# A ratiometric $Al^{3+}$ ion probe based on the coumarin-quinoline FRET system

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## ABSTRACT

A coumarin-quinoline based fluorescence resonance energy transfer (FRET) system (**TCQ**) has been synthesized and employed as a ratiometric fluorescence probe. The selective fluorescent response of the probe **TCQ** toward $Al^{3+}$ was devised by employing a quinoline moiety as a FRET energy donor with a coumarin moiety as an energy acceptor. The quinoline emission at 390 nm decreased and the coumarin emission at 480 nm increased concurrently on addition of $Al^{3+}$ under excitation wavelength at 253 nm. The **TCQ** probe exhibited high selectivity for $Al^{3+}$ as compared to other tested metal ions and the ratiometric sensing of $Al^{3+}$ was determined by plotting the fluorescence intensity ratio at 480 nm and 390 nm *versus* $Al^{3+}$ ion concentration. Moreover, test strips based on **TCQ** were fabricated, which were found to act as a convenient and efficient $Al^{3+}$ ion detection kit. Furthermore, this system has been used for imaging of $Al^{3+}$ in living cells.

**Keywords:**

Coumarin; Quinoline; FRET; Ratiometric fluorescent probe; $Al^{3+}$

## 1. Introduction

Many fluorescent probes have been synthesized and subsequently employed for biosensing, bioimaging, and in environmental detection. This widespread use is due to a number of desirable features such as real time monitoring of fluorescence with high selectivity and sensitivity, non-destructive analysis and simple instrumentation [1]. As

we all known, metal ions are ubiquitous and play a fundamental role in a wide range of chemical and biological processes. Undoubtedly, the understanding of metal ion homeostasis in biology has significantly benefited from advancements in the development of fluorescent probes for monitoring metal ions. Aluminum is the third most prevalent element and the most abundant metal in the earth's crust, and has been widely used in many fields [2]. Excess levels of $Al^{3+}$ in the human body can cause adverse physiological effects and leads to lots of diseases such as microcytic hypochromic anemia, bone softening, encephalopathy, myopathy and Alzheimer's disease [3]. So, highly sensitive and selective bioimaging of $Al^{3+}$ in the cell is required to understand the underlying mechanism about how aluminum ions cause aluminum-induced human diseases. Thus, detection of $Al^{3+}$ is very significant to monitor the concentration level in the environment and minimize direct effect of the $Al^{3+}$ ion on human health [4]. Numerous probes for $Al^{3+}$ have been reported which displayed a decrease or increase in the emission intensity [5-18]. Most of them are fluorescence intensity-based probes, which mean that ion detection depends on a simple change of fluorescence intensity arising from metal-binding, and tends to be significantly influenced by the excitation power, detector sensitivity and instrument environmental factors, especially at very low ions concentrations. To alleviate the above problems, ratiometric measurements were developed for precise and quantitative analysis of biological events occurring under complex conditions by simultaneously recording fluorescence intensities at two wavelengths and then calculating their ratios [19]. However, despite many potential advantages, examples of ratiometric fluorescence probes for $Al^{3+}$ [20, 21], $Fe^{3+}$/$Al^{3+}$/$Cr^{3+}$ [22], $Al^{3+}$/$Zn^{2+}$ [23, 24], and $Al^{3+}$/$Hg^{2+}$ [25] are scant.

The application of fluorescence resonance energy transfer (FRET) should be an efficient approach to the design of ratiometric fluorescence probes, since they can emit at two different wavelengths at a single excitation source. FRET is a distance-dependent interaction between the electronic excited states of two different fluorescent groups in which excitation is transferred from a donor moiety to an acceptor moiety without photoemission. The efficiency of FRET is primarily

controlled by the scope of spectral overlap between donor emission with acceptor absorption and the distance between donor and acceptor [23, 24]. Because the pseudo Stokes shifts of FRET based probes are larger than the Stokes shifts of either the donor or acceptor dyes, the possibility of self-quenching as well as fluorescence detection errors due to backscattering effects of the excitation source will be efficiently avoided [28].

Given the high flexibility in the design of FRET probes and the wide choice of fluorophore available, FRET has been widely utilized for the design of ratiometric probes, such as for pH [29], $Fe^{3+}$ [30], $Cu^{2+}$ [31, 32], $Hg^{2+}$ [33], $H_2S$ and sulfide/sulfite [34, 35].

Notably, so far only a few FRET-based probes for the detection of $Al^{3+}$ have been reported [36-38]. These probes have demonstrated some promising attributes such as high specificity and sensitivity; however, there are still a number of limitations and most of them are based on rhodamine. Therefore the development of new ratiometric FRET-based probes for $Al^{3+}$ ions with improved detection limits in biological systems are desirable.

The important challenge is selectivity of suitable “Donor-Acceptor” pairs, because a requirement for Forster energy transfer is that the emission spectrum of the donor must overlap with the absorption spectrum of the acceptor [39]. Owing to their excellent photochemical and photophysical properties, rhodamine and coumarin derivatives have often been used to construct FRET-based probes for detecting different species, typically $Hg^{2+}$/ $Cu^{2+}$/ $Fe^{2+}$/ $Fe^{3+}$ [40], $Cu^{2+}$ [41], $H_2S$ [42-44], pH [45], $Fe^{3+}$ [46], $Cu^{2+}$/$Hg^{2+}$ [47, 48], HOCl [49].

Coumarin and quinoline dyes have been widely used as fluorophores owing to their favourable biocompatibility and photophysical properties [50,51]. In addition, coumarin dyes, for which the emission profiles are tunable from the blue to near-infrared region by simply modifying the coumarin core structure, have been extensively applied in fluorescent probes [52]. Moreover, the emission spectrum of coumarin and the excitation spectrum of quinaldine have substantial overlap, which would fulfill an

important requirement for the FRET process.

Herein, we report the design, synthesis, and application of a FRET-based ratiometric $Al^{3+}$ probe (**TCQ**) by combining the coumarin fluorophore in a quinoline derivative using a triazole linker. In this sensing system, the quinoline moiety was chosen as the energy donor, since its fluorescence spectra can match well with the absorption spectra of coumarin (the energy acceptor). Furthermore, fluorescence imaging by **TCQ** for $Al^{3+}$ in living cells was demonstrated.

## 2. Experimental

### 2.1. Materials and equipments

Deionized water was used throughout the experiments. Other chemicals were purchased from Alfa Aesar Co., Tianjin, China and used without further purification. The solutions of metal ions were prepared from their nitrate salts which were analytical grade.

