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8-Aminoquinoline-based ratiometric zinc probe: Unexpected binding mode and its application in living cells

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ABSTRACT

PMQA, an 8-aminoquinoline-based ratiometric fluorescent sensor, demonstrates the Zn^{2+}-induced red-shift of emission (85 nm), and was successfully applied to image zinc in living cells. Compared to 2:1 stoichiometry in **PMQA**–Zn^{2+}, **PMQA**–Cu^{2+} shows 1:1 composition. Both nitrogen atoms from the aminoquinoline are missing in binding of zinc, while they are critically involved in Cu^{2+} chelation. The structure difference between **PMQA**–Zn^{2+} and **PMQA**–Cu^{2+} might shed light in designing novel zinc probes without suffering from copper interference.

Zinc, the second most abundant *d*-block metal ion in human body, is an essential element and plays multiple roles in various biological processes such as maintaining brain function, regulating gene expression, and keeping mammalian reproduction.[1] Disturbance of the zinc homeostasis in living organisms is critically related to a series of disease.[2] Therefore, the detection of zinc has attracted increasing interests.[3] Among numerous methods, fluorescent sensors, due to the simplicity, high sensitivity, and super biocompatibility, are a powerful tool in detection of zinc both in vitro and in vivo.[3,4] Particularly, ratiometric fluorescent probes permit signal rationing to detect target molecules by measuring the ratio of fluorescence intensities at two different wavelengths, which could be independent of environmental effects.[4c,d,5] And thus, the past decades have witnessed a fruitful acquisition from different groups.[3,4] However, a majority of these sensors suffer from diverse drawbacks, such as selectivity, sensitivity, water solubility as well as cell membrane permeability, which prevent them from further application in living cells or animals.

8-Aminoquinoline (AQ) was commonly employed as fluorophore for metal ion sensors based on internal charge transfer (ICT), which is one of the most important signaling mechanism in designing metal ion sensors.[5b,6] In this sense, our previous probe, 2-(bis(2-(ethylthio)ethyl)amino)-*N*-(quinolin-8-yl)acetamide (**BEAQA**), bearing N/S/S heteroatoms as a receptor had designed and synthesized, and successfully applied in imaging both exogenous and endogenous zinc in living HeLa cells.[6f] However, the stability of the probe has harassed us for its broad application since the two sulfide groups in the molecule are prone to oxidation during storage. To surmount this shortage, a new fluorescent probe **PMQA** (Scheme 1) was prepared. The synthesis of sensor **PMQA** is depicted in Scheme 1 according to the reported procedures with some modifications.[5b] The probe **PMQA**, obtained in two steps with 62.4% overall yield starting from readily available AQ, was fully characterized (Fig. S1–S6).

The absorbance and emission spectra of **PMQA** were examined by UV–vis absorbance and fluorescence titrations. Two bands (~240 nm & ~302 nm) were both red-shifted with the addition of Zn^{2+} (0–1.0 equiv) in the absorption spectra, accompanying three isosbestic points at 245, 280 and 325 nm (Fig. 1A). The absorption spectra is saturated by 0.5 equiv of Zn^{2+} (inset in Fig. 1A), which is different from our previous zinc probe **BEAQA**, whose spectra gets saturated by 1 equiv of Zn^{2+}. Fluorescence spectra of Zn^{2+} titration were shown in Figure 1B. **PMQA** has a very weak fluorescence emission at about 415 nm with excitation at 325 nm. Upon addition of Zn^{2+}, it displays a 85 nm red-shift of emission to 500 nm and a remarkable enhancement of green fluorescence intensity with a concomitant decrease of the weakly intrinsic fluorescence, displaying a typically ratiometric fluoresce character. The quick changes of the emission wavelength as well as intensity upon zinc addition can be explained by a nearly planar molecule conformation and a large π-electron conjugation system of **PMQA**–Zn^{2+} complex (vide infra), which further enhance the ICT process. The inset in Figure 1B shows the fluorescence intensity ratio (F_{500}/F_{415}) as a function of equivalent of Zn^{2+}. Again, the values of F_{500}/F_{415} reach a plateau by ~0.5 equiv of Zn^{2+}. Both absorbance and emission spectral titrations suggest **PMQA** might form a 2:1 complex with zinc. It is worth noting that this fluorescence titration experiment was performed in a pure aqueous solution, which is improved from other probes determined in organic solvent

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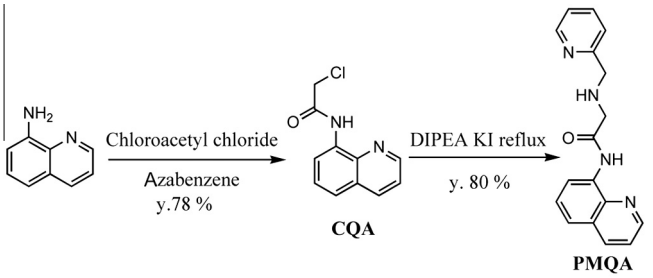

Scheme 1. Synthesis of **PMQA**.

mixed solutions,[5b,6a,c,e,7] and thus has potential application in biological imaging. The solubility of the probe **PMQA** in Tris–HCl (50 mM, pH 7.4) buffer (TB) was determined to be more than 1 mM (3 mg/10 ml) by directly suspending the solid powder in the aqueous solution.

