Dye-Assembled Nanocomposites for Rapid Upconversion Luminescence Sensing of Cu²⁺

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ABSTRACT: An upconversion luminescence probe for the detection of Cu²⁺ is constructed by assembling meso-tetra(4-sulfonatophenyl)porphine dihydrochlorid (TSPP) on SiO₂-encapsulated β-NaYF₄:Yb,Er,Gd nanorods which were synthesized by hydrothermal reaction and encapsulated with a layer of ultrathin silica through a water-in-oil reverse microemulsion strategy. Under excitation of 980 nm, the nanorods emit upconversion lights at 545 nm and 659 nm, respectively. While in the presence of Cu²⁺, Cu²⁺ forms complex with TSPP, which shifts the maximum absorption of TSPP from 515 to 545 nm. As the maximum absorption of TSPP-Cu²⁺ is overlapped with the emission at 545 nm of the nanorods, the luminescence energy of the nanorods can be transferred to TSPP-Cu²⁺ complex, resulting in a quench of the emission at 545 nm, while the upconversion emission at 659 nm is not affected by Cu²⁺, and can be used as a reference. Cu²⁺ was therefore quantified based on the ratio of the upconversion luminescence at 545 to that at 659 nm. Using the ratiometric

upconversion luminescence as detection signal, the nanoprobe shows high selectivity and sensitivity towards Cu^{2+} with a limit of detection of 2.16 μM , and a quick response time of within 10 s. The analysis of Cu^{2+} in Hela cell lysates indicates the potential applications of this nanoprobe in the near-infrared fluorescent imaging of Cu^{2+} in cells.

KEYWORDS: upconversion luminescence, LRET, Cu^{2+}

1. Introduction

Copper is a transition element essential to human health. In particular, Cu^{2+} is a cofactor for many protein enzymes and it plays a significant role in numerous physiological processes in the human body [1-4]. However, excessive Cu^{2+} intake is highly toxic to human health, which can result in liver damage and other neurodegenerative diseases such as Alzheimer's, Parkinson's, Wilson's and Menkes diseases [5-8]. The highest Cu^{2+} level in drinking water is 1.3 ppm ($\sim 20 \mu\text{M}$) according to the U.S. Environmental Protection Agency (EPA) [9-10]. In addition, along with the widespread use of copper in industry and agriculture, Cu^{2+} has been a serious environmental pollutant [11]. In view of the current situation above, realistic methods with high sensitivity and selectivity for the detection of Cu^{2+} in environmental and organisms at cellular level are crucial.

Luminescence resonance energy transfer (LRET) biosensors, which generally are composed of an acceptor and a donor fluorophore, have been used for biosensing [12], bioimaging [13], bioassay [14], photodynamic therapy [15] and drug delivery [16]. Among those LRET sensors, lanthanide-doped upconversion nanoparticles (UCNPs) have emerged as alternatives over conventional down-shifting fluorescence probes (organic dyes, quantum dots, etc.) [17-22]. Rare-earth doped upconversion

nanomaterials can convert long-wavelength near-infrared (NIR) light to short-wavelength UV or visible light [23,24] with intriguing advantages, such as a large anti-Stokes shift, a narrow emission band, lower toxicity, no photobleaching [25], reinforced light penetration depth in tissue [26] and no autofluorescence (noise) from biosamples [27], which make upconversion nanomaterials particularly useful for sensing and bioimaging in vivo. Various nanoprobe containing upconversion nanomaterials and organic chromophores were proposed for biosensing as well as detection of special ions [28-35], small molecules [36,37], radical [38], viruses [39], amino acid [40], protein [41], DNA [42] and pH [43] based on the LRET process. Generally, upconversion nanomaterials serve as the energy donors while the organic chromophores as energy acceptors. Additionally, special interest has been paid to lanthanide ion photoluminescence [44-45].

