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Design and Synthesis of a Ratiometric Fluorescent Chemosensor for Cu(II) with a Fluorophore Hybridization Approach

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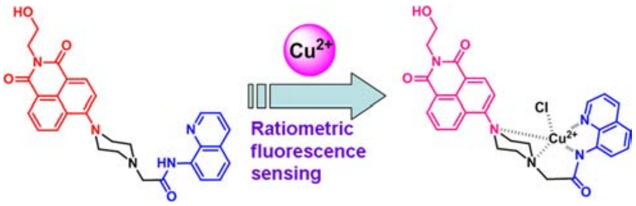
ABSTRACT



A new ratiometric fluorescent sensor for Cu^{2+}, WLN, has been developed via integrating a 1,8-naphthalimide fluorophore with 8-aminoquinoline. WLN exhibits a highly selective ratiometric response to Cu^{2+} over other transition metal ions in aqueous media. Moreover, its practical ratiometric imaging ability for intracellular Cu^{2+} has been confirmed in human breast adenocarcinoma cells (MCF-7 cells) using a confocal microscope.

Development of fluorescent sensors for chemical species of biological and environmental significance is currently an attractive field for scientists.[1] As the third most abundant transition metal ions in the human body, Cu^{2+} plays vital roles in various biological processes, and its homeostasis is critical for the metabolism and development of living organisms.[2] The Cu^{2+} disorder in its uptake, storage, and trafficking was proposed to be associated with certain diseases such as Menkes syndrome,[3] Wilson's disease,[4] and Alzheimer's disease[5] owing to the aberrant oxidative and nitrosative stress induced by Cu^{2+}. Moreover, long-term exposure to high levels of Cu^{2+} has been reported to induce liver and kindey damage.[6] According to the U.S. Environmental Protection Agency (EPA), the maximum acceptable level of Cu^{2+} in drinking water is ~20 μM.[7] Therefore, there is considerable interest in developing specific fluorescent sensors for sensitive Cu^{2+} detection, especially in environmental and physiological conditions.

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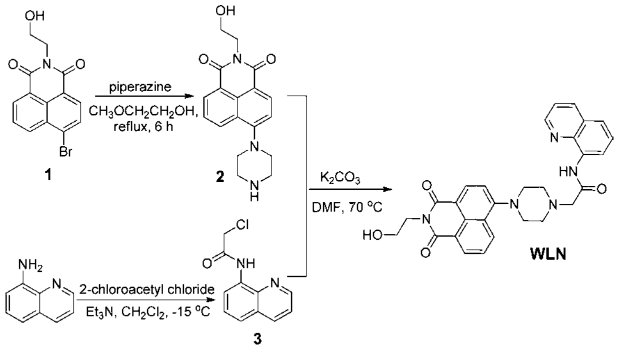
A number of fluorescent Cu^{2+} sensors have been reported, and some of them have been successfully applied both in biological and in environmental samples.[2d,8] Most of these reported sensors sense Cu^{2+} via the mechanism of Cu^{2+} induced chemical reactions or Cu^{2+} coordination. Due to the paramagnetic nature of Cu^{2+}, Cu^{2+} coordination often results in fluorescence quenching, and very few systems have been found to exhibit fluorescence enhancement.[8a,b] To overcome the quenching nature of Cu^{2+}, several turn-on chemodosimeters have been developed via the specific Cu^{2+}-induced reaction to form a fluorophore.[8c−h] For example, Cu^{2+}-induced opening of the spirolactam of xanthenes and related derivatives have been well developed for the construction of turn-on sensors for Cu^{2+}.[8h] However, most of these fluoresent chemodosimeters displayed a slower and irreversible response to Cu^{2+} when compared with those from the Cu^{2+} coordination mechanism. This drawback limited the application of these chemodosimeters especially in the real-time detection of Cu^{2+} flunctuation in biological samples, and reversible fluorescent Cu^{2+} sensor functioning directly via Cu^{2+} coordiantion is more appealing. However, quantitative Cu^{2+} detection in living systems requires a sensor displaying a ratiometric response for Cu^{2+} to reduce the interference ascribed to deviations in detecting parameters and microenvironments. Different approaches have been proposed to realize ratiometric Cu^{2+} sensing and overcome the emission quenching nature of Cu^{2+}, and a few ratiometric Cu^{2+} sensors have been reported.[8i−p] However, diversified and complicated biological systems demand ratiometric Cu^{2+} sensors of differnt natures to satisfy Cu^{2+} imaging in differnt microenvironments, and exploring a new rationale to construct ratiometric Cu^{2+} fluorescent sensors of different properties for quantitative Cu^{2+} detection is of great significance and challenging. In this communication, we describe a new ratiometric fluorescent Cu^{2+} sensor, **WLN**, which was constructed by integrating a 4-amino-1,8-naphthalimide (ANP) fluorophore with an 8-aminoquinoline (AQ) fluorophore.