Fluorescence spectra measurements were performed on a Cary Eclipse fluorescence spectrophotometer (Varian) equipped with a xenon discharge lamp using a 1 cm quartz cell. UV-vis spectra were recorded on a UV-1800 spectrophotometer (Beijing General Instrument Co., China). IR spectra were obtained using a Vertex 70 FT-IR spectrometer (Bruker). Melting points were determined on an X-5 binocular microscope (Beijing Tech Instrument Co., China). $^{1}H$ and $^{13}C$ NMR spectra were measured on a Nova-400 NMR (Varian) and WNMR-I 500 NMR spectrometer (at the Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences) using TMS as an internal standard. ESI-MS spectra was recorded on a HPLC-MSD-Trap-VL spectrometer (Agilent) and MALDI-TOF mass spectra was collected on a BIFLEX III ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometer (Bruker) with α-cyano-4-hydroxycinnamic acid as matrix. ITC experiments were performed using a Nano isothermal titration calorimeter (TA). The cell imaging test was carried out with an eclipse Ti-U (Nikon, Japan) inverted fluorescence microscopy.

2.2. Solution preparation

The **TCQ** stock solution (1 mM) was prepared using a 100 mL volumetric flask, with 43 mg of **TCQ** dissolved in EtOH and then diluting to the mark.

The $Al^{3+}$ stock solutions (2 mM) were prepared in a 50 mL volumetric flask by dissolving 40.0 mg $Al(NO_3)_3$ in $H_2O$, and then diluting to the mark with $H_2O$. The standard stock solution of other metal ions (2 mM) $Li^+$, $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Ba^{2+}$, $Sr^{2+}$, $Hg^{2+}$, $Pb^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Ag^+$, $Fe^{3+}$, $Cr^{3+}$, $Ga^{3+}$, $In^{3+}$ and $As^{3+}$ were prepared by dissolving an appropriate amount of their perchlorate or nitrate in water and adjusting the volume to 100 mL in a volumetric flask.

The Tris-HCl buffer stock solution was prepared in water solutions (1 mM, pH 7) with the requisite amount of HCl.

The $^1H$ NMR spectroscopic experiments used analytical grade $Al(ClO_4)_3$.

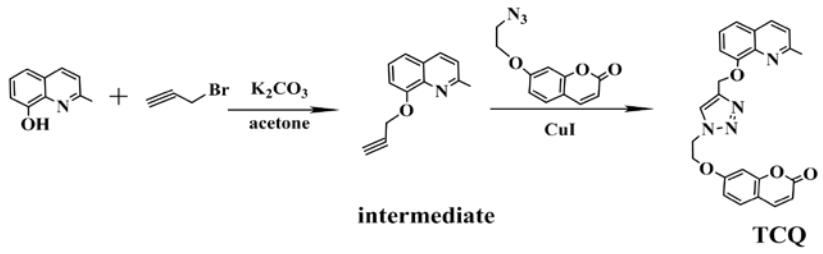
The ITC experiment consisted of 25 consecutive injections (10 μL) of $Al^{3+}$ ion solution (1 mM) into the microcalorimetric reaction cell (1 mL) charged with a solution of **TCQ** (0.1 mM). The heat of reaction was corrected for the heat of dilution of the ion solution determined in the separate experiments. All solutions were degassed prior to the titration experiment by sonication. Computer simulations (curve fitting) were performed using the Nano ITC ananlyze software.

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) and PC3 cells were grown in Roswell Park Memorial Institute-1640 (RPMI-1640), respectively, supplemented with 10 % fetal bovine serum, 100 U /mL penicillin and 100 μg/mL streptomycin at 37 ºC and 5 % $CO_2$. One day before imaging, the cells were seeded in 6-well flat-bottomed plates. The next day, cells were incubated with 10 μM **TCQ** for 60 min at 37 ºC in a humidified environment of 5 % $CO_2$ and then washed with fresh culture medium three times to remove the remaining **TCQ**. Before incubating with 50 μM $Al^{3+}$ for another 50 min, cells were rinsed with fresh culture medium three times again, then the fluorescence imaging of intracellular $Al^{3+}$ was observed using an inverted fluorescence microscope (excited with UV light). Cells which were only incubated with 10 μM **TCQ** for 60 min at 37 ºC under 5 % $CO_2$ were used as a blank

control.

2.3. Synthesis of probe **TCQ**

The synthetic route of **TCQ** was carried out as outlined in Scheme 1:




**Scheme 1.** Synthesis of **TCQ**.

To a solution of 8-hydroxyquinaldine (1.00 g, 6.29 mM) in acetone solution (50 mL) was added $K_2CO_3$ (1.30 g, 9.44 mM). The mixture was heated under reflux for 0.5 h. After cooling, 3-bromopropyne (3.70 g, 31.45 mM) was added, and then the mixture was heated at reflux for 18 h. The reaction mixture was filtered and the solvent was removed under vacuum. The crude product was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (v/v, 4/1) as the eluent to give the target intermediate compound (1.05 g, 5.33 mM) in 85.0 % yield.

A solution of the intermediate compound (1.00 g, 5.08 mM), 7-(2-azido-ethoxy)-coumarin (2.34 g, 10.15 mM) and CuI (catalytic amount) was reacted in THF/$H_2O$ (v/v, 10/1) at room temperature for 10 h. The crude product was purified by column chromaography on silica gel using petroleum ether/ethyl acetate (v/v, 1/1) as the eluent to afford 1.73 g probe **TCQ** as a pale white solid 70 % yield. m.p. 141 ~ 143 $^oC$; $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$: 2.78 (s, 3H, -$CH_3$), 4.42 (t, $J$= 7.0 Hz, 2H, -$CH_2CH_2$-), 4.79 (t, $J$= 7.0 Hz, 2H, -$CH_2CH_2$-), 5.58 (s, 2H, -$CH_2$-), 6.27 (d, J= 10.0 Hz, 1H, ArH), 6.72 ~ 6.75 (m, 2H, ArH), 7.25 (d, $J$= 2.0 Hz, $J$= 10.0 Hz, 1H, ArH), 7.31 ~ 7.37 (m, 4H, ArH), 7.61 (d, $J$= 10.0 Hz, 1H, ArH), 7.95 (s, 1H, ArH), 8.02 (d, J= 5.0 Hz, 1H, ArH); $^{13}C$ NMR ($CDCl_3$, 400 MHz) δ (ppm): 161.71, 160.56, 158.02, 155.39, 153.09, 144.33, 143.06, 139.61, 136.01, 128.79, 127.53, 125.45, 124.45, 124.24, 20.07, 113.44, 112.98, 112.23, 110.17, 101.49, 66.46, 62.19, 49.28, 25.52; MS (positive mode, *m/z*): Calcd. 428.15, Found 429.174 for $[M+H]^+$. (Figs S1~S4).

## 3. Results and Discussion

### 3.1. Fluorescence spectra behavior of **TCQ**

The skeleton of the FRET platform was composed of a coumarin moiety (energy acceptor) and a quinoline moiety (energy donor), which were further covalently connected by a triazole linker unit. The emission spectrum of quinoline and the absorption spectrum of coumarin have substantial overlap, which fulfills a favourable condition of the FRET process (Fig. 1, shaded area). The FRET efficiency between the donor and acceptor moieties calculated as 71.1 % according to the literature [53-55].