However, most of these upconversion nanomaterials are synthesized in organic solvents or at high temperature [46] so that obtained nanocrystals are hydrophobic and difficult to dissolve in water. The direct use of these nanocrystals for biological applications is limited owing to the low solubility in aqueous solution and lack of functional chemical groups or appropriate sites for attachment of biomolecules. Thus, further surface modification of these nanocrystals is much required. More often than not, silica-coated core-shell structures are considered to be an ideal method for the modification of the upconversion nanocrystal surface because of the facile grafted other functional groups and good solubility in water [47,48].

In the present study, a new Cu^{2+} -responsive upconversion luminescence (UCL) nanoprobe was fabricated by loading meso-tetra(4-sulfonatophenyl)porphine dihydrochlorid (TSPP) [49-51] into a layer of ultrathin silica encapsulated upconversion $\beta\text{-NaYF}_4\text{:Yb,Er,Gd}$ nanorods, denoted as $\beta\text{-NaYF}_4\text{:Yb,Er,Gd@SiO}_2\text{-TSPP}$. In this LRET nanoprobe, $\beta\text{-NaYF}_4\text{:Yb,Er,Gd}$ nanorods worked as the energy donor, ultrathin silica shell as spacer, and Cu^{2+} -responsive TSPP as the acceptor. In the presence of Cu^{2+} , green UCL emission decreased concomitantly, while the red UCL emission only changed a little, which was used as the reference. The proposed $\beta\text{-NaYF}_4\text{:Yb,Er,Gd@SiO}_2\text{-TSPP}$ was a UCL

turn-off nanoprobe for Cu^{2+} .

2. Experimental Section

2.1. Chemicals and materials

$\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%), $\text{YCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%), $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$ (99.99%), $\text{ErCl}_3 \cdot 6\text{H}_2\text{O}$ (99.9%) were purchased from Energy Chemical. TSPP ($\geq 95.0\%$), igepal CO-520, (3-Aminopropyl)triethoxysilane (APTES, 97%) and tetraethyl orthosilicate (TEOS, 99.999%) were purchased from Aladdin. Tryptophan (Trp), arginine (Arg), aspartic acid (Asp), valine (Val), leucine (Leu), tyrosine (Tyr), lysine (Lys), cysteine (Cys), glutathione (GSH), bovine serum albumin (BSA) and ovalbumin (OVA) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). NaOH (98+%), NH_4F (98+%), oleic acid (90%) and other reagents of analytical reagent grade were all obtained from commercial sources and used as starting materials without further purification. All ultrapure water was prepared with Mill-Q water (Millipore, 18.2 $\text{M}\Omega\cdot\text{cm}$ resistivity) and used throughout the experiment.

2.2. Apparatus

The morphology of the samples was characterized with transmission electron microscopy (TEM) on a JEM 2100 (JEOL, Japan) electron microscope. These as-prepared samples were dispersed in cyclohexane or water and dropped on the surface of a copper grid for TEM test. Elemental analyses were carried out on an energy-dispersive X-ray analysis spectrometer (EDXA). Fourier transform infrared (FT-IR) spectra ($4000\text{--}500\text{ cm}^{-1}$) in KBr were recorded on a spectrometer (Nicolet-5700, American). UV-vis absorption spectra were obtained on a Cary 60 spectrophotometer (Agilent Technologies). A Mettler-Toledo Delta 320 pH meter was used to measure pH values. UCL measurements were carried out on a Hitachi-F4600 fluorescence spectrometer (Tokyo, Japan) under the excitation of a 980 nm laser. The crystalline phases of the samples were characterized by an X-ray diffractometer (XRD, M21X, MAC Science Ltd., Japan) with $\text{Cu K}\alpha$ irradiation source and a scanning

speed of 2°/min in the 2 θ range of 20-80°.

