Sensors and Actuators B 177 (2013) 956–963

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Highly selective and sensitive pH-responsive fluorescent probe in living Hela and HUVEC cells

Hong-Shui Lv[a,1], Jing Liu[b,1], Jing Zhao[b], Bao-Xiang Zhao[a,*], Jun-Ying Miao[b,*]

[a] Institute of Organic Chemistry, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, PR China
[b] Institute of Developmental Biology, School of Life Science, Shandong University, Jinan 250100, PR China

ARTICLE INFO

Article history:
Received 29 August 2012
Received in revised form
26 November 2012
Accepted 3 December 2012
Available online 13 December 2012

Keywords:
Rhodamine B
Fluorescent probe
Intracellular pH
Living cell
Lysosome

ABSTRACT

A new fluorescent probe has been synthesized, characterized and evaluated in living cells as an intracellular pH indicator. The probe is designed based on the fluorescence switching character of rhodamine B dye. The probe exhibits highly selective and sensitive response to acidic pH. The fluorescence intensity of the probe gradually increases about 46-fold from pH 6.7 to 4.4 with a pK_a value of 5.05 which is suitable for studying acidic organelles in living cells. Moreover, the fluorescence intensity of the probe is linearly proportional ($R = 0.98809$) to pH values in the range of 4.7–5.7. The probe can monitor intracellular pH in living Hela and HUVEC cells. The fluorescence of the probe in cells weakened as the addition of bafilomycin A1 or chloroquine that could induce pH value increase in lysosomes. The results demonstrate that the probe can selectively mark lysosomes and is valuable to monitor intracellular pH in living cells.

1. Introduction

Intracellular pH is an important factor in cell biology and plays a pivotal role in many cellular events, such as cellular metabolism [1,2], signal transduction [3], cell growth [4,5], proliferation [6], apoptosis [7] and autophagy [8]. Under normal physiological conditions pH values are maintained within very narrow limits. Abnormal pH values in organelles result in cellular dysfunction and affect human physiology to cause serious diseases such as cancer [9] and Alzheimer's disease [10]. It was reported that the pH of the tumor extracellular space (pH 6.2–6.9) is lower than that of normal tissues (pH 7.4) [11]. Some tumors were characterized by acidic extracellular pH compared with normal tissues, which may influence the outcome of tumor therapy. In addition, lysosomes and lysosome-related organelles provide another acidic microenvironment in the cytoplasm (pH 4.5–5.5) in which a large number of enzymes and secretory proteins exist and exert a variety of functions [12,13].

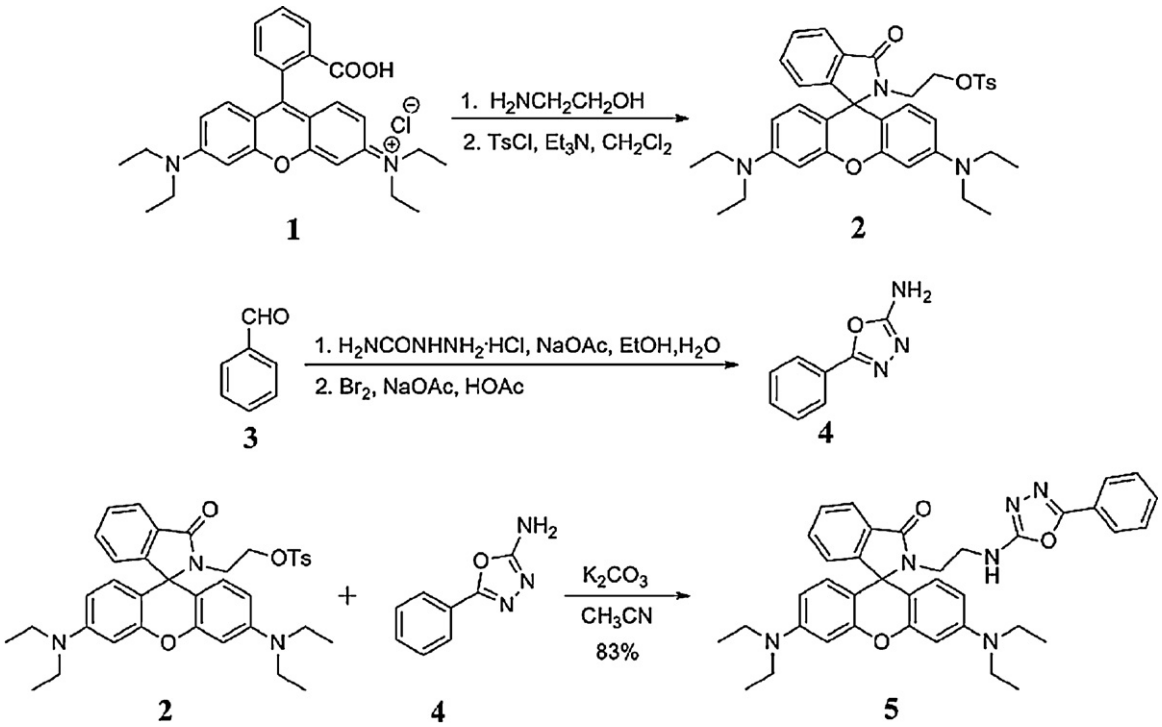
Therefore, monitoring pH changes inside living cells is important for exploring cellular functions and understanding physiological and pathological processes. There are many methods, including microelectrodes, nuclear magnetic resonance (NMR), absorbance spectroscopy and fluorescence spectroscopy, for measuring the intracellular pH. Among them, fluorescence techniques have attracted much attention due to their high selectivity and sensitivity. Up to now, a variety of fluorescent pH probes have been developed, some of which are commercially available [14].