Scheme 1. Synthesis of **WLN**



In **WLN**, ANP was adopted as a fluorophore due to its long emission wavelength, large Stokes shift, and pH innertness under near-neutral conditions.[9] The second fluorophore 8-aminoquinoline (AQ) was incoporated into ANP as a Cu^{2+} ionophore via a piperazine linker. Since there is a large overlap between the excitation spectra of ANP (340−420 nm) and AQ (300−410 nm),[10] the same excitation wavelength would excitate the emission of ANP and AQ fluorophores simutaneously. It was envisioned that Cu^{2+} coordination to AQ might alter the emission ratio of ANP and AQ, offering the Cu^{2+} ratiometric sensing ability. The synthesis of **WLN** is depicted in Scheme 1. **WLN** was synthesized through a three-step procedure from the starting materials 4-bromo-1,8-naphthalimide and AQ. **WLN** was well characterized by ^1H, ^{13}C NMR, ESI-MS, and elemental analysis (see Supporting Information).

As shown in Figure 1, free **WLN** in HEPES buffer (50 mM HEPES; ethanol/H_2O = 35:65, v/v; pH 7.2) shows two absorption bands centered at 252 nm (band **A**, $\varepsilon = 2.3 \times 10^4\ M^{-1}\ cm^{-1}$) and 403 nm (band **B**, $\varepsilon = 1.0 \times 10^4\ M^{-1}\ cm^{-1}$), which can be assigned respectively as a $\pi-\pi^*$ transition and an ICT band. Upon Cu^{2+} addition (0−1.5 equiv), band **A** increased gradually, while broad band **B** underwent a slight decrease and minor hyposochromic shift, which can be ascribed to the decrease of electron-donating ability induced by Cu^{2+} coordination. The linear increase of absorbance at 252 nm with $[Cu^{2+}]_{total}$ up to a molar ratio ($[Cu^{2+}]_{total}$/[**WLN**]) of 1:1 and the stable spectrum at even higher $[Cu^{2+}]_{total}$ implied a 1:1 Cu^{2+} binding stoichiometry of **WLN**.

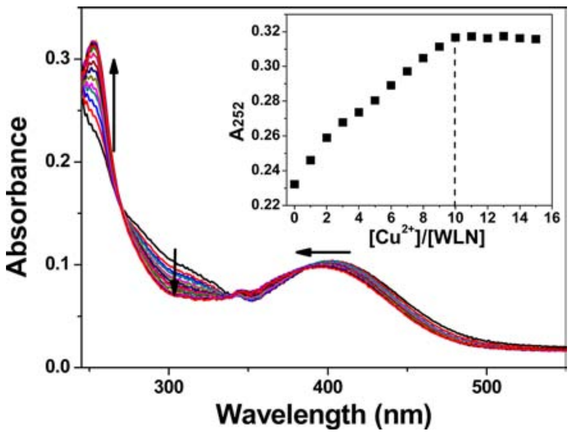


Figure 1. Absorption spectra of **WLN** (10 μM) in HEPES buffer (50 mM, pH 7.2) obtained by adding aliquots of 25 μL of $CuCl_2$ (1.2 mM) solution. Inset, the titration profile based on the absorbance at 252 nm.