2.3. Synthesis of OA-Capped β -NaYF₄:Yb,Er,Gd (18, 2, 60 mol%) nanorods.

The upconversion β -NaYF₄:Yb,Er,Gd (18, 2, 60 mol%) nanorods with high monodispersity were synthesized by a rational hydrothermal method using oleic acid as a stabilizing agent [46]. Nanorods rather than nanoparticles were chosen because of their relatively simpler synthesis method, more intense luminescence and upconversion efficiency than nanoparticles [46,47,52,53]. In a typical procedure for the synthesis of lanthanide-doped β -NaYF₄:Yb,Er,Gd nanorods, 3 mL NaOH solution (0.2 g/mL) was mixed with 20 mL oleic acid in ethanol (1:1 v/v) under stirring. To the resulting mixture were added 4 mL of RECl₃ (0.2 M, RE = Y, Yb, Er and Gd) and 2 mL of NH₄F (2 M). The solution was then transferred into a 50 mL Teflon-lined autoclave and heated at 200 °C for 2 h. The obtained nanorods were collected by centrifugation, washed with ultrapure water and ethanol several times, redispersed in cyclohexane in the end.

2.4. Synthesis of β -NaYF₄:Yb,Er,Gd@SiO₂-NH₂ nanorods.

The β -NaYF₄:Yb,Er,Gd@SiO₂-NH₂ nanorods were synthesized through a water-in-oil reverse microemulsion strategy [54]. 9 mL of the as-prepared β -NaYF₄:Yb,Er,Gd solution dispersing in cyclohexane was added into a mixture solution containing 1 mL Igepal CO-520 and 20 mL cyclohexane. After stirring for 3 h, 140 μ L NH₃·H₂O (30%) was added and the mixture was stirred for another 2 h. Then, 1 mL TEOS solution in cyclohexane (1:4 v/v) was introduced into the system within 1 h. The mixture was hermetically stirred for 36 h. To graft amino-groups, 50 μ L APTES was added into the mixture and stirred for another 5 h. Then 30 mL hexane was added to precipitate the product which was collected by centrifugation. The as-prepared nanorods were dispersed in ethanol under ultrasonic treatment, precipitated with excess hexane, and collected by centrifugation. This procedure was repeated for three times to remove excess Igepal CO-520. The as-obtained nanorods were finally dispersed in 20 mL ultrapure water.

2.5. Assembly of TSPP on β -NaYF₄:Yb,Er,Gd@SiO₂-NH₂ nanorods.

1 mL TSPP solution (1 mM) in water was added dropwise into 9 mL β -NaYF₄:Yb,Er,Gd@SiO₂ solution (1 mg/mL). After 10 min of ultrasonication, the mixture solution was then stirred overnight. Redundant TSPP was removed by centrifugation. The precipitate was washed several times with water and ethanol by a repetitive dispersion/precipitation cycle. The as-obtained hybrid materials (β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP) were dispersed in water by a brief sonication to form a homogeneous solution and stored at 4 °C.

2.6. Analysis of Cu²⁺ in the HeLa cell lysate.

The β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP (1 mg/mL), Cu²⁺ (15, 30, 45, 60, 75 μ M) were incubated with HeLa cell lysate (100,000 cells/mL) in the 0.05 M Tris-HCl solution (pH=7.4) with a total volume of 100 μ L. After incubation for 10 min, the mixture solutions were ready for measurement.

3. Results and discussion

3.1. Characterization of β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP Upconversion Nanocomposites.

The upconversion nanorods were characterized by transmission electron microscopy (TEM), X-ray powder diffraction (XRD), energy-dispersive X-ray analysis (EDXA), and Fourier transform infrared spectroscopy (FT-IR).

The TEM image of Fig. 1A shows that the β -NaYF₄:Yb,Er,Gd nanorods were well-dispersed and not aggregated with an average diameter of 17 nm and a mean length of 109 nm. The inset in Fig. 1A shows that the nanorods are uniformly encapsulated with a thin layer of silica at a thickness of ~5 nm. The as-prepared β -NaYF₄:Yb,Er,Gd nanorods are pure hexagonal phase characterized by XRD pattern of Fig. 1C (JPCDS No. 16-0334). The immobilization of TSPP on the surface of the as-prepared β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP was confirmed by EDXA (Fig. 1E) in which elements S and N are characterized, while the EDXA of β -NaYF₄:Yb,Er,Gd@SiO₂ nanorods shows no S and N (Fig. 1D).