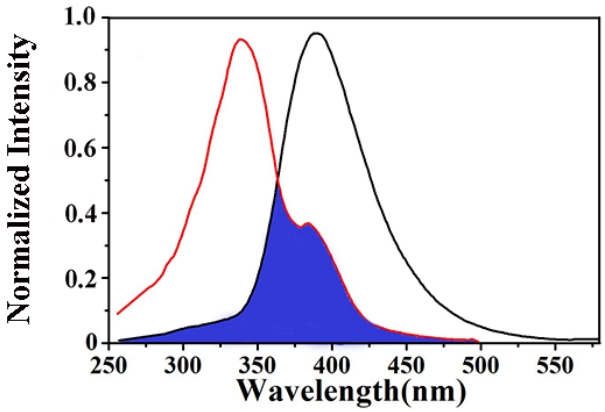



**Fig. 1.** Spectral overlap between the energy donor 8-hydroxyquinaldine emission (black) and acceptor 7-hydroxycoumarin absorption (red).

The fluorescence spectroscopy of **TCQ** (10 μM) was recorded in EtOH/$H_2O$ (2/3, v/v, buffer pH 7) solution. When excited at 253 nm, **TCQ** exhibited a strong emission for the quinoline moiety at 390 nm ($\Phi$ = 0.41, *versus* quinine sulfate as reference material, $\Phi$ = 0.55), and no emission at long wavelength was observed. Upon addition of $Al^{3+}$ to **TCQ**, the emission at 390 nm decreased, whilst a new emission for the coumarin moiety at 480 nm appeared and gradually increased, showing a pseudo Stokes shifts of 230 nm (Fig. 2a). Thus, probe **TCQ** exhibited a ratiometric fluorescent response upon the addition of $Al^{3+}$ ion and the $Al^{3+}$-triggered FRET process occurred. The matched fluorophores (quinoline and coumarin), the appropriate distance between the energy donor and energy acceptor, together with the binding sites provided by the nitrogen atom of the linker unit (triazole) and oxygen and nitrogen atoms of the 8-hydroxyquinaldine, demonstrated that the

quinoline-coumarin base FRET system had been developed. This system effectively avoided the emission spectra overlap and ensured accuracy and high resolution in determining the presence of $Al^{3+}$.

The fluorescence intensity ratio ($F_{480\ nm}$/ $F_{390\ nm}$) varied from 0.11 to 1.25, corresponding to a 12-fold enhancement. Additionally, marked fluorescent color changes in the solutions, which ranged from dark blue to light blue, could be distinguished (Fig. 2a, inset). The fluorescence titration of **TCQ** was treated with $Al^{3+}$ (0 ~ 20 μM) to obtain the relationship of intensity ratio *versus* $Al^{3+}$ concentration. When the amount of $Al^{3+}$ was greater than 1 equivalent of **TCQ**, the ratio emission reached saturation. The stoichiometry of the interaction revealed a 1:1 ratio between **TCQ** and $Al^{3+}$ (Fig. 2b and Fig. 2c). On the basis of the fluorescence intensity ratio of **TCQ** as a function of $Al^{3+}$ concentration, the association constant for **TCQ** with $Al^{3+}$ was calculated to be $5.6\times10^4$ $M^{-1}$ using a Benesi-Hildebrand plot (Fig. S8).

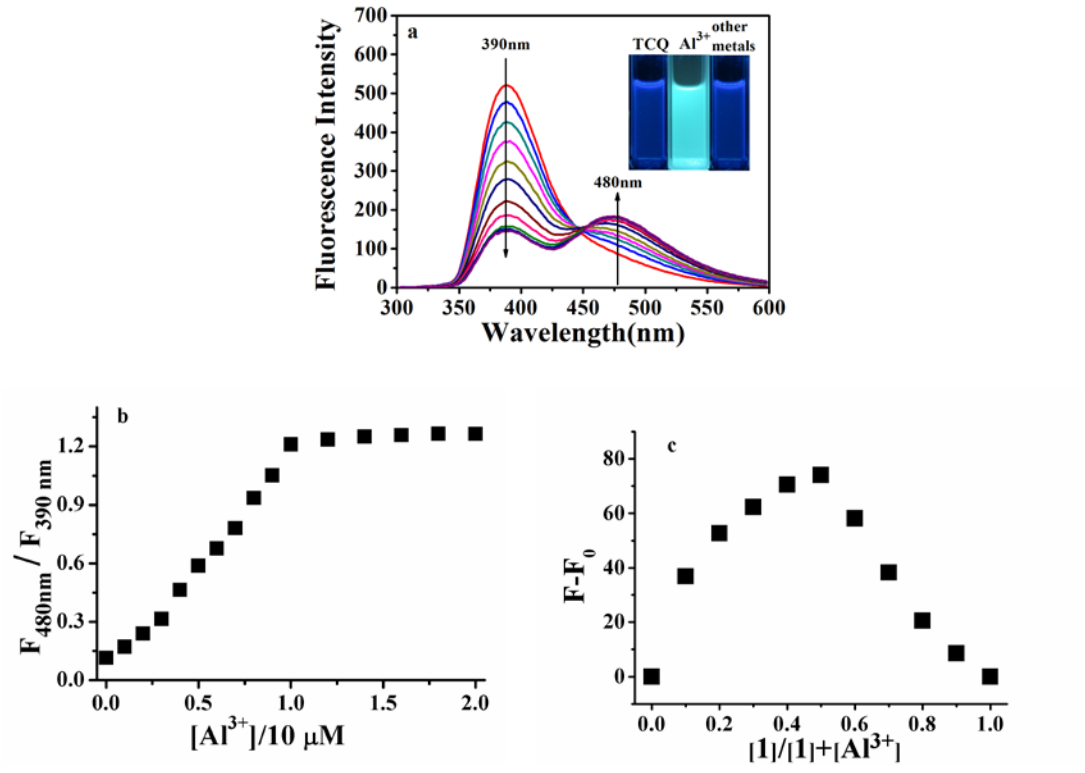



**Fig. 2.** (a) Fluorescence spectral changes of probe **TCQ** (10 μM, EtOH/$H_2O$, v/v, 2/3, pH 7) solution upon addition of $Al^{3+}$ (0 ~ 20 μM). Inset: fluorescent color changes of **TCQ** upon addition of $Al^{3+}$ (2 eq.) at 365 nm using a handheld UV lamp. (b) The plot of fluorescence

intensity ratio of **TCQ** as a function of $Al^{3+}$ concentration. (c) The Job's plot data. $\lambda_{ex}$ = 253 nm, $F_{480\ nm}/ F_{390\ nm}$.

