Lysosome-targeting NIR ratiometric luminecent upconversion nanoprobe toward arginine

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Near-infrared (NIR) fluorescent sensors have been actively pursued due to organisms exhibiting little auto-fluorescence in near-infrared regions. Capable of emitting luminescence under 980 nm excitation, upconversion nanoparticles have shown promise as optical agents for in vivo bioimaging. Based on luminescence resonance energy transfer (LRET), a NIR luminescent Arg-nanoprobe was developed by the decoration of upconversion nanoparticles (UCNPs) (NaYF4:Yb,Er) with organic molecules. The as-developed nanoprobe could quantitatively detect concentrations within the range of 528–1250 μM, and had high sensitivity (the limit of detection, LOD, being 15.6 μM) and good photostability. Because the probe could target lysosomes, intracellular detection of Arg behavior was demonstrated via upconversion luminescence (UCL) imaging.

1. Introduction

For quite a long time, Arg has attracted the attention of scientists due to its indispensability to the human body. Arg is responsible for protein synthesis and the production of many substances. Moreover, Arg has an important role in many life activities, such as wound healing, cell division, and hormone release [1,2]. Therefore, it is crucial to develop an effective method for selective and sensitive recognition of Arg.

Fluorescent molecular probes have extensive applications in numerous fields, such as disease diagnosis and environmental detection [3–8]. As a result, many organic molecular probes have been developed for examining metal ions [9–13], anions [14–17], pH [18–21], and biological molecules [22–27]. Although Arg is significant for humans, only a few molecular probes have been developed for its recognition [28–31].

Ratiometric detection has been regarded as a way to overcome the interference effects of instrumental parameters, photobleaching, the microenvironment around the probe molecule, etc. [32–34]. LRET, which has been considered to be a satisfactory luminescence recognition mode, has been widely adopted in ratiometric detection [35,36]. Up to now, very few LRET-based ratiometric luminescent probes for Arg have been reported and thus it is very significant to design LRET-based Arg probes.

Currently, Arg probes excited with UV–vis light are most reported [28–30]. A hopeful strategy for the design of luminescent Arg-nanoprobes is the use of upconversion nanoparticles, which exhibit upconversion luminescence when excited with NIR light, as well as outstanding light penetration depth in tissues, and can eliminate spontaneous fluorescence from biological samples [37–39]. At 980 nm excitation, the upconversion nanoparticles (UCNPs) were applied as LRET donors; the organic dyes, as LRET acceptors [40–42].

UCNPs-1 is the first upconversion luminescence (UCL) probe developed for ratiometric bioimaging of Arg. Fig. 1a illustrates the mechanisms involved in Arg sensing. With this UCNPs-organic molecule architecture, the probe demonstrates several merits. First, its excitation at 980 nm makes it capable of offering greater depth of penetration in tissues. Second, as a LRET-based Arg probe, it can provide more accurate detection in biosystem and improve signal-to-noise ratios. Third, it displays good photostability, selectivity, and anti-interference ability. Lastly, due to its lysosome-targeting ability, the ratiometric probe allows accurate intracellular tracking of Arg.

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2. Experimental

2.1. Materials and methods

Iodomethane, \( p \)-hydrazinobenzoic acid hydrochloride, 3-amino phenol, \( \text{K}_2\text{CO}_3 \), dimethylacetone, 1,4-dibromobutane, Pd-C catalyst, ethyl pyruvate, \( \text{SeO}_2 \), \( \text{DMF} \), \( \text{AcOH} \), \( \text{AcONa} \), 1,4-dioxane, \( \text{DMF} \), \( \text{AcOH} \), \( \text{AcONa} \), 4-Hydroxybenzaldehyde, alanine (Ala), isoleucine (Ile), methionine (Met), threonine (Thr), tryptophane (Trp), cysteine (Cys), homocysteine (Hcy), glucose (Glc), \( \text{H}_2\text{O}_2 \), \( \text{NaCl} \), \( \text{MgCl}_2 \), \( \text{KCl} \), \( \text{CaCl}_2 \), \( \text{MnCl}_2 \), \( \text{CoCl}_2 \), \( \text{ZnCl}_2 \), \( \text{Na}_2\text{SO}_3 \), \( \text{NaHS} \), \( \text{NaClO}_3 \), \( \text{NaF} \), \( \text{Na}_2\text{C}_2\text{O}_4 \), \( \text{NaHSO}_3 \), \( \text{Na}_2\text{SO}_4 \), \( \text{NaNO}_3 \), \( \text{NaClO}_4 \), \( \text{NaHCO}_3 \), \( \text{Na}_2\text{HPO}_4 \), \( \text{NaAc} \), \( \text{NaBr} \), \( \text{NaN}_2 \),...
NaH$_2$PO$_4$, C$_{17}$H$_{35}$COOH, polyacrylic acid (PAA, M$_{w}$ = 1800), Y$_2$O$_3$, Yb$_2$O$_3$, and Er$_2$O$_3$ were used directly unless otherwise specified.