Fig. 1B displays the Fourier-transform infrared (FT-IR) spectra of β -NaYF₄:Yb,Er,Gd, β -NaYF₄:Yb,Er,Gd@SiO₂, TSPP and β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP. For β -NaYF₄:Yb,Er,Gd, the peaks at 2925 and 2855 cm⁻¹ are assigned to the asymmetric and symmetric stretch vibrations of methylene (-CH₂-) in the long alkyl chain of OA. The band at 1553 cm⁻¹ is attributed to carbonyl group (-C=O-). These results indicate that OA was coated on the surface of the β -NaYF₄:Yb,Er,Gd nanorods. As for β -NaYF₄:Yb,Er,Gd@SiO₂, the strong and broad absorption band at 1046 cm⁻¹ is attributed to Si-O-Si asymmetric stretching vibration, which suggests the formation of β -NaYF₄:Yb,Er,Gd@SiO₂. At the same time, FT-IR spectra show characteristic vibration bands at 1640 cm⁻¹ for amine groups, and bands at 739 and 632 cm⁻¹ for TSPP on the surface of silica shell. The FT-IR data indicate the successful assembly of the complex TSPP on the surface of the as-prepared upconversion β -NaYF₄:Yb,Er,Gd@SiO₂ nanorods through electrostatic attraction.

3.2. The UCL detecting of Cu²⁺.

The detection of Cu²⁺ relies on a LRET process between the β -NaYF₄:Yb,Er,Gd nanorods working as the energy donor and the Cu²⁺-responsive TSPP working as the acceptor (Scheme 1). Upon excitation of 980 nm laser, the β -NaYF₄:Yb,Er,Gd nanorods emit intense upconversion emission at 521 nm, 545 nm and 659 nm (Fig. 2, curve a), which can be assigned to ²H_{11/2} - ⁴I_{15/2}, ⁴S_{3/2} - ⁴I_{15/2}, ⁴F_{9/2} - ⁴I_{15/2} transitions of Er³⁺, respectively [54]. From the UV-vis absorption spectrum of β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP in the absence and presence of Cu²⁺ (Fig. 2, curves b and c), one can notice that the addition of Cu²⁺ results in a significant red shift of the maximum absorption of TSPP from 515 to 545 nm, due to the formation of TSPP-Cu²⁺ complex. As the maximum absorption at 545 nm is overlapped with the upconversion emission at 545 nm of the β -NaYF₄:Yb,Er,Gd@SiO₂ nanorods, a LRET can be happened between the β -NaYF₄:Yb,Er,Gd@SiO₂ nanorods and the TSPP-Cu²⁺ complex, resulting in a decrease in the UCL at 545 nm (Fig. 2, curve d).

While in the absence of Cu^{2+} , the maximum absorption of $\beta\text{-NaYF}_4\text{:Yb,Er,Gd@SiO}_2\text{-TSPP}$ (which is actually the absorption of TSPP) is around 515 nm, the LRET is weak. As TSPP- Cu^{2+} complex has no absorption around 659 nm (Fig. 2, curve c), the addition of Cu^{2+} induces little change in the UCL at 659 nm (Fig. 2, curves d and e).

Comparing the UCL intensity of $\beta\text{-NaYF}_4\text{:Yb,Er,Gd@SiO}_2$ (Fig. 2, curve a) with that of $\beta\text{-NaYF}_4\text{:Yb,Er,Gd@SiO}_2\text{-TSPP}$ (Fig. 2, curve e), one can see that the immobilization of TSPP results in a little decrease in the UCL intensity at 545 nm, the U_{545}/U_{659} is down from 1.22 to 0.98.