From the fluorescence titration, a linear relationship between the ratio emission intensity ($F_{480\ nm}$ / $F_{390\ nm}$) of **TCQ** and $Al^{3+}$ concentration ($R^2$ = 0.990, n = 11, 0.2 ~ 10 μM) was observed. The limit of detection (LOD = 3σ / slope) was 0.024 μM (Fig. S9), which is suitable for the quantitative detection of $Al^{3+}$ ions.

The fluorometric behavior of **TCQ** towards other metal ions was studied (see Fig. 3a), and no significant spectral changes of **TCQ** occurred in the presence of 20 eq. of $Li^+$, $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Ba^{2+}$, $Sr^{2+}$, $Hg^{2+}$, $Pb^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Ag^+$, $Fe^{3+}$, $Cr^{3+}$, $Ga^{3+}$, $In^{3+}$ and $As^{3+}$. Only the addition of $Al^{3+}$ resulted in a significant change of the ratio emission intensity ($F_{480\ nm}/ F_{390\ nm}$) under the identical conditions employed. Moreover, as shown in Fig. 3b, in the presence of miscellaneous competitive metal ions, the addition of $Al^{3+}$ also resulted in the changes of ratio emission intensity. All these results indicated that the selectivity of probe **TCQ** for $Al^{3+}$ over other competitive metal ions was remarkably high.

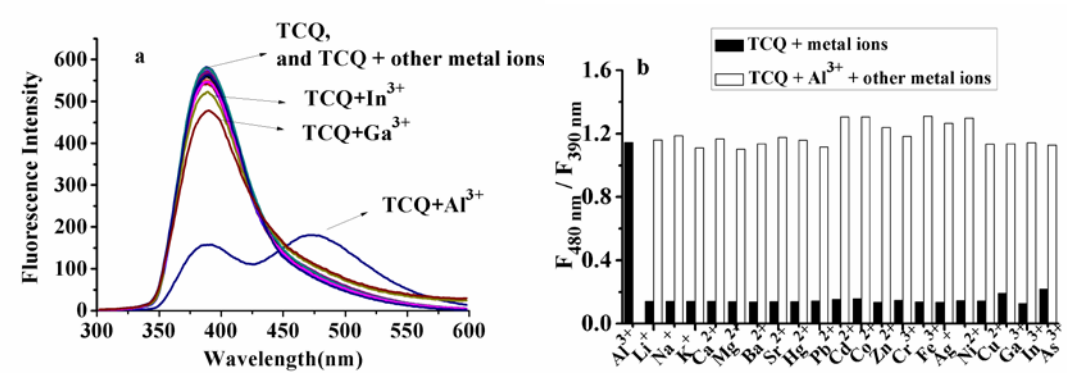



**Fig. 3.** (a) Fluorescence spectra of **TCQ** (10 μM, EtOH/$H_2O$, v/v, 2/3, pH 7) in the presence of 20 eq. of metal ions. (b) Ratio emission intensity of **TCQ** (10 μM). Black bars: ratio emission intensity of **TCQ** with the addition of the respective metal ions (20 eq.). White bars: ratio emission intensity of **TCQ** with the addition of the respective competing ions (20 eq.) and $Al^{3+}$ (20 eq.). Metal ions including $Al^{3+}$, $Li^+$, $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $Ba^{2+}$, $Sr^{2+}$, $Hg^{2+}$, $Pb^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Ag^+$, $Fe^{3+}$, $Cr^{3+}$, $Ga^{3+}$, $In^{3+}$ and $As^{3+}$. $\lambda_{ex}$ = 253 nm, $F_{480\ nm}/ F_{390\ nm}$.

## 3.2. The complexation of **TCQ** with $Al^{3+}$

3.2.1. Characteristics using isothermal titration calorimetry

Isothermal titration calorimetry (ITC) can provide complete thermodynamic parameters, offers a direct means of characterizing the thermodynamic properties between the interactions of the probe and ion. The binding of **TCQ** with $Al^{3+}$ was investigated by ITC in EtOH solution at 288.15 K (Fig. S5a) together with the integrated heats per mole of injectant (Fig. S5b). When $Al^{3+}$ concentration increased, the bonding process was shown to be exothermic and then tended to saturate. The binding enthalpy and entropy change ($\Delta H°$ = -23.43 ± 2.32 kJ·$M^{-1}$, $T\Delta S°$ = 6.74 ± 0.36 kJ·$M^{-1}$) indicated that the complexation of **TCQ** with $Al^{3+}$ was driven by both enthalpy and entropy. The values of $\Delta G°$ = -(30.17 ± 1.65) kJ·$M^{-1}$ are less than zero, which indicates that the binding processes are spontaneous. The stoichiometry of **TCQ** bound to $Al^{3+}$ was about 1.06 ± 0.026, and the binding constant (K) up to (1.61 ± 0.027) × $10^4$ $M^{-1}$. The experimental results of ITC are consistent with those of the fluorescence spectra.

3.2.2. $^1$H NMR and IR spectra

The formation of the **TCQ**·$Al^{3+}$ complex was monitored by $^1$H NMR spectroscopic titration. Fig. 4 shows the partial $^1$H NMR spectra of **TCQ** in $CD_3CN$ recorded in the presence of $Al^{3+}$. The resonances associated with the protons Ha, Hb, Hc, Hd, He and Hf revealed a gradual downfield shift of 0.19, 0.80, 0.37, 0.23, 0.10 and 0.08 ppm, respectively. All the protons of the quinoline moiety were also shifted downfield simultaneously, indicative of effective binding of $Al^{3+}$ to the nitrogen on the quinoline and nitrogen donor groups on the triazole ring. The significant downfield shifts of the methylene of quinaldine bridged triazole (Hd) protons (Δδ = 0.23 ppm) suggested strong coordination of the oxygen atoms with the $Al^{3+}$ ions. These overall shifts in the proton signals were observed upon addition of 2.0 eq. of $Al^{3+}$ to the solution of **TCQ**. These results suggested that two nitrogen atoms and one O atom provided a good binding site for $Al^{3+}$.

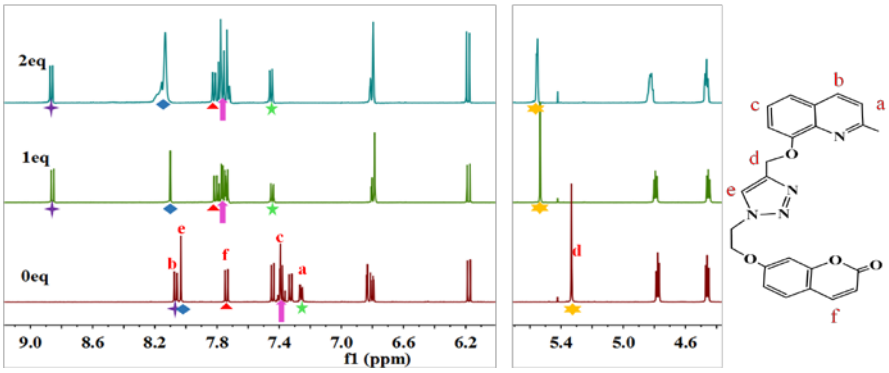



**Fig. 4.** Partial $^{1}$H NMR spectra in the absence and presence of $Al^{3+}$ for **TCQ**.

In order to demonstrate the stoichiometry between **TCQ** and $Al^{3+}$ ion, MALDI-TOF mass spectrometry was conducted (Fig. 5). Mass peaks at m/z 429.271 (calcd 429.15) corresponding to [**TCQ**+$Al^{3+}$]$^{+}$, and 456.228 (calcd 456.15) corresponded to [**TCQ**+H]$^{+}$ were clearly observed when $Al^{3+}$ was added to **TCQ**, which provided evidence for the formation of a 1:1 complex, and the results are consistent with the spectral data *vide infra*.

