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# A novel dual-emission ratiometric fluorescent nanoprobe for sensing and intracellular imaging of $Zn^{2+}$

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ABSTRACT

The integration of unique characteristics of nanomaterials with highly specific recognition elements, such as biomolecules and organic molecules, are the foundation of many novel nanoprobes for bio/chemical sensing and imaging. In the present report, branched polyethylenimine (PEI) was grafted with 8-chloroacetyl-aminoquinoline to synthesize a water-soluble and biocompatible quinoline-based $Zn^{2+}$ probe PEIQ. Then the PEIQ was covalently conjugated to $[Ru(bpy)_3]^{2+}$-encapsulated SiNPs to obtain the ratiometric fluorescence nanoprobe which exhibits a strong fluorescence emission at 600 nm and a negligible fluorescence emission at 500 nm in the absence of $Zn^{2+}$ upon a single wavelength excitation. After the addition of different amounts of $Zn^{2+}$, the fluorescence intensity at 500 nm increased continuously while the fluorescence intensity at 600 nm remained stable, thus changing the dual emission intensity ratios and displaying continuous color changes from red to green which can be clearly observed by the naked eye. The nanoprobe exhibits good water dispersivity, biocompatibility and cell permeability, high selectivity over competing metal ions, and high sensitivity with a detection limit as low as 0.5 μM. Real-time imaging of $Zn^{2+}$ in A549 cells has also been realized using this novel nanoprobe.



## 1. Introduction

As the second most abundant transition metal in the human body, zinc always occurs as a divalent cation [Zn (II)] and plays pivotal roles in biological systems (Falchuk, 1998; O'Halloran, 1993). For example, $Zn^{2+}$ serves as catalytic and structural cofactor when it is bound to some specific proteins, thus facilitating enzyme regulation, gene expression and neural related signal transmission (Vallee and Falchuk, 1993). Although it remains unclear about its functional role, growing evidence suggests the correlations between the disorder of $Zn^{2+}$ metabolism and many neurological diseases, including Alzheimer's disease, infantile diarrhea, epilepsy and cerebral ischemia (Adlard and Bush, 2006; Koh et al., 1996). Because of its important biological roles, research interests to develop highly sensitive and selective detection and monitoring of $Zn^{2+}$ under physiological conditions are growing unabated (Lim et al., 2004). Fluorescence is believed to be the most effective way to determine the concentration, together with visualization of subcellular distribution of $Zn^{2+}$ in living cells (Hanaoka et al., 2004; Jiang and Guo, 2004; Walkup et al., 2000; Xie et al., 2012). Among the fluorescent probes for $Zn^{2+}$ ions detection, quinoline based molecules, especially 8-aminoquinoline and 8-hydroxy-quinoline, have been studied extensively because they are pH insensitive, ready to form strong inter-molecular hydrogen bonds with surrounding water molecules, and their metal-coordination. Moreover, quinoline based $Zn^{2+}$ sensors are suitable candidates for photofunctional molecular devices because their sensing mechanism is mainly on the basis of photo-induced electron transfer and photo-induced charge transfer processes (Du et al., 2010; Xie et al., 2011; Zhou et al., 2010). However, most of these quinoline-based probes targeting $Zn^{2+}$ ions still suffer from poor solubility, bad cell permeability, and weak sensitivity. And a few examples of ratiometric fluorescence methods for sensing and intracellular imaging of $Zn^{2+}$ have been reported (Jiang and Guo, 2004; Liu et al., 2012; Raje et al., 2013).