One may wonder if there is LRET between the $\beta\text{-NaYF}_4\text{:Yb,Er,Gd@SiO}_2$ and Cu^{2+} because Cu^{2+} also portrays a broad absorbance spectrum. The upconversion luminescence of the $\beta\text{-NaYF}_4\text{:Yb,Er,Gd@SiO}_2$ nanorods (without loading of TSPP) was measured in the presence of Cu^{2+} (Fig. 2, curve f). The addition of 0.2 mM Cu^{2+} results in an insignificant decrease in the UCL of less than 0.8% (Fig. 2, curves a and f), indicating that the LRET between $\beta\text{-NaYF}_4\text{:Yb,Er,Gd@SiO}_2$ and Cu^{2+} is ignorable.

The influence of pH (from 4.0 to 9.0) on the upconversion luminescence of the $\beta\text{-NaYF}_4\text{:Yb,Er,Gd@SiO}_2\text{-TSPP}$ with/without Cu^{2+} was also investigated shown in Fig. S1. The results show that the effect of pH on the responses in the range of 5.0-8.0 is insignificant no matter in the presence or absence of Cu^{2+} , indicating that this sensor is suitable for applications in physiological conditions. To make this probe feasible and biocompatible, pH=7.4 was selected for the detection of Cu^{2+} .

Fig. 3 shows the changes of UCL spectra upon continuous addition of Cu^{2+} , the UCL intensity at 545 nm significantly decreases while the UCL intensity at 659 nm just changes a little. As the UCL intensity at 659 nm is independent on the Cu^{2+} concentration while the UCL intensity at 545 nm is dependent on the Cu^{2+} concentration, the UCL intensity at 659 nm can be therefore used as the reference. Cu^{2+} can be quantified based on the ratio of UCL intensity at 545 nm to that at 659 nm (U_{545}/U_{659}). The changes (ΔR) of the UCL intensity ratio relative to the blank was used to quantify Cu^{2+} :

$$\Delta R = (U_{545}/U_{659})_0 - (U_{545}/U_{659})$$

Where $(U_{545}/U_{659})_0$ is the blank response without Cu^{2+} .

As shown in Fig. 4 the changes of UCL intensity ratio are linear related to the Cu^{2+} concentration in 0-0.16 mM. The limit of detection (LOD) given by the three times noise was calculated to be 2.16 μM with a noise of 0.005 calculated from blank measurements ($n = 10$) (Fig. 4, inset).

3.3. Selectivity.

For an excellent detection system, high selectivity is of great importance. To validate the selectivity of the $\beta\text{-NaYF}_4\text{:Yb,Er,Gd@SiO}_2\text{-TSPP}$ nanoprobe, the responses to some common metal ions in the presence of 0.2 mM Cu^{2+} were investigated, including Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Fe^{3+} , Fe^{2+} , Ni^{2+} , Co^{2+} , Mn^{2+} , Cd^{2+} , Cr^{3+} , Pb^{2+} , Hg^{2+} , and Ag^+ (Na^+ , 10 mM; K^+ , 5.0 mM; Ca^{2+} , 20 mM; Mg^{2+} , 20 mM; Fe^{3+} , 5.0 mM; other metal ions, 1 mM). As shown in Fig. 5A, the proposed probe shows a high selectivity toward Cu^{2+} . As high as 100 times some coexisting interferents result in only upto 2.5% deviation in the responses. The high selectivity is due to the high thermodynamic affinity of the N, O-chelate ligands to Cu^{2+} and fast metal-to-ligand binding kinetics [5]. As shown in Fig. 5B, the addition of metal ions except Cu^{2+} results in insignificant changes in the upconversion luminescence spectra, only Cu^{2+} can induce a significant decrease in the UCL intensity at 545 nm. From the UV-vis absorption spectra in Fig. 6, one also can see the addition of the investigated ions only Cu^{2+} results in red shift of the maximum absorption at 515 nm.

