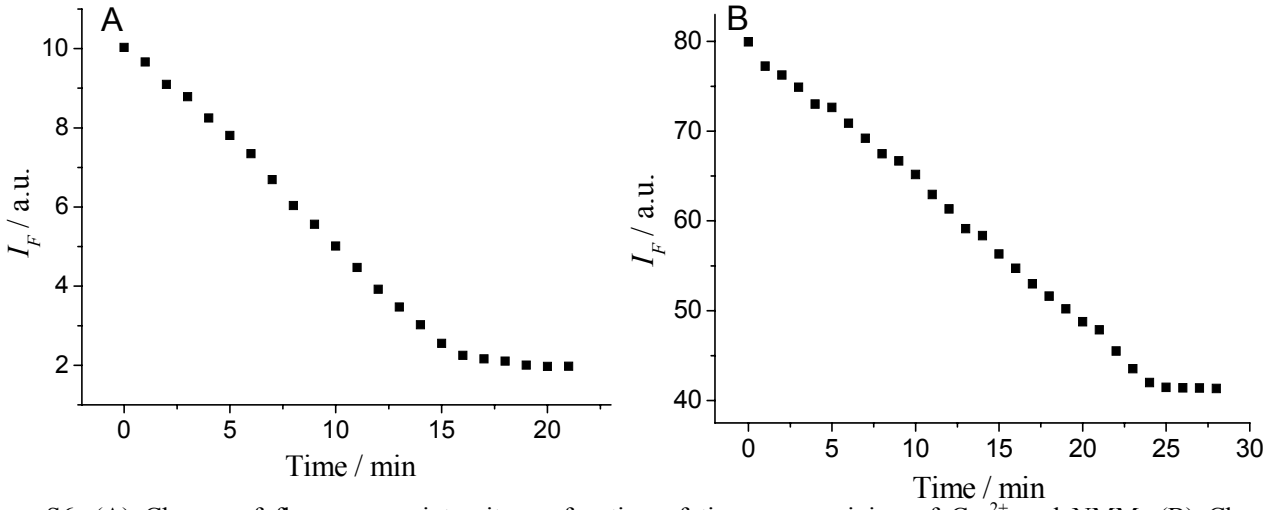

Figure S6. (A) Change of fluorescence intensity as function of time upon mixing of Cu^{2+} and NMM. (B) Change of fluorescence intensity as function of time upon mixing of Cu^{2+} and NMM+24GT .

Table S2 Analytical results for Cu^{2+} in water samples.

Sample	Content (μM, n=3)*	ICP-MS (μM)	Added Cu^{2+} (μM)	Found Cu^{2+} (μM)	Recovery (%)
Lake water 1	0.476 ± 0.029	0.481	2.00	1.92	96.0
Lake water 2	0.405 ± 0.019	0.400	2.00	1.89	94.5
Tap water 1	0.636 ± 0.022	0.630	2.00	2.04	102
Tap water 2	0.858 ± 0.024	0.857	2.00	2.01	100

* measured by our chemosensor.

Table S3 Performance comparison of this work with other homogeneous Cu^{2+} sensors

Type	Detection limit	Probe synthesis	Linear range	Solvent	Temperature	Ref.
Fluorescence turn-off	83 nM	Convenient (label-free)	0.083-10 μM	H_2O	Room temperature	TW[a]
Off-on fluorescent chemosensor	0.3 μM	Difficult (organic synthesis)	0.8-10 μM	H_2O/EtOH (8:2, v/v)	Room temperature	[1]
Fluorescent excimer–monomer switching	NR[b]	Difficult (organic synthesis)	NR	CH3CN/HEPES (1:1, v/v)	Room temperature	[2]
Allosteric dual-DNAzyme-based colorimetric	1 μM	Inconvenient (catalyzeH2O2-mediated oxidation of TMB)	0.001-1 mM (nonlinear correlation)	H_2O	Room temperature	[3]
Visual detection	50 μM	Difficult (organic synthesis)	NR	H_2O	Room temperature	[4]
Ratiometric displacement approach	~2 μM	Complex (two kinds of ligands)	NR	H_2O	Room temperature	[5]
Copper(II)-dependent self-cleaving DNAzymes	290 nM	Insensitivity (Au NPs-based colorimetric)	0.625-15 μM	H_2O	Room temperature	[6]
Fluorescence anisotropy	~1 nM	Expensive (dual labeling)	0.001-10 μM	H_2O	Room temperature	[7]
Au NP-based colorimetric	20 μM	Expensive (dual labeling)	20-100 μM	H_2O	Increasing temperature	[8]
Fluorescence quenching	42 nM	Difficult (organic synthesis)	0.05-100 μM	EtOH/ H_2O (1:1, v/v)	Room temperature	[9]
DNAzyme Catalytic Beacon Sensor	35 nM	Expensive (dual labeling)	0.035-20 μM	H_2O	Room temperature	[10]

[a] TW stands for this work.
[b] NR stands for not reported.

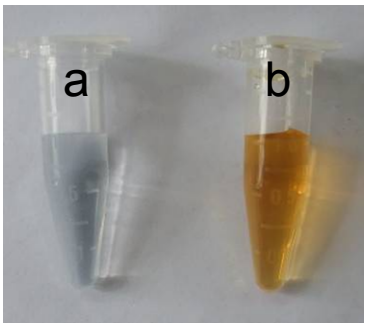

Figure S7. The stock aqueous solutions (10mM) for $CrCl_3$ (a) and $FeCl_3$ (b) are clear during the last 6 months.

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