Recently, the conjugation of fluorescent probes with nanoparticles has emerged as an attractive strategy to develop new chemosensors for intracellular monitoring of $Zn^{2+}$, since nanoparticles can shelter the probes from interferences and can transport the probes neglecting their intrinsic solubility (Li et al., 2011; Lu et al., 2012; Rastogi et al., 2011; Ruedas-Rama and Hall,

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2008; Pal et al., 2011; Teolato et al., 2007; Xu et al., 2013). Due to their high luminescence quantum yield, good photostability and water dispersivity, biocompatibility and versatile surface modification chemistry, $[Ru(bpy)_3]^{2+}$-encapsulated silica nanoparticles (SiNPs) become an excellent choice for developing bio/chemo nanosensors and diagnostic nanoprobes in bio/chemical analysis (Santra et al., 2001; Shi et al., 2013a, 2013b; Wang et al., 2013). Herein, in the present report, branched polyethylenimine (PEI) was firstly grafted with 8-chloroacetyl-aminoquinoline to synthesize a water-soluble and biocompatible quinoline-based $Zn^{2+}$ probe PEIQ. Then PEIQ was covalently conjugated onto the surface of $[Ru(bpy)_3]^{2+}$-encapsulated SiNPs to develop a selective and sensitive ratiometric strategy for the sensing and monitoring of $Zn^{2+}$ both *in vitro* and *in vivo*. The nanoprobe exhibits high selectivity over competing metal ions, high sensitivity with a detection limit as low as 0.5 μM, and suitability of real-time $Zn^{2+}$ imaging in A549 cells. Notably, the reported nanoprobe possesses some remarkable features: (1) the recognition moiety is synthesized by grafting branched PEI with quinoline derivative, thus reducing the possible cytotoxicity of PEI and increasing water dispersivity and cell permeability of the as-prepared $Zn^{2+}$ probe, PEIQ; (2) the internal standard is encapsulated into SiNPs and the recognition moiety is covalently conjugated onto SiNPs surface, thus providing a reliable reference signal and a stable nanoprobe; (3) the large amount of PEIQ units on the outer surface of an individual nanoparticle enable the signal amplification, hence making the immediate and highly sensitive detection of $Zn^{2+}$ possible.

## 2. Materials and methods

### 2.1. Apparatus and reagents

Morphology of the nanoparticles was examined by a transmission electron microscope (TEM, JEOL JEM-1400). DLS measurements were performed at 25 °C using a Malvern Zetasizer NanoZS90 instrument. UV–vis, FTIR, and fluorescence spectra were obtained on a Beckman DU730 UV–vis spectrometer, a VERTEX 70 spectrometer (Bruker), and a PTI Quanta-Master QM4CW spectrofluorometer, respectively. $^1H$ NMR spectra were recorded with a Varian Mercury-400 spectrometer with $Me_4Si$ as the internal standard. The fluorescence images were acquired by an inverted fluorescence microscope (OLYMPUS IX71). The metal ions solutions were prepared from NaCl, KCl, $MgCl_2$, $CaCl_2$, $FeCl_3$, $Pb(NO_3)_2$,$CoCl_2$, $NiCl_2$, $ZnCl_2$, $HgCl_2$, $CrCl_3$ and $CuSO_4$ in distilled water with a concentration of 0.01 M or 0.001 M.

### 2.2. Synthesis of 8-chloroacetylaminoquinoline

8-chloroacetylaminoquinoline was synthesized according to a previously reported method (Zhou et al., 2010). 288 mg of 8-aminoquinoline (2 mM) and 202 mg (2.1 mM) of $N(Et)_3$ were added into 10 mL of $CH_2Cl_2$ and mixed in a round flask for 20 min at 0 °C. Then 246 mg (2.2 mM) of chloroacetyl chloride was added dropwise. The mixture was warmed to room temperature and allowed to react overnight. The solvent was evaporated in vacuum. The crude product was further purified by column chromatography (silica gel, PE/EA at 3:1) and a pale white solid was obtained.

### 2.3. Synthesis of PEIQ

A mixture of PEI (MW = 1800) (500 mg, 0.28 mM) and $K_2CO_3$ (38.4 mg, 0.28 mM) in 5 mL $CH_3CN$ was added to 20 mL of 8-chloroacetylaminoquinoline (44 mg, 0.2 mM) in $CH_3CN$ and refluxed for 8 h under $N_2$. Then the solvent was evaporated in vacuum. The crude product was purified by co-precipitation with ether to give yellow oil.