The IR spectra of **TCQ** and **TCQ**·$Al^{3+}$ mixtures were measured in EtOH medium (Fig. S6). On addition of $Al^{3+}$, the C=C bands for the quinoline ring at 1468 cm$^{-1}$, 1510 cm$^{-1}$ and 1610 cm$^{-1}$ clearly changed. The C-O band for the ether subunit of the quinoline moiety at 1098 cm$^{-1}$, 1130 cm$^{-1}$ and 1298 cm$^{-1}$ also changed dramatically, which indicated the participation of the ether subunit on the quinoline in the complexation of the probe with the ion. In addition, a band for N-$Al^{3+}$ appeared at 622 cm$^{-1}$, which further hinted at that the participation of the nitrogen atoms on the quinoline and nitrogen ring in the complexation of the probe with the ion. When combined with the $^{1}$H NMR and IR spectroscopy and the MS results, the observations were all consistent with the proposal that the oxygen and nitrogen atoms on the 8-hydroxyquinaldine and nitrogen moiety participated in the chelation with $Al^{3+}$, and all demonstrated the formation of the **TCQ**·$Al^{3+}$ complex.

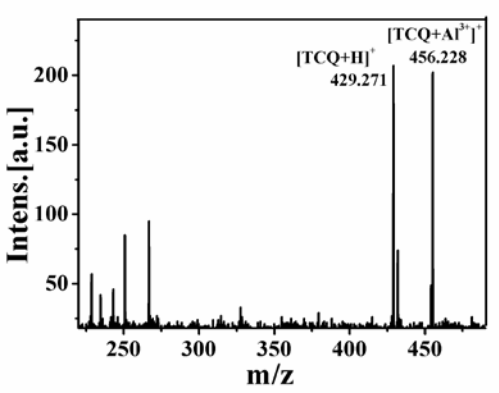
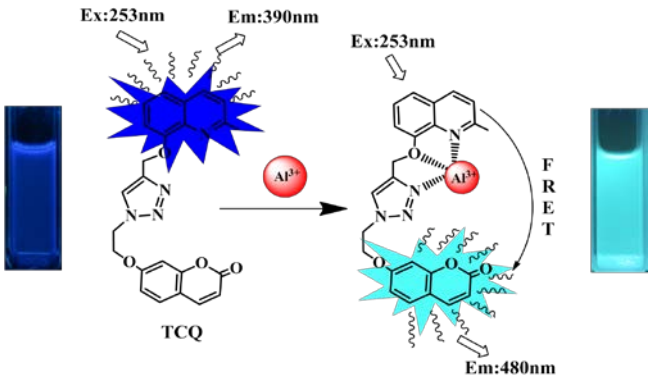



**Fig. 5.** MALDI-TOF spectrum spectrum of **TCQ** (2 mM, EtOH) in the presence of 2.0 equiv $Al^{3+}$.

### 3.3. The proposed mechanism

According to the above experiments, the FRET process was proposed to explain the fluorescence responses of the probe **TCQ** to $Al^{3+}$ as shown in Scheme 2. At 253 nm as excitation wavelength, before addition of $Al^{3+}$, the probe **TCQ** only showed an emission band for quinoline at 390 nm, whereas upon addition of $Al^{3+}$, because of the nitrogen and oxygen atoms on the 8-hydroxyquinaldine and triazole linker moiety participated in the chelation with $Al^{3+}$, a new emission band with a maximum at 480 nm appeared. This was ascribed to an $Al^{3+}$ induced FRET process from quinoline to coumarin. Thus, the excitation energy is passed from the quinoline moiety to the coumarin moiety, namely, the FRET process of **TCQ** is triggered by $Al^{3+}$. As a result, the emission at 390 nm for the quinoline decreased, and an enhancement of the characteristic fluorescence of coumarin at 480 nm emerged.




**Scheme 2.** Fluorescent **TCQ** for $Al^{3+}$ detection based on FRET.

3.4. The practical applicability of TCQ

3.3.1. Cell imaging

The fluorescence imaging of **TCQ** as an $Al^{3+}$ probe in living cells was examined. Incubation of HeLa or PC3 cells with 10 μM of **TCQ** for 60 min at 37 ºC gave almost no intracellular fluorescence as monitored by fluorescence microscopy. Fig. 6a, d are Bright-field, Fig. 6b, e are fluorescence measurements. When HeLa or PC3 cells were incubated with 10 μM **TCQ** in growth medium for 60 min, similar treatment with 50 μM $Al^{3+}$ for 50 min. generated remarkable intracellular fluorescence (Fig. 6c, f). The fluorescence imaging measurements after treatment with **TCQ** and $Al^{3+}$ confirmed that the cells were viable throughout the imaging experiments. The results suggested that **TCQ** can penetrate the cell membrane in a short time and can monitor intracellular $Al^{3+}$ in HeLa or PC3 cells by *in vitro* imaging and potentially *in vivo*.

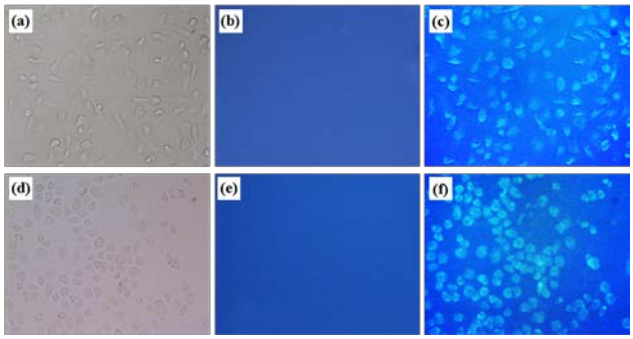



**Fig. 6.** (a, d) Bright field image of cells incubated with **TCQ** (10 μM, pH 7); cells incubated with **TCQ** in the absence of $Al^{3+}$ (b, e) and presence (c, f) of 50 μM of $Al^{3+}$ fluorescence image from the blue channel. a ~ c: HeLa cells; d ~f: PC3 cells.