However, among the fluorescent pH probes reported, most are practical for near-neutral pH range (pH 6.8–7.4). Only a few probes were reported for monitoring pH changes inside lysosomes (pH 4.5–5.5) [15–20]. DND dyes, such as DND-160, DND-167 and DND-189, are commercially available "lysosensors" from Life Technologies which can indicate acidic pH in lysosomes. A disadvantage of these dyes is that some of them have short absorption and emission wavelengths which can lead to cell damage. Moreover, these commercial lysosensors and some reported probes are usually weak bases containing aliphatic amines which can cause an "alkalizing effect" on lysosomes. Lysosomal pH will increase after longer incubation with these probes. Another commercial pH sensitive probe pHrodo (from Life Technologies) has long absorption and emission wavelengths and a relatively wide pH sensitivity range, but the changes in fluorescence intensity of pHrodo do not have a linear relationship with pH in the sensitivity range, which is problematic for microscopy based measurements [21]. Thus, to develop efficient acidic fluorescent probes, which are useful for studying acidic organelles functioning in the pH range of 4.5–6.0, are still desirable.

Rhodamine derivatives have been widely used as "turn-on" metal ion fluorescence probes [22–24] because of their excellent

* Corresponding authors. Tel.: +86 531 88366425; fax: +86 531 88564464.
E-mail addresses: bxzhao@sdu.edu.cn, sduzhao@hotmail.com (B.-X. Zhao), miaojy@sdu.edu.cn (J.-Y. Miao).
[1] These authors contributed equally.

0925-4005/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2012.12.014



Scheme 1. Synthesis of the probe **5**.

photophysical properties, such as high quantum yields, relatively long absorption and emission wavelengths in the visible region, large extinction coefficients and great photostability. Rhodamine-based fluorescent probes usually contain a spirocyclic structure which is high sensitive to the pH of the solutions. Under basic conditions, it remains the spirocyclic form that is non-fluorescent and colorless. While in acidic solutions, the ring-opened form performs strong fluorescence and pink color. Thus rhodamine derivatives are superior candidates which can be used to measure acidic pH and some rhodamine-based pH probes have been developed [16,17,25–32]. Unfortunately, many of them may cause "alkalizing effect" [16,27,28,30,32]. Thus, it is necessary to develop new rhodamine-based pH probes that do not contain aliphatic amines.

Here, we designed and synthesized a new rhodamine-based probe which is more sensitive to acidic pH in the range of 4.4–6.7. The probe with pK_a 5.05 is suitable for studying acidic organelles in living cells. Moreover, it can avoid "alkalizing effect" on lysosomes.