Considering the application in the analysis of Cu^{2+} in organisms, selectivity was investigated against those biological compounds that existing in organisms, including some natural amino acids: tryptophan (Trp), arginine (Arg), aspartic acid (Asp), valine (Val), leucine (Leu), tyrosine (Tyr), lysine (Lys), cysteine (Cys), and peptides: glutathione (GSH), as well as proteins: bovine serum albumin (BSA) and ovalbumin (OVA). As shown in Fig. 5A, coexisting of 0.5 mM above amino acids, 0.5 mM peptides and 2 mg/mL proteins with 0.2 mM Cu^{2+} results in only upto 2.1% deviations in the responses.

3.4. Determination of Cu²⁺ in the HeLa cell lysates.

The proposed method was compared with some published works in terms of the sensitivity and linear range, with results shown in Table 1. The proposed method exhibited a comparable sensitivity with the published works. The proposed nanoprobe has a quick response of less than 10 s upon the addition of Cu²⁺. In addition, the nanocomposite has good water solubility and biocompatibility, which makes the nanoprobe possible to be applied in biological fields.

The practical application of this nanoprobe was evaluated through the determination of Cu²⁺ spiked in HeLa cell lysates. The recovery experiment was summarized in Table 2, giving recoveries ranging from 98% to 102% and the RSD below 5.0%. These results show a good agreement with those for Cu²⁺ detection in buffer solutions, further confirming the sensor is promising for the detection of Cu²⁺ in real biological samples.

4. Conclusion

Dye-assembled nanocomposites β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP were developed for rapid upconversion luminescence sensing of Cu²⁺ based on luminescence resonance energy transfer with the β -NaYF₄:Yb,Er,Gd nanorods as the energy donor, and the Cu²⁺-responsive TSPP as the energy acceptor. The formation of TSPP-Cu²⁺ complex resulted in the quench of nanocomposite emission at 545 nm, and Cu²⁺ was detected based on the decrease in emission intensity at 545 nm. A linear response was obtained in the range of 5 μ M to 0.16 mM with a LOD of 2.16 μ M. The proposed probe shows a high selectivity. As the prepared β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP can be well-dispersed in aqueous and is based on upconversion luminescence, the proposed probe can be applied in assay of Cu²⁺ in biological systems. The analysis of Cu²⁺ in biological systems was evaluated by the determination of Cu²⁺ spiked in Hela cell lysates with recoveries ranging from 98% to 102% and RSD below 5.0%.

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Figure Captions

Fig. 1. TEM images of β -NaYF₄:Yb,Er,Gd (18, 2, 60 mol%) nanorods (A) and **inset is** β -NaYF₄:Yb,Er,Gd@SiO₂ nanorods. FT-IR of β -NaYF₄:Yb,Er,Gd, β -NaYF₄:Yb,Er,Gd@SiO₂, TSPP, and β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP (**B**). X-ray powder diffraction pattern of the as-prepared β -NaYF₄:Yb,Er,Gd nanorods, showing that all peaks can be well indexed in accordance with pure hexagonal β -NaYF₄ (JCPDS card no.16-0334) (**C**). The EDXA of β -NaYF₄:Yb,Er,Gd@SiO₂ (**D**) and β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP (**E**).

Scheme 1. Schematic illustration of synthesis of the TSPP-modified upconversion β -NaYF₄:Yb,Er,Gd nanorods (β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP), and their LRET process towards Cu²⁺.

Fig. 2. **The UCL spectrum of β -NaYF₄:Yb,Er,Gd@SiO₂ without (blue line, curve a) and with 0.2 mM Cu²⁺ (purple line, curve f); as well as the UCL spectrum of β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP with (orange line, curve d) and without 0.2 mM Cu²⁺ (gray line, curve e). UV-vis absorption spectra of β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP without (black line, curve b) and with 0.2 mM Cu²⁺ (red line, curve c).**

Fig. 3. Upconversion luminescence spectra of β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP in 0.05 M Tris-HCl solution (pH=7.4) upon gradual addition of Cu²⁺ (0-0.4 mM).