Mass spectra were measured on a Tesla FTMS-MALDI/DHB mass spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker (400 MHz) instrument. Low-resolution TEM were measured on a JEOL-JEM 2100 transmission electron microscopy operated at 200 kV. High resolution TEM was carried out on a JEOL-JEM 2100 F field emission transmission electron microscope. XRD patterns were determined on a Rigaku D/MAX-2000 diffractometer.

### 2.2. Synthesis of rare earth stearate precursor

Y$_2$O$_3$ (880.7 mg, 3.9 mmol), Yb$_2$O$_3$ (394.1 mg, 1.0 mmol) and Er$_2$O$_3$ (38.3 mg, 0.1 mmol) were added to 20 mL concentrated nitric acid and heated to volatilize excessive nitric acid. The rare earth nitrate was obtained as white powder, in which the mole ratio of Y$_3^+$, Yb$^{3+}$, and Er$^{3+}$ was 0.78:0.20:0.02. The mixture of 80 mL ethanol solution of the above salts and 8.5344 g (30 mmol) stearic acid were gradually heated to 78°C and refluxed, and then 20 mL ethanol solution of NaOH (1.1900 g, 30 mmol) was titrated and refluxed for 1 h. The mixture was filtrated, and the filter cake was washed with twice water and once with ethanol. The stearic acid precursor was gained after drying the filter cake for 12 h in vacuum drying box.

### 2.3. Synthesis of NaYF$_4$:Yb,Er [43]

The as-prepared stearate precursor (957.7 mg, 1.0 mmol) and NaF (209.9 mg, 5 mmol) were added to 15 mL ethanol, 5 mL OA, and 10 mL H$_2$O. The reaction solution was stirred for 15 min and then transferred to a hydrothermal synthesis reactor. After reacting for 1 d at 150°C, the mixture was cooled. The mixture of CHCl$_3$ and EtOH (v/v = 1:6) was added to the precipitate and the crude product was obtained after centrifugal separation as white powder. After washing thrice with water-ethanol (v/v = 1:2) and once with ethanol, the white powder was dispersed in CHCl$_3$.

### 2.4. Preparation of PAA modified upconversion nanoparticles [44,45]

The saturated dichloromethane solution of NOBF$_4$ was titrated to 30 mL CHCl$_3$ colloidal solution of hydrophobic NaYF$_4$:Yb,Er nanoparticles and the mixture was stirred for 15 min. By centrifugal separation, the precipitate was washed with ethanol and dissolved in DMF. After precipitated in toluene, the precipitate was collected by filtration and dissolved in DMF. 300 mg PAA was added slowly to the above DMF solution and then 60 mL acetone was added. After stirring for 12 h, centrifugation (18,000 rpm, 15 min), and washing with water, the PAA-modified nanoparticles were collected and redispersed in water.

### 2.5. Preparation of UCNPs-1 [46]

For the preparation of upconversion nanoparticles, a mixture of compound 1 (45.72 mg) in DMSO and UCNPs (5 mg/mL) in 20 mL water was stirred for 12 h. After centrifuging, the resulting precipitate
was collected and washed three times with water. After heating the as-prepared solid at 60 °C in vacuum drying box, the nanocomposite material (UCNPs-1) was obtained.

2.6. Detection of Arg in solution phase

All the detection experiments were carried out in 8:2 (v/v) water/DMSO solution, the concentration of compound 1 was 6.6 × 10^{-6} M and the amount of compound 1 in the hybrid material was 7.51% its weight. UV–vis absorption spectra were obtained with a TU-1901 spectrophotometer. Photoluminescence (PL) and PL excitation spectra were measured on a Hitachi F-4600 spectrometer with 980 nm pump laser source.