3.3.2. Detection of $Al^{3+}$ in aqueous solutions

A practical application of **TCQ** was evaluated, namely the detection of $Al^{3+}$ in lake water samples under the optimized conditions (**TCQ**, 10 μM, EtOH/$H_2O$, 2/3, v/v, pH 7). All the measurements were performed three times. The results showed satisfactory recovery and R.S.D. values for all of the samples (Fig. S7, Tab. S1). These results demonstrated that the proposed probe **TCQ** meets the monitoring sensitivity as well as selectivity requirements necessary for screening environmental water samples.

3.3.3. Test strips of $Al^{3+}$

Furthermore, a filter paper of probe **TCQ** was prepared by immersing the filter paper into the solution of **TCQ** in EtOH (1 mM) and then exposing it to air to evaporate the solvent. After immersing in the $Al^{3+}$ aqueous solution for several seconds and drying in air (Fig. 7) the test strips showed an increase in fluorescence intensity with the increase concentration of $Al^{3+}$ under a UV lamp (365 nm). The detection limit of the test strips was $1\times10^{-6}$ M. Therefore, the test strips could conveniently detect $Al^{3+}$ in solution.

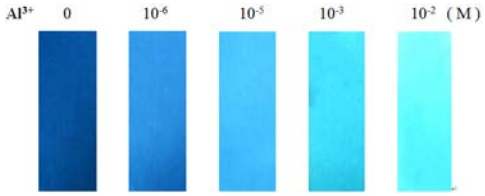



**Fig. 7.** Fluorescence changes of **TCQ** (1 mM, EtOH) paper test strips for detecting $Al^{3+}$ in aqueous solution with different $Al^{3+}$ concentrations ($10^{-6}$ M ~ $10^{-2}$ M, EtOH/$H_2O$, v/v, 2/3). The test papers were excited at 365 nm using a hand-held UV lamp.

## 4. Conclusion

A ratiometric fluorescent probe **TCQ** for response to $Al^{3+}$, which is based on an intramolecular FRET mechanism from the quinoline to coumarin moiety, has been designed and synthesized. The properties of **TCQ** and the previously studied $Al^{3+}$ probes are compared and listed in the table (Table S2). As expected, the ratiometric response of the $Al^{3+}$ probe exhibited clear variations in the fluorescence ratio, and signaled the binding event through occurrence of FRET mediated emission at 480 nm of the coumarin chromophore with enhancement in the fluorescence intensity. Concurrently, the characteristic emission of the quinoline donor at around 390 nm gradually decreased. This small-molecule fluorescent probe **TCQ** is not only suitable for fluorescent imaging of $Al^{3+}$ in living cells based on $Al^{3+}$ induced FRET response, but also detects $Al^{3+}$ with a very low detection limit in neutral aqueous medium. Furthermore, a simple and convenient test paper may provide an easy way to detect $Al^{3+}$ in daily life, which makes this a good candidate system for $Al^{3+}$-sensing. In this regard, a practical and universal FRET system to realize simultaneous selective and quantitative detection of $Al^{3+}$ ions was developed, mainly because of the good

complexion ability for metal ions and simple synthetic procedure employed. Moreover, this work may stimulate further development of new FRET probes possessing excellent performance.

## Acknowledgement

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# A ratiometric $Al^{3+}$ ion probe based on the coumarin-quinaldine FRET system