The selectivity of **PMQA** toward different metal ions was investigated as shown in Figure 2. There is no significant influence by addition of 10 equiv of NaCl, $KClO_4$, $CaCl_2$, $MgCl_2$, $Al(ClO_4)_3$ or 1 equiv of $AgClO_4$, $Ni(ClO_4)_2$, $Fe(ClO_4)_3$, $Mn(ClO_4)_2$, $HgCl_2$, $CrCl_3$, $Co(ClO_4)_2$, $Cu(ClO_4)_2$, $Zn(ClO_4)_2$. Addition of $Cd(ClO_4)_2$ caused a slight enhancement of the intensity (~threefold), as zinc and cadmium are in the same group of the periodic table and have similar properties. Compared to a 15-fold increase of emission induce by zinc, **PMQA** easily distinguishes zinc from cadmium. Also, cadmium is an ultratrace element, and has extremely low concentration in human bodies. Taken together, the trivial interference caused by cadmium in vitro should not prevent the application of probe **1** for imaging zinc in vivo. Next, we determined if **PMQA** can recognize zinc when it coexists with other metal ions. As shown in Figure 2, Co^{2+} and Cu^{2+} almost completely quench the fluorescene signal, while other ions have little interference. This is probably due to the displacement of zinc by Co^{2+} and Cu^{2+} since nitrogen-containing lignands usually have higher affinity for them. Many zinc probes exhibited similarly unfavorable responses due to the competition from those ions.[6c,8,11] Copper and cobalt belong to essential elements. However, their concentrations are usually more than 10 times lower than that of zinc in vivo,[1c] and their influence could be neglected. The probe **PMQA** is also a sensitive sensor for other zinc metal sources demonstrated in Figure S7.

Quenching of emission by Cu^{2+} is a depressing yet very common drawback for diverse zinc sensors, which is usually caused by displacement of zinc from the sensors by copper.[6a,6c,8b–f,9,11] Subsequently, we examined the fluorescence response of **PMQA**–Zn^{2+} complex toward Cu^{2+} in detail. Remarkable fluorescence quenching of **PMQA**–Zn^{2+} complex was observed dose-dependently upon

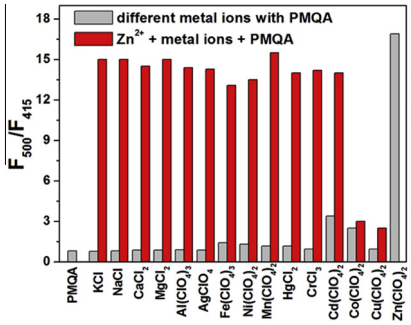

Figure 2. Metal ion selectivity of **PMQA**. Light grey bars: fluorescence responses of **PMQA** (10 μM) and upon the addition of different metal ions in TB. Dark grey bars (red bars): fluorescence responses of **PMQA** (10 μM) with different metal ions followed by addition of Zn^{2+} (10 μM), λ_{ex} = 325 nm.

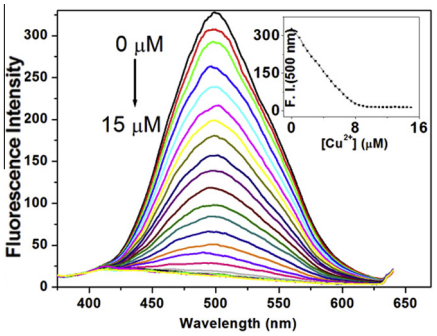

Figure 3. Fluorescence titration spectra of **PMQA**–Zn^{2+} complex (5 μM) in TB upon addition of increasing concentrations of $Cu(ClO_4)_2$ (0–1.5 equiv calculated based on the amount of **PMQA**). The inset shows decay of fluorescence intensity at 500 nm with increasing [Cu^{2+}], λ_{ex} = 325 nm.

addition of Cu^{2+} (0–1.5 equiv calculated based on the amount of **PMQA**), and complete quenching achieved when more than 1.0 equiv of Cu^{2+} was added (Fig. 3), indicating a 1:1 complex formed between **PMQA** and Cu^{2+}, which is consistent with its crystal structure (vide infra).

To further determine the binding stoichiometry of **PMQA**–Zn^{2+} complex, we adapted the Job's plot based on the fluorescence titration. The maximal fluorescence intensity appears at about 0.39 mol fraction (Fig. S8), supporting that the **PMQA**–Zn^{2+} complex has 2:1 stoichiometry. The most convincing evidence was provided by the crystal structure of **PMQA** complexed with zinc (Fig. 4A), which unambiguously demonstrates that two molecules of **PMQA** in a head-to-tail orientation, acting as a hexadentate ligand via N atoms

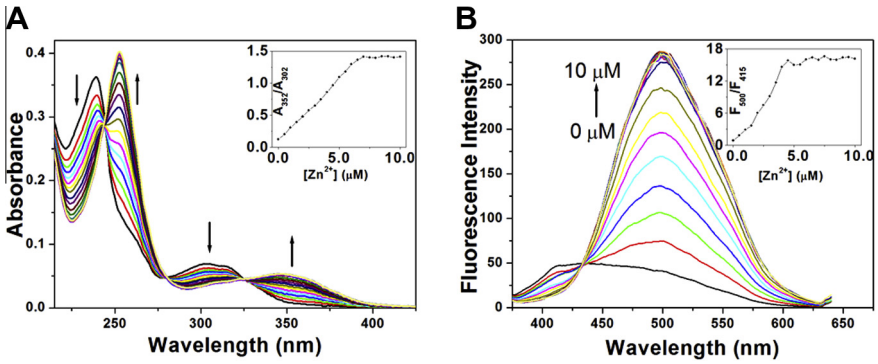

Figure 1. Absorption (A) and fluorescence (B) titration spectra of **PMQA** (10 μM) in TB with increasing concentrations of $Zn(ClO_4)_2$ (0–1.0 equiv), inset: the titration profile based on the absorbance ratio at $A_{352\ nm}/A_{302\ nm}$ and the fluorescence intensity ratio at $F_{500\ nm}/F_{415\ nm}$, λ_{ex} = 325 nm.