Fig. 4. Change of UCL intensity ratio at 545 to 659 nm, $\Delta R = (U_{545}/U_{659})_0 - (U_{545}/U_{659})$ upon addition of 0-0.4 mM Cu²⁺, inset is the linear fitting in the concentration range of 0-0.16 mM.

Fig. 5. (A) **The effect of coexisting ions and biological compounds on the signal (ΔR) of the ratiometric probe in the presence of Cu²⁺. Final concentrations: Cu²⁺, 0.2 mM; Na⁺, 10 mM; K⁺, 5.0 mM; Ca²⁺, 20 mM; Mg²⁺, 20 mM; Fe³⁺, 5.0 mM; other metal**

ions, 1 mM; 0.5 mM above amino acids; 0.5 mM peptides and 2 mg/mL proteins. $\Delta R = (U_{545}/U_{659})_0 - (U_{545}/U_{659})$, where $(U_{545}/U_{659})_0$ is the blank response. (B) UCL spectra of β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP solutions (1 mg/mL) in the presence of different metal ions (Cu²⁺, 0.2 mM; Na⁺, 10 mM; K⁺, 5.0 mM; Ca²⁺, 20 mM; Mg²⁺, 20 mM; Fe³⁺, 5.0 mM; other metal ions, 1 mM).

Fig. 6. Absorption spectra of TSPP after reacting with common metal ions in 0.05 M Tris-HCl solutions (pH=7.4) (Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe³⁺, Fe²⁺, Ni²⁺, Co²⁺, Mn²⁺, Cd²⁺, Cr³⁺, Pb²⁺, Hg²⁺, Ag⁺, and Cu²⁺).

Table 1. Comparison of the principles and analytical parameters of different Cu²⁺ probes.

Table 2. Recovery study for Cu²⁺ using β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP sensor with HeLa cell lysates.

Table 1. Comparison of the principles and analytical parameters of different Cu²⁺ probes.

Method	Probe	Detection mechanism	Analytical range	LOD	Reference
UCL	β -NaYF ₄ :Yb,Er,Gd@SiO ₂ -TSPP	LRET	5 μ M – 0.16 mM	2.16 μ M	This work
UCL	UCNPs -RB-hydrazide	FRET	1 μ M – 10 μ M	1 μ M	[5]
UCL	β -NaYF ₄ :Yb ³⁺ /Er ³⁺ :Rd-NH ₂	ET	2 μ M – 14 μ M	—	[10]
Colorimetric	Allosteric Dual-DNAzyme Unimolecular	Molecular engineering	1 μ M – 1.0 mM	1 μ M	[55]
Colorimetric	Triazolyl monoazo derivative	Stabilization of LUMO	—	13.6 μ M	[56]
Optical chemical	H ₂ TPBPy optode membrane	ET	0.2 nM – 10 μ M	5 nM	[57]
Optical	Calmagite immobilized agarose membrane	Complexation reaction	0.4 – 200 μ g l ⁻¹	0.07 μ g l ⁻¹	[58]
Electrochemical	TPCBZ/Nafion/GCE	Conjugated complex	1.0 nM – 1.0 mM	0.84 nM	[59]

Table 2. Recovery study for Cu²⁺ using β -NaYF₄:Yb,Er,Gd@SiO₂-TSPP sensor with HeLa cell lysates.

Sample number	Add (μ M)	Found (μ M) ^a	Recovery (%) ^a	RSD(%) ^a
1	15	14.85	99	5.0
2	30	29.54	98	3.2
3	45	45.37	101	1.2
4	60	61.07	102	1.5
5	75	75.86	101	2.1

^a the average value of three parallel determinations.

Dye-Assembled Nanocomposites for Rapid Upconversion Luminescence Sensing of Cu^{2+}

Fig.1A

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