2.7. Cell viability

Cell viability was determined with 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) assay. HeLa cells were seeded into 96-well plates at a density of 5 × 10^5 cells per well in DMEM (10% FBS) in six replicates and incubated for 24 h. After that the cells were exposed to 0, 8.3, 13.6, 27.3, 54.5, and 109.1 μg/mL UCNPs-1 for 1 h, then medium was discard and replaced with 100 μL fresh medium for 24 h, then 10 μL MTT (5 mg/mL) was added, and the cells were further incubated for 5 h at 37 °C. The medium was removed and the formazan crystals were dissolved in DMSO. The absorbance at 570 nm and the background value at 690 nm were measured with enzyme labelling instrument, and then the cell survival rate was calculated.

2.8. Detection of Arg in cells

The HeLa cells were incubated with 6 μg/mL UCNPs-1 for fluorescence imaging. The fluorescent images were recorded with 660 nm filters after the cells incubated with probe UCNPs-1 (6 μg/mL, 37 °C) for 30 min. After adding 2.0 × 10^{-5} M and 2.8 × 10^{-5} M Arg respectively and incubation for 30 min, the fluorescent images were taken at the same situation. The excitation was performed at 980 nm.

3. Results and discussion

To fabricate the recognition system, organic compound was synthesized as the recognition part and energy acceptor. The synthetic routes are shown in Scheme S1. The synthetic details and characterization data can be found in our previous work [47,48].

The peaks at 650 and 475 nm, respectively, represented the maximum absorption of 1 without and with Arg in 8:2 (v/v) water/DMSO solution. The main emission peaks of UCNPs (0.50 mg/mL) were located approximately at 525, 575 and 660 nm in water solution (excited at 980 nm, Fig. 1b). The significant spectral overlap of NIR excitation source and the absorption of 1 indicated LRET between Yb³⁺–Er³⁺ pairs and 1. Hence, the NIR 980 nm excitation source can be selected to
The luminescence of UCNPs was absorbed by compound 1 completely. After the addition of Arg, an emission band centered at 660 nm was recovered. The absorbance of compound 1 at 525 nm was almost the same with or without Arg. That is to say, the emission at 525 nm hardly changes whether 1 is with or without Arg.

Based on the absorption spectra of UCNPs-1 in the solution (the 4th line of Fig. S2), the concentration of the loading compound 1 could be determined (6.6 × 10⁻⁸ M) (Fig. S2 in Supporting information). In the following experiments, the concentration of compound 1 was 6.6 × 10⁻⁸ M and the amount of compound 1 in the hybrid material was 7.51% its weight.

Figures S3 and S4 (Supporting information) present the absorption spectral response of 1 and upconversion emission spectral change of PAA-UCNPs upon titration of Arg water solution. It is clear that the absorbance of 1 at 650 nm decreased dramatically as Arg concentration increased (Fig. S3). Arg induced the decomposition of compound 1 (Fig. 1b) [48]. The upconversion luminescence (UCL) spectrum hardly changes whether 1 completely. After the addition of Arg, an emission band centered at 525 nm was 7.51% its weight.

An aqueous solution (containing 20% DMSO) was utilized as the medium to determine the luminescence spectral change of UCNPs-1 at different Arg concentrations. As shown in Fig. 1c, when the Arg concentration increased from 0 to 2.0 × 10⁻³ M and the UCNPs-1 were excited at 980 nm, the peak centered at 660 nm increased gradually and the peak centered at 525 nm shifted little in intensity. The changes in the upconversion spectrum can be ascribed to the Arg-induced decomposition of compound 1 and LRET between the energy donor (UCNPs) and the energy acceptor (compound 1). In Fig. 1d, a good linear relationship between UCL₆₆₀/UCL₅₂₅ and Arg concentration, varying from 525 μM to 1250 μM, was observed. The linear calibration was shown in Fig. 1d, and the detection limit of the probe UCNPs-1 was 15.6 μM according to the signal-to-noise ratio [49, 50], thus showing that UCNPs-1 makes an excellent ratiometric Arg UCL probe.