2. Experiments

2.1. Materials and reagents

All reagents and solvents were purchased from commercial sources and used without further purification. The solutions of metal ions were prepared from nitrate salts which were dissolved in deionized water. Britton–Robinson (B–R) buffers was mixed by 40 mM acetic acid, boric acid, and phosphoric acid. Dilute hydrochloric acid or sodium hydroxide was used for tuning pH values.

2.2. Apparatus

Thin-layer chromatography (TLC) was conducted on silica gel 60F_{254} plates (Merck KGaA). ^{1}H NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer, using d_6-DMSO as solvent and tetramethylsilane (TMS) as internal standard. Melting points were determined on an XD-4 digital micro melting point apparatus. IR spectra were recorded with an IR spectrophotometer VERTEX 70 FT-IR (Bruker Optics). HRMS spectra were recorded on a Q-TOF6510 spectrograph (Agilent). UV–vis spectra were recorded on a U-4100 (Hitachi). Fluorescent measurements were recorded on a Perkin Elmer LS-55 luminescence spectrophotometer. The pH measurements were performed on a PHS-3C digital pH-meter (YouKe, Shanghai, China).

2.3. Preparation of 3′,6′-bis(diethylamino)-2-(2-((5-phenyl-1,3,4-oxadiazol-2-yl)amino) ethyl)spiro[isoindoline-1,9′-xanthen]-3-one (**5**)

The synthetic route of **5** is shown in Scheme 1. The compound 2-(3′,6′-bis(diethylamino)-3-oxospiro[isoindoline-1,9′-xanthen]-2-yl)ethyl 4-methylbenzenesulfonate 2 and

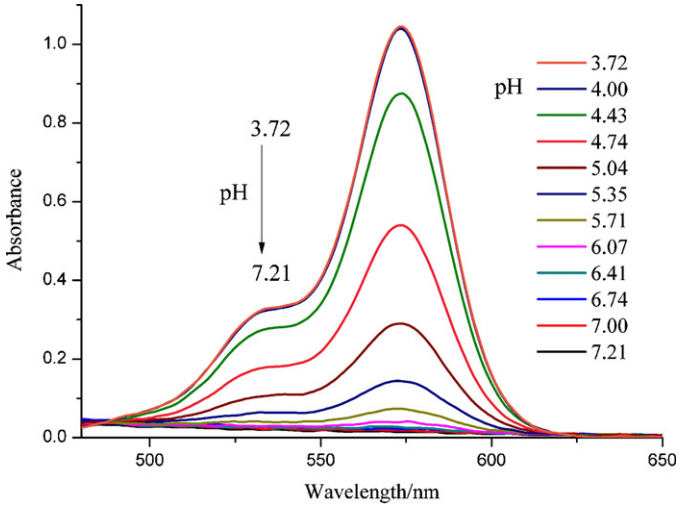


Fig. 1. Absorption spectra of 5 (10 μM) in aqueous solution (9:1, buffer–EtOH, v/v, 0.1 M NaCl) with different pHs.