When excited at its excitation maximum of 395 nm, **WLN** showed two characteristic fluorescence bands centered at 435 and 526 nm, which can be assigned to the emission band of AQ and ANP, respectively. Cu^{2+} titration demonstrated a distinct emission decrease of the band centered at 526 nm, while the emission band of 435 nm remained almost intact. The emission ratio at 435 and 526 nm (F_{435}/F_{526}) increased linearly with $[Cu^{2+}]_{total}$ from 0.15 to 0.41 until the $[Cu^{2+}]_{total}$/[**WLN**] ratio reached 1:1.

After that, the emission spectrum of **WLN** became stable. The Cu^{2+} titration profile according to F_{435}/F_{526} is consistent with a 1:1 Cu^{2+} binding stoichiometry disclosed by UV−vis titration (Figure 2). The association constant was calculated to be $2.9 \times 10^4\ M^{-1}$ according to the fluorescence titration profile (Figure S4).

The sensing selectivity of **WLN** toward Cu^{2+} was evaluated by adding 1 equiv of various metal ions including Hg^{2+}, Cd^{2+}, Pd^{2+}, Cu^{2+}, Co^{2+}, Ag^{+}, Ni^{2+}, Mn^{2+}, Fe^{2+}, Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+}, respectively. As shown in Figure 3, the addition of other metal cations did not distinctly alter the emission ratio (F_{435}/F_{526}) of **WLN** except for the addition of Cu^{2+}. Moreover, the ratiometric sensing behavior of **WLN** to Cu^{2+} experienced no interference by the presence of other metal ions.

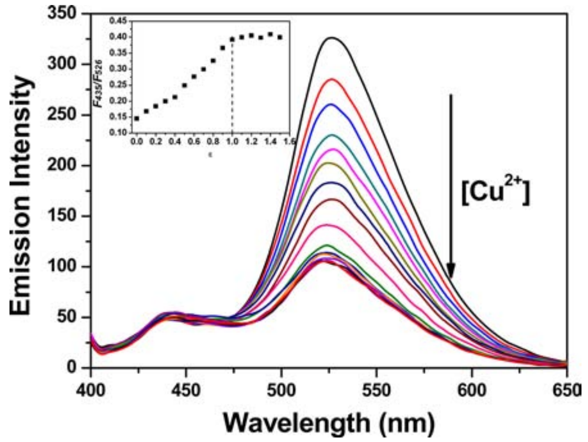


Figure 2. Emission spectra of **WLN** (10 μM) in HEPES buffer (50 mM, pH 7.2) obtained by adding aliquots of 25 μL of $CuCl_2$ (1.2 mM) solution. Inset, the titration profile based on the emission ratio at 435 and 526 nm, F_{435}/F_{526}. Excitation was at 395 nm.

The practical ratiometric imaging application of **WLN** to track Cu^{2+} levels was investigated in MCF-7 cells stained by **WLN** *via* a dual emission imaging mode. Therefore, two series of confocal fluorescence images were obtained respectively from the green channel of band path 420−470 nm and red channel of band path 480−580 nm, and ratiometric images were obtained by mediating the image from the green channel with the corresponding one from the red channel. The bright fluorescence inside the cells shown in both green and red channel images indicated that **WLN** can be loaded into cells in 1 h, displaying the fine membrane permeability of **WLN**. The ratiometric imaging of cells loaded with **WLN** showed very low levels of the background intracellular emission ratio, indicating the low Cu^{2+} level inside MCF-7 cells (Figure 4a). When exogenous Cu^{2+} was introduced via incubation with $CuCl_2$ solution, an intensive blue to green color change was observed inside the cell, displaying an enhanced intracellular Cu^{2+} level (Figure 4b). Treatment with the metal ion chelator TPEN for 1 min at 25 °C reduced the emission ratio enhancement distinctly (Figure 4d), implying **WLN** can monitor intracelluar Cu^{2+} flunctuation reversibly.