Although the UCNPs were developed with a ligand exchange process [51–53], the NaYF₄:Yb,Er particles were produced using a previously reported procedure [54, 55]. The TEM image (Fig. 2a) reveals that the particles had a diameter ranging between 21 and 63 nm (Fig. S7). The HRTEM photographs show a distinct lattice distance of 0.30 nm, matching the (110) plane of hexagonal phase NaYF₄:Yb,Er nanoparticles (Fig. 2b). The TEM image of PAA-UCNPs (Fig. 2c) indicates that there was a layer of organic membrane outside the nanoparticles, implying that PAA was successfully coated on the surface of the nanoparticles. Fig. 2d demonstrates the XRD patterns of as-prepared lanthanide-doped upconversion nanoparticles (The patterns of OA-UCNPs and 1-PAA-UCNPs coincide with each other.), which were identified with the standard pattern (JCPDS 28–1192) and indicate that surface modification does not change the crystal phase of the nanoparticles. In the FTIR spectra (Fig. S1 in Supporting information), the peaks at 1578, 1622, and 1722 cm⁻¹ are corresponding compound 1.

The kinetic curves (Fig. 3a) showed that the reaction between UCNPs-1 and Arg completely finished within 5 min at which the Arg concentration was 6.6 × 10⁻⁴ M. In the photostability experiment, the luminescence intensity ratio of UCL₆₆₀ to UCL₅₂₅ (UCL₆₆₀/UCL₅₂₅) did not shift with irradiation time (Fig. 3b), indicating excellent photo-stability. Whereas, for the thermal stability experiment, in which the temperature was increased from 30 to 48 °C, the UCL ratio shifted slightly, indicating good thermal stability. It can therefore be concluded that the UCNPs-1 as an Arg probe has excellent photostability and better thermal stability.

Various species, including nucleophilic biological molecules (Pro, Tyr, Val, Ser, Glu, Gly, Met, Trp, Lys, Thr, Phe, Ala, Asp, Ile, Leu, Cys, Hcy, Glc, and H₂O₂), viability assay for HeLa cells treated with probe UCNPs-1 in dark (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

**Fig. 4.** (a) UCL responses of UCNPs-1 (0.50 mg/mL in 8.2 (v/v) water/DMSO) under 980 nm excitation upon addition of different species (2.0 × 10⁻³ M) (green bars) and UCL responses of the mixture of UCNPs-1 and Arg (2.0 × 10⁻³ M in water) under 980 nm excitation after addition of an excess of the indicated species (2.0 × 10⁻³ M) (red bars). The reaction time was 40 min. UCL₆₆₀ and UCL₅₂₅ represent the UCL intensity at 660 nm and 525 nm. The species used were Ala, Asp, Glu, Gly, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val, Cys, Hcy, Glc, and H₂O₂. (b) Viability assay for HeLa cells treated with probe UCNPs-1 has low cytotoxicity and good biocompatibility to the cultured cells and can be used to image Arg in living cells.

**UCNPs-1** was applied to image intracellular Arg-targeting lysosomes. The cells stained with 6 μg/mL UCNPs-1 (5 h, 25 °C) were further co-stained with a commercially available lysosome-specific dye Neutral Red (2.0 mM, 30 min). The pictures indicate that the red image acquired upon 980 nm was almost exactly same as the purple one gained upon excitation at 543 nm (Fig. S). These results suggest that UCNPs-1 has the ability to target lysosomes ascribing to the tertiary
The amino group of compound 1 [48,56].

The confocal UCL microscopy cellular imaging experiments of HeLa cells after incubation with UCNPs-1 upon 980 nm excitation are shown in Fig. 5. UCNPs-1 nanoparticles were incubated with HeLa cells for 5 h at 37 °C. Luminescence was not detected at the red channel, verifying excellent cell permeability. After subsequent addition of different concentrations of Arg (2.0 × 10⁻⁵ M and 2.8 × 10⁻⁵ M), an obvious gradual increase of luminescence at the red channel was recorded. The cells before and after treatment with Arg did not change throughout bright-field imaging experiments.
4. Conclusion

In summary, a LRET-based NIR ratiometric UCL Arg nanoprobe was developed. The NIR LRET-based ratiometric nanoprobe for Arg displayed excellent photostability, good selectivity, strong anti-interference ability, low cytotoxicity, and lysosome-targeting abilities. Overall, this research may provide a new approach for constructing NIR ratiometric Arg probes of improved accuracy.

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Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi:https://doi.org/10.1016/j.snb.2018.10.057.

References