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# 1. Spectra copies

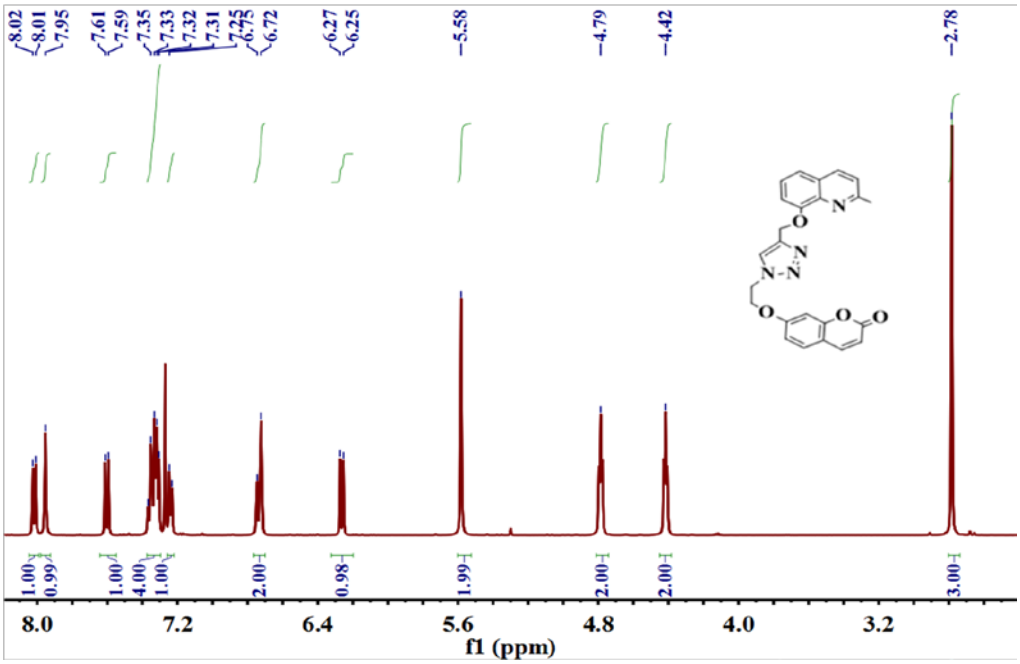


**Fig. S1.** $^{1}$H-NMR spectra of probe **TCQ** $CDCl_3$.

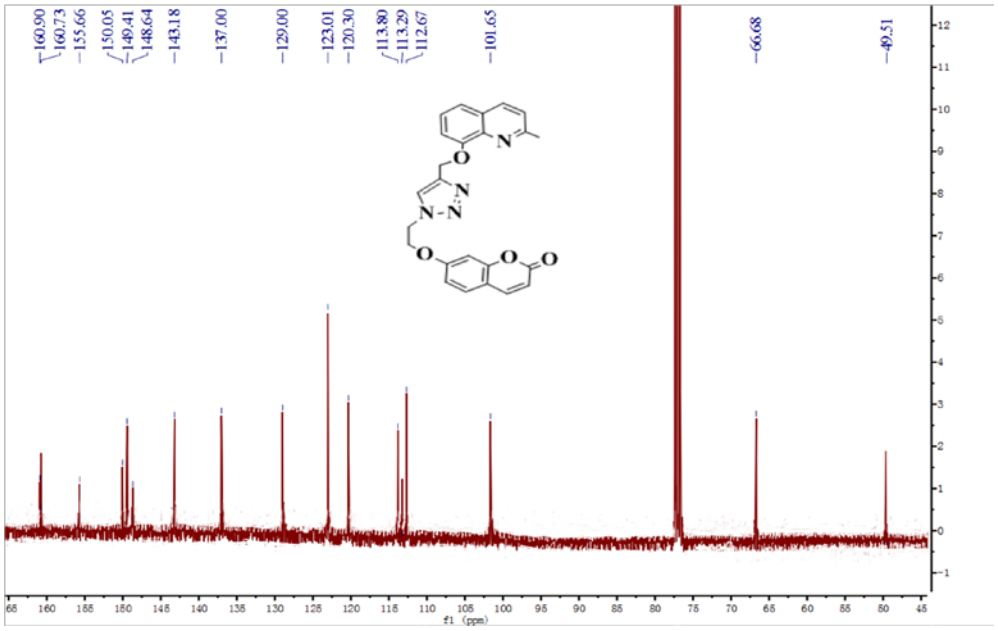


**Fig. S2.** $^{13}$C-NMR spectra of probe **TCQ** $CDCl_3$.

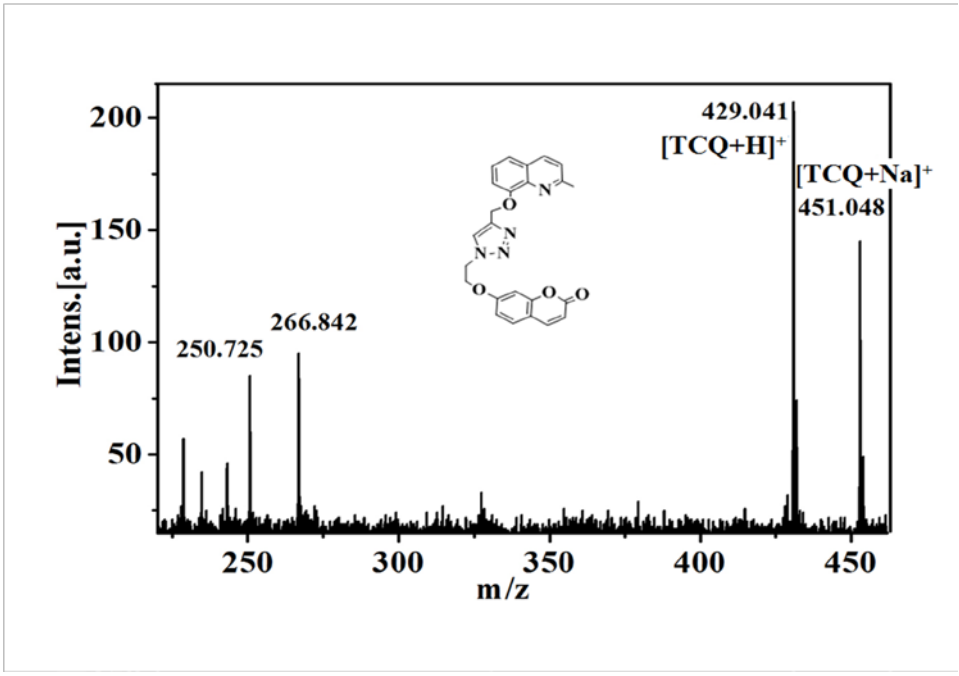


**Fig. S3.** MALDI-TOF mass spectra of probe **TCQ** with a-cyano-4-hydroxycinnamic acid as matrix.

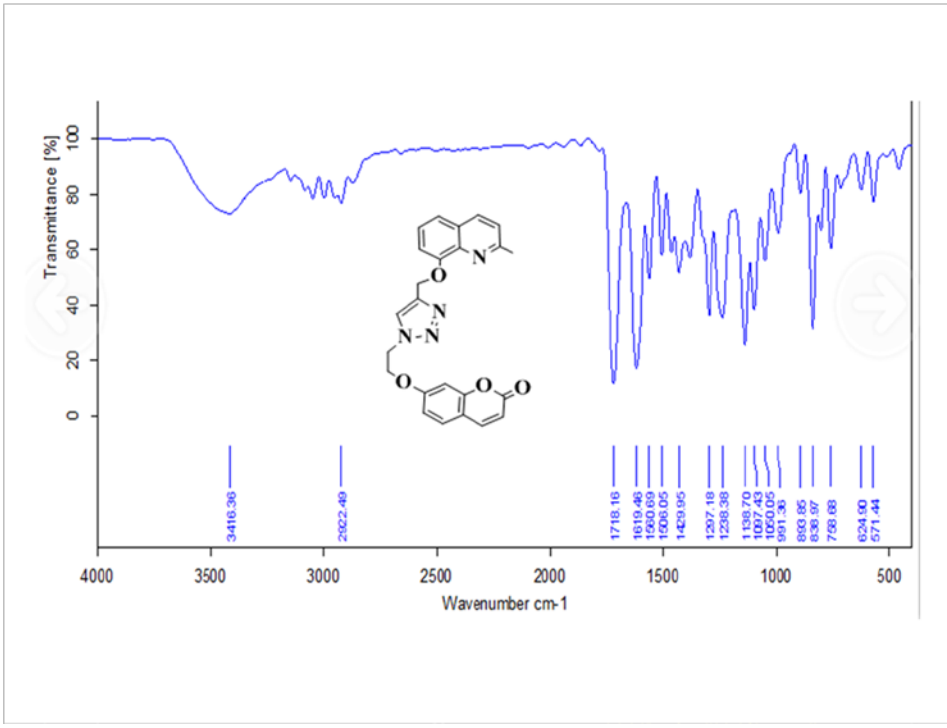


**Fig. S4.** IR spectra of probe **TCQ** EtOH.

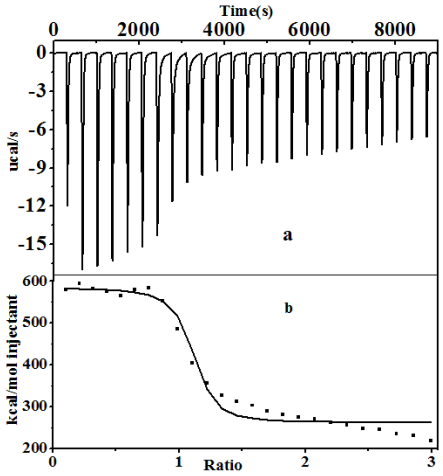


**Fig. S5.** ITC profile (a) for the binding of probe **TCQ** (0.1 mM, EtOH) to $Al^{3+}$ ions (1 mM) and its non-linear curve fitting (b).