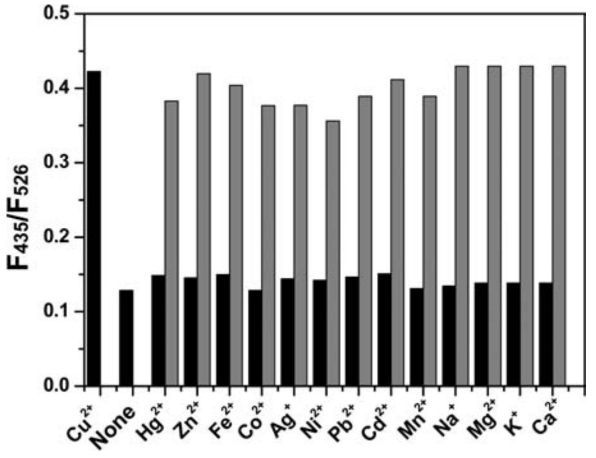


Figure 3. Emission ratio at 435 and 526 nm (F_{435}/F_{526}) of **WLN** (10 μM) in HEPES buffer (50 mM, pH 7.2) induced by different metal cations. Black bars represent the F_{435}/F_{526} ratio of free sensor or in the presence of 1 equiv of Cu^{2+}, Hg^{2+}, Zn^{2+}, Fe^{2+}, Co^{2+}, Ag^{+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+}, Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}. Red bars, the F_{435}/F_{526} ratio of **WLN** determined after the addition of 1 equiv of indicated metal ions followed by addition of 1 equiv of Cu^{2+}. λ_{ex}, 395 nm.

In summary, we have developed a new ratiometric Cu^{2+} sensor (**WLN**) via a fluorophore hybridization approach. **WLN** showed a specific Cu^{2+}-induced deviation in the ratio of its two emission bands due to the different quenching effects of Cu^{2+} on its two constituent fluorophores. The specific ratiometric sensing ability for Cu^{2+} implied **WLN** as a potential imaging candidate for intracelluar Cu^{2+} ratiometric imaging. Indeed, the imaging experiment clearly confirmed the ratiometric imaging ability of **WLN** to monitor Cu^{2+} levels in living cells.

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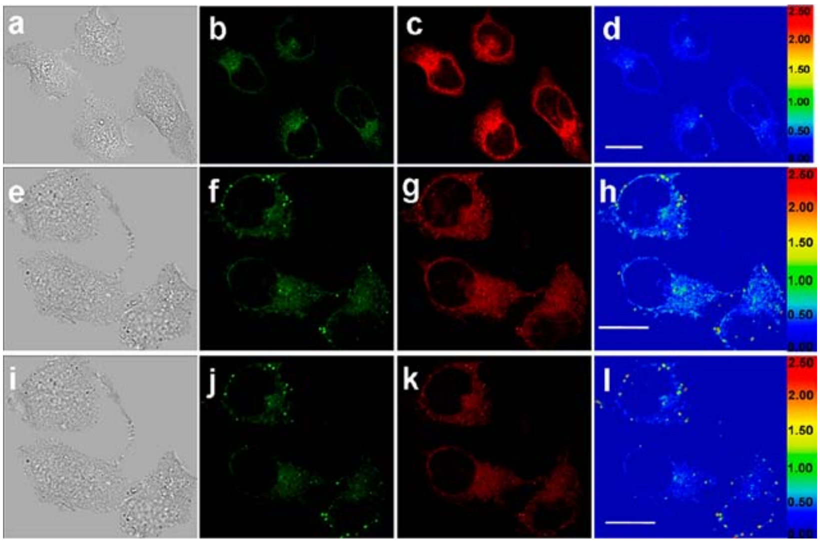

Figure 4. Confocal fluorescence images of intracellular Cu^{2+} in MCF-7 cells with **WLN**-staining. MCF-7 cells incubated with **WLN** (10 μM) at 25 °C for 1 h (a, b, c, d). The stained cells were exposed to 200 μM $CuCl_2$ solution at 37 °C for 20 h, followed by washing with **WLN** solution (e, f, g, h); the cells in (e) were further treated by TPEN solution (100 μM, 30 min, i, j, k, l). (a, e, i) Bright-field transmission images. (b, f, j) Fluorescence images obtained according to the emission collected by the green channel (band path 420−470 nm). (c, g, k) Fluorescence images obtained from the red channel (band path 480−580 nm). (d, h, l) Ratiometric images generated from (b, f, j) and (c, g, k). λ_{ex}, 405 nm; bar = 20 μM.

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Supporting Information Available. Synthesis of **WLN**; ^1H, ^{13}C NMR and ESI-MS spectra of **WLN**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.