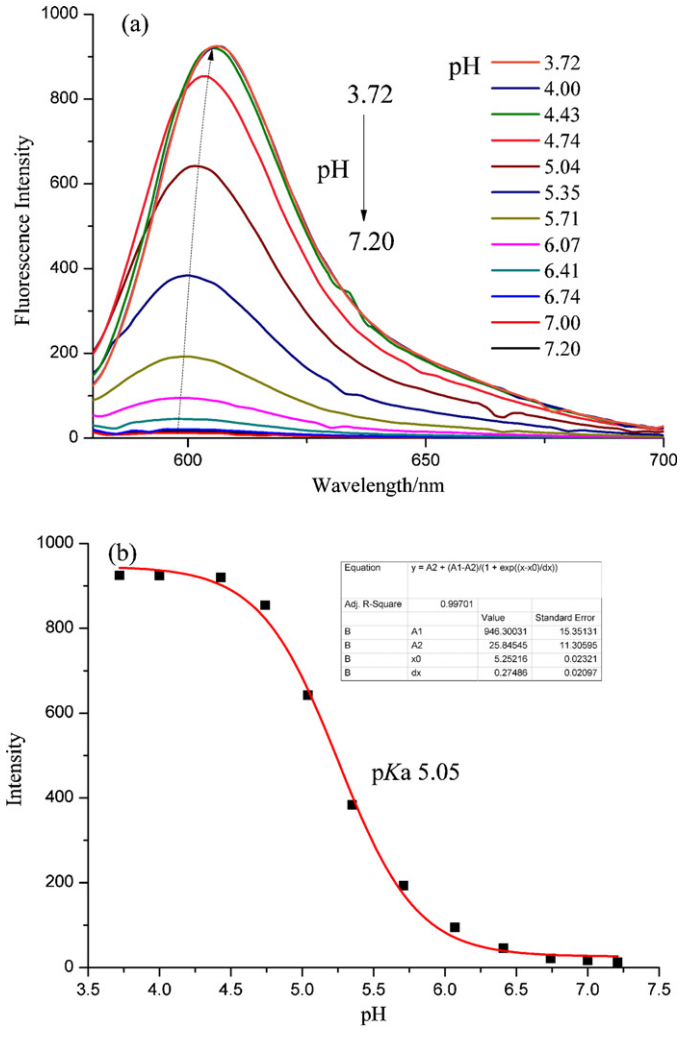


Fig. 2. (a) Fluorescence spectra of 5 (10 μM) in aqueous solution (9:1, buffer–EtOH, v/v, 0.1 M NaCl) with different pH; (b) fluorescence intensity at 603 nm *versus* pH according to the fluorescent pH titration (pH 3.72–7.20), λ_{ex} = 565 nm.

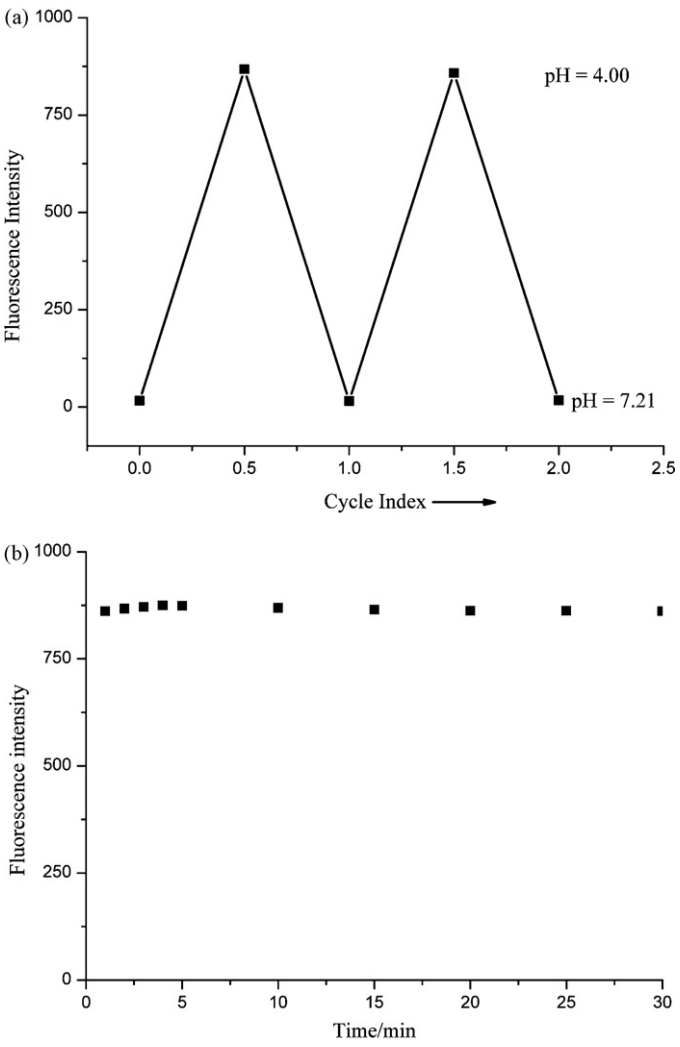


Fig. 3. (a) pH reversibility study of probe **5** between pH 7.2 and 4.0. (b) The time course of fluorescence intensity of probe **5** in buffer solution with pH 4.74 at room temperature.