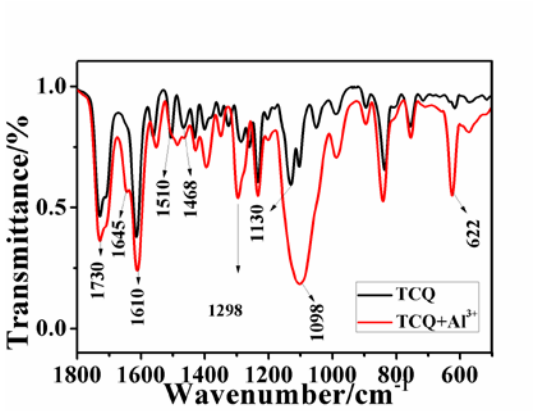


**Fig. S6.** IR spectrum of **TCQ** (2 mM, EtOH) in the presence of 2.0 equiv $Al^{3+}$.

## 2. Additional spectra

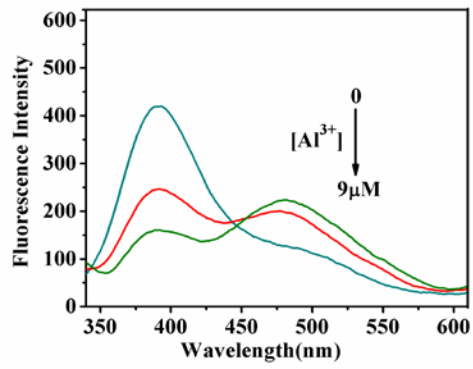


**Fig. S7.** Probe **TCQ** detect the fluorescence spectrum of $Al^{3+}$ in the lake water samples.

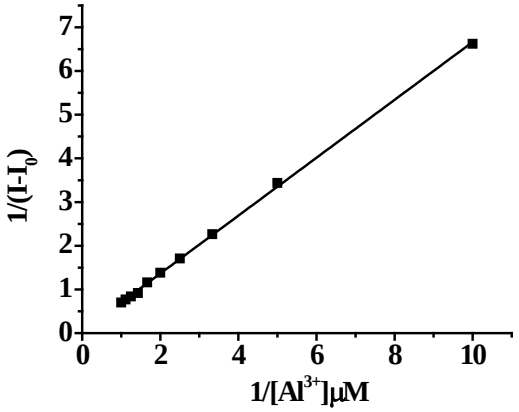


**Fig. S8.** Benesi-Hildebrand plot of Sensor **TCQ** assuming a 1:1 stoichiometry for association between Sensor **TCQ** and $Al^{3+}$ in EtOH/$H_2O$ (2/3, v/v, pH 7) solution by fluorescence spectroscopy. $\lambda_{ex}/\lambda_{em}$ = 253/480 nm, Y = 0.187 + 0.859 × $10^{-5}$ X, R = 0.987, K = A/B = 2.2× $10^4$ $M^{-1}$.

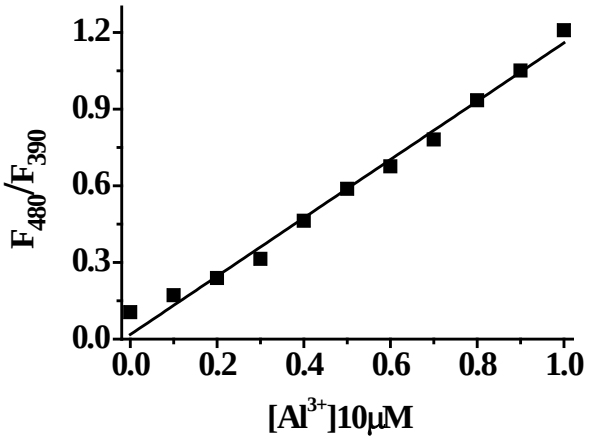


**Fig. S9.** The fluorescence intensity ratio of **TCQ** (10 μM, EtOH/$H_2O$, 2/3, v/v, pH 7) as a function of $Al^{3+}$ concentration. $\lambda_{ex}$ = 253 nm, $F_{480}/F_{390}$.

**Table S1.** Fluorescence spectrum determination of trace amounts of $Al^{3+}$ by **TCQ** in aqueous solution

| Sample | Amount of $Al^{3+}$ (μM) | Determination of $Al^{3+}$ (μM) | Recovery rate (%) | RSD (% n = 3) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Lake water samples* | 0.00 | 2.94 | 98 | 1.7 |
| | 3.00 | 6.22 | 104 | 2.8 |
| | 6.00 | 9.03 | 100 | 1.9 |
| | 9.00 | 11.88 | 99 | 3.6 |

* Deriving from Hua Xi river of Gui Zhou province.

**Table S2.** Comparison of **TQC** and others Probes in literatures

| | Our work (**TQC**) | Ref.[5] | Ref. [6] | Ref. [9] | Ref. [10] |
|---|---|---|---|---|---|
| Detection ions | $Al^{3+}$ | $Al^{3+}$ | $Al^{3+}$ | $Al^{3+}$ | $Al^{3+}$ |
| Detection solvent (v/v) | $C_2H_5OH/H_2O$, 2/3 | $C_2H_5OH/H_2O$, 95/5 | $CH_3OH$ | $C_2H_5OH$ | $C_2H_5OH$ / $H_2O$, 9/1 |
| Yield (%) | 70 | NA | 73 | 77.24 | 73 |
| Quantum yield | 0.41 | 0.102 | NA | NA | 0.12 |
| Detection limit (μM) | 0.24 | NA | NA | NA | 5.2 |
| Detection Method | ratiometric fluorescence Stoke's shift (~90 nm) | single wavelength | ratiometric fluorescence Stoke's shift (~37nm) | single wavelength | single wavelength |
| Recognition mechanism | FRET | PET | PET | PET | PET |
| Application | PC3 Cells, HeLa Cells, lake water samples, Test strips | NA | HeLa cells | NA | NA |

| | Ref. [11] | Ref.[13] | Ref. [14] | Ref. [19] | Ref. [33] |
|---|---|---|---|---|---|
| Detection ions | $Al^{3+}$ | $Al^{3+}$ | $Al^{3+}$ | $Al^{3+}$, $Fe^{3+}$, $Cr^{3+}$ | $Al^{3+}$, $Fe^{3+}$, $Cr^{3+}$ |
| Detection solvent (v/v) | $CH_3CN$ | $C_2H_5OH$ | DMSO/ $H_2O$,1/4 | $CH_3CN$/ $H_2O$, 1/1 | $C_2H_5OH$/ $H_2O$,4/1 |
| Yield (%) | 74 | 53 | 16 | 65 | NA |
| Quantum yield | 0.32 | NA | 0.49 | NA | 0.19 |

| | | | | | |
|---|---|---|---|---|---|
| Detection limit(μM) | NA | 0.22 | 0.19 | NA | 0.032 |
| Detection Method | single wavelength | single wavelength | single wavelength | ratiometric fluorescence Stoke's shift (~51 nm) | ratiometric fluorescence Stoke's shift (~85 nm) |
| Recognition mechanism | NA | PET process | NA | FRET system | FRET system |
| Application | NA | NA | Pollen cells Candida cells | W138 cells | Allamanda puberula cells |

NA= Not Available