### 2.4. Synthesis and functionalization of fluorescent SiNPs

The fluorescent $[Ru(bpy)_3]^{2+}$-encapsulated SiNPs were synthesized by a water-in-oil reverse micelle method as described in detail elsewhere. Typically, 7.5 mL of cyclohexane, 1.77 mL of Triton X-100, 1.8 mL of hexanol, and 0.34 mL of $H_2O$ were stirred for 20 mins to generate the microemulsion system, followed by the addition of 80 μL of 0.1 M $[Ru(bpy)_3]^{2+}$ and 100 μL of tetraethoxy orthosilicate (TEOS). After being stirred for 30 min, silica polymerization was initiated by the addition of 60 μL aqueous ammonia and allowed to react for 24 h. Then, in order to prepare carboxylated SiNPs (SiNP-COOH), 50 μL TEOS and 50 μL carboxyethylsilanetriol sodium salt (CTES) were added to the mixture and the reaction was allowed to continue for another 24 h. Finally, acetone was added to destabilize the micro-emulsion system. The fluorescent SiNPs were isolated *via* centrifugation and washed in sequence with ethanol and D.I. water to remove any surfactant and unreacted reactants.

Carbodiimide chemistry was employed to covalently conjugate PEIQ onto SiNPs. Briefly, 0.1 g of SiNP-COOH and 0.05 g of PEIQ were suspended in 20 mL of 0.01 M phosphate-buffered saline (PBS) buffer (pH = 7.4) containing 5 mM N-hydroxy-succinimide (NHS) and 2 mM 1-ethyl-3-(3-dimethyl- aminopropyl) carbodiimide (EDC). Then the mixture was allowed to react at room temperature for another 16 h under gentle shaking. The final nanoprobes were obtained *via* centrifugation and were washed in sequence with D.I. water to remove any of the unreacted reactants.

### 2.5. Sensitivity and selectivity of the SiNPs–PEIQ nanoprobe

For the detection of $Zn^{2+}$, different concentrations of $Zn^{2+}$ (0, 1.0, 2.0, 4.0, 6.0, 10.0, 15.0, 20.0, 30.0, 50.0, and 100.0 μM) were mixed with the nanoprobe (2.0 μg $mL^{-1}$) in 1.2 mL of distilled water under gentle shaking. Photographs and fluorescence spectra were taken after $Zn^{2+}$ reacted with the nanoprobe for 5 mins. To evaluate the selectivity of the nanoprobe, 20.0 μM of amino acids or 50.0 μM of competing metal ions was mixed with the SiNP–PEIQ (2.0 μg $mL^{-1}$) under gentle shaking. Fluorescence spectra were taken after the metal ions or amino acids reacted with the dispersed nanoprobe for 5 mins.

### 2.6. Intracellular imaging of $Zn^{2+}$

The A549 cells were provided by Cells Bank of the Chinese Academy of Science (Shanghai, China). Cells were grown in H-DMEM (high glucose) supplemented with 10% FBS in an atmosphere of 5% $CO_2$ at 37 °C. Cells ($10^5$/well) were plated on 6 mm glass coverslips and allowed to adhere for 24 h. Intracellular imaging of $Zn^{2+}$ were performed in the same medium supplemented with 50 μM $ZnCl_2$ for 0.5 h. A549 cells were washed with PBS and incubated with the nanoprobe (10.0 μg $mL^{-1}$) at 37 °C for 2 h. Cell imaging was performed with an inverted fluorescence microscope after washing the cells with PBS.

## 3. Results and discussions

### 3.1. Synthetic routes for the SiNPs–PEIQ nanoprobe and its sensing mechanism

As illustrated in Scheme 1, 8-chloroacetyl-aminoquinoline firstly reacted with branched PEI to synthesize a novel receptor that selectively binds $Zn^{2+}$, namely PEIQ. Then PEIQ was covalently conjugated to fluorescent SiNPs by the reaction between