5-phenyl-1,3,4-oxadiazol-2-amine 4 were synthesized according to the literature methods respectively [33,34].

In a 25 mL round-bottomed flask was placed compound **2** (320 mg, 0.5 mmol), compound **4** (80 mg, 0.5 mmol), potassium carbonate (104 mg, 0.75 mmol) and dry acetonitrile (10 mL). The reaction mixture was refluxed for 2 h. Then it was cooled and filtered. The filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography using petroleum ether/ethyl acetate (2: 1, v/v) as an eluent. The target compound **5** was obtained as light yellow solid in 82.7% yield (265 mg). mp: 133–135 °C. IR (KBr) ν: 3406 (N—H), 1615 (C=O) cm^{-1}; ^1H NMR (d_6-DMSO, 400 MHz)) δ (ppm): 1.10 (t, 12H, *J* = 6.9 Hz, CH_3), 3.04–3.11 (m, 2H, CH_2), 3.19–3.25 (m, 2H, CH_2), 3.34 (q, 8H, *J* = 6.9 Hz, CH_2), 4.67 (t, 1H, *J* = 5.6 Hz, NH), 6.36 (d, 2H, *J* = 9.5 Hz, Xanthene-H), 6.40–6.44 (m, 4H, Xanthene-H), 7.09 (d, 1H, *J* = 7.7 Hz, Ar—H), 7.44 (t, 1H, *J* = 7.5 Hz, Ar—H), 7.53 (t, 1H, *J* = 7.5 Hz, Ar—H), 7.55–7.61 (m, 3H, Ar—H), 7.93 (d, 1H, *J* = 7.7 Hz, Ar—H), 7.95–8.01 (m, 2H, Ar—H); HRMS calcd for $[M+H]^+$ $C_{38}H_{41}N_6O_3$: 629.3240, found 629.3214.

2.4. Cell culture and imaging

Hela cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% (v/v) calf bovine serum (HyClone, USA) at 37 °C in humidified air and 5% CO_2. Human umbilical vein endothelial cells (HUVECs) were obtained as described previously [35]. The cells were routinely cultured in M199 medium (Gibico, USA) supplemented with 20% (v/v) fetal bovine serum (HyClone, USA) and 2 μg/mL fibroblast growth factor (FGF) in the same incubator environment. For fluorescence imaging, both types of cells ($4–5 \times 10^4$ cells/mL) were seeded into 24-well plates, cells were treated with or without bafilomycin A1 (0–40 nM) or chloroquine (0–32 μM) in the presence of probe (3 μM), respectively, then incubated for 12 h. The fluorescent images were photographed using Nikon Eclipse TE2000-U fluorescence inverted microscope. We chose the optical filter G-2A, the parameters of which are EX 510-560, DM 575, and BA 590.

3. Results and discussion

3.1. Synthesis of the probe (*5*)

The probe was designed based on the excellent photophysical properties of rhodamine B and its switching property responding to pH. Firstly, the compound 2-(3′,6′-bis(diethylamino)-3-oxospiro[isoindoline-1,9′-xanthen]-2-yl)ethyl 4-methylbenzenesulfonate 2 and 5-phenyl-1,3,4-oxadiazol-2-amine 4 were synthesized according to the literature respectively [33,34].

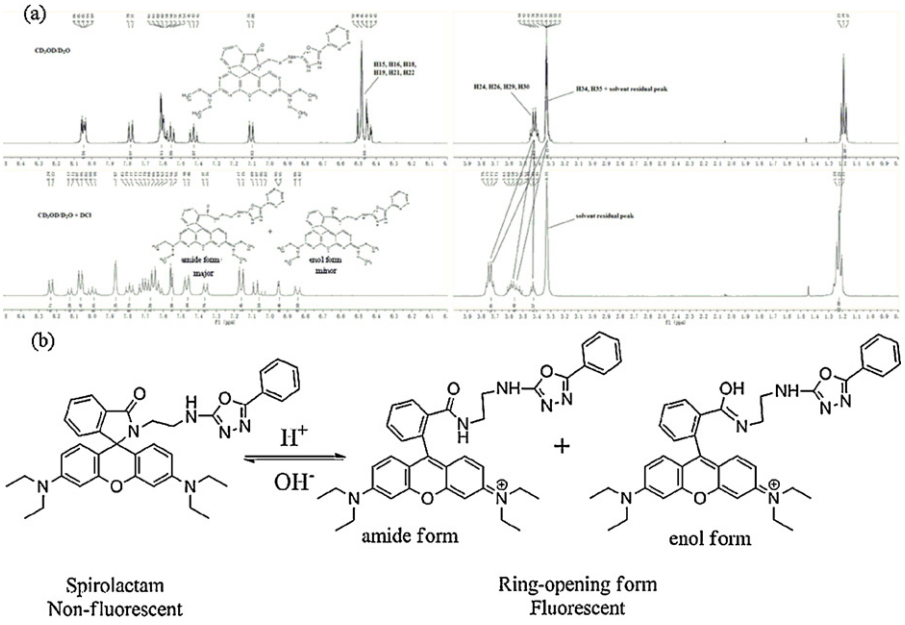


Fig. 4. (a) ^{1}H NMR spectra of probe **5** under neutral and acidic conditions; (b) spirolactam ring-opening process of the probe **5** upon addition of H^+.

Then, compound **5** was obtained by the reaction of compound **2** and **4** under the condition of refluxing acetonitrile in 83% yield. The structure of **5** was confirmed by ^{1}H NMR, IR and HRMS. Compound **5** has a spirocyclic structure and is non-fluorescent. Therefore, compound **5** is expected to act as a signal switcher, which is envisioned to turn on when the target proton is bound in acidic solution.

3.2. Spectroscopic properties and optical responses to pH

All samples were performed in aqueous solution (9:1, buffer–EtOH, v/v, 0.1 M NaCl) and waited for 1 h before measurement. The UV–vis pH titration of **5** was shown in Fig. 1. As we expected, free **5** in neutral aqueous solution exhibited very weak absorbance above 500 nm. With the decrease of pH values, the absorbance enhanced significantly and a new peak at 574 nm appeared indicating that the spirolactam ring of rhodamine was opened. The absorbance reached the maximum value at pH = 4.0. Moreover, color of the solution changes from colorless to pink (Fig. S1) which suggested probe **5** can serve as a "naked-eye" pH indicator.

The fluorescence emission spectra of probe **5** at different pH were shown in Fig. 2(a). Probe **5** was non-fluorescent at weak basic pH. When the value of pH falls to less than 7.0, the fluorescence intensity gradually increased (about 46-fold from 6.7 to 4.4) and a pink color appeared, indicating that this probe is very sensitive to acidic pH. Meanwhile, the maximum emission wavelength had a red shift from 595 nm to 603 nm. The quantum yield of the probe **5** was determined to be 0.71 in acidic condition (pH = 4.5) and 0.09 in neutral condition (pH = 7.0) with rhodamine B (Φ = 0.69 in ethanol) as a standard [36]. Fig. 2(b) shows the fluorescence intensity at 603 nm *versus* pH according to the fluorescent pH titration (pH

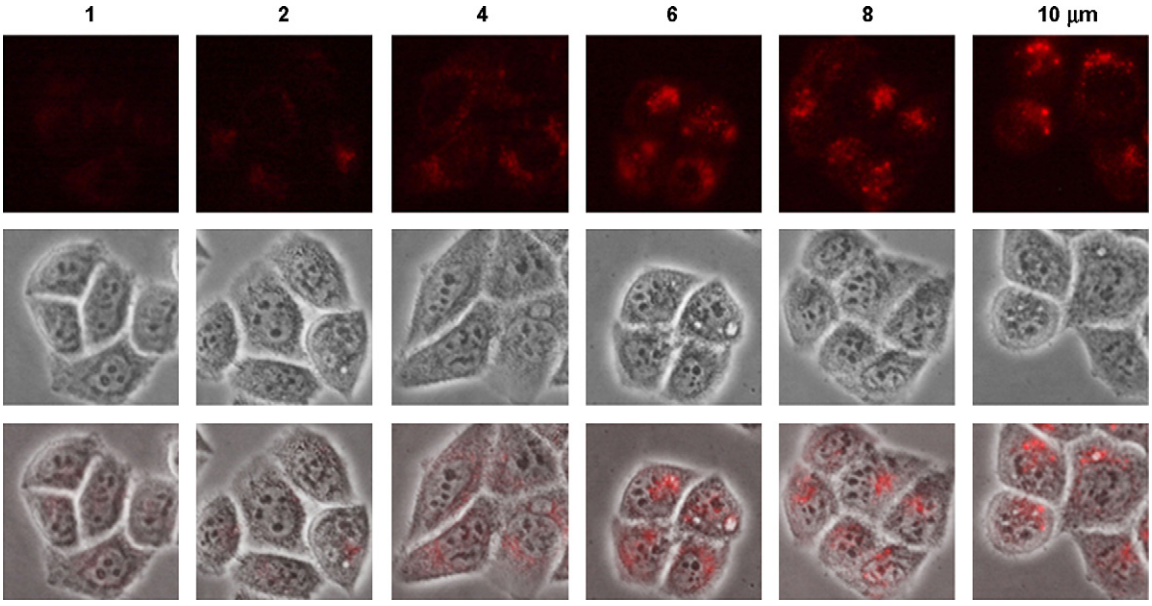


Fig. 5. Fluorescence microscope images of living Hela cells with different concentrations of **5** for 12 h at 37 °C.

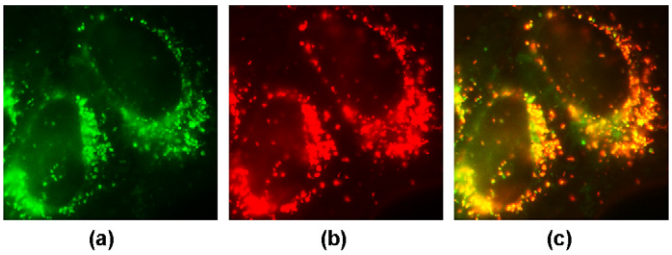


Fig. 6. Fluorescence microscope images of living Hela cells co-stained with 6 μM probe **5** and 1 μM LysoSensor® Green. (a) Green emission from LysoSensor® Green; (b) red emission from probe **5**; (c) overlay of (a) and (b), areas of co-localization appear in yellow. (Fluorescence images were acquired using the Zeiss Axio Observer. A1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.72–7.20, λ_{ex} = 565 nm). According to the Henderson–Hasselbach-type mass action equation, the pK_a value calculated is 5.05, which indicated probe **5** is very suitable for studying acidic organelles. The fluorescence intensity of probe **5** was linearly proportional (R = 0.98809) to pH values in the range of 4.7–5.7 as shown in Fig. S2. Moreover, the reversibility of probe **5** is excellent between pH 4.0 and pH 7.2 (Fig. 3(a)). The time course of fluorescence intensity of probe **5** in buffer solution with pH 4.74 at room temperature demonstrated that this probe is very sensitive to acidic pH and is stable to environmental factors such as light, air and medium (Fig. 3(b)). The fluorescence enhancement multiples of probe **5** at different concentrations at pH = 4.0 and pH = 7.2 are shown in Fig. S3, which indicate that the probe can be used at a lower concentration than some reported rhodamine-based pH probes [26,28].

The intracellular environment is very complex. It contains a variety of metal ions, such as Ca^{2+}, Mg^{2+}, Zn^{2+}, and Cu^{2+}, which can bind with amines [37–39]. The binding may cause ring-opening reaction of rhodamine B-based probes. In order to confirm probe **5** can be used for cell imaging, the influence of metal cations was studied at pH = 4.74 (Fig. S4) and pH = 7.21 (Fig. S5). The results demonstrate that these metal ions have no influences on the response of this probe to acidic pH. Even when the abundant cations in cells, Na^+, K^+, Ca^{2+} and Mg^{2+}, are added at mM range, the pH sensing behavior of probe **5** is not interfered. All these results suggest that probe **5** has specific fluorescent response to acidic pH and is suitable for fluorescent intracellular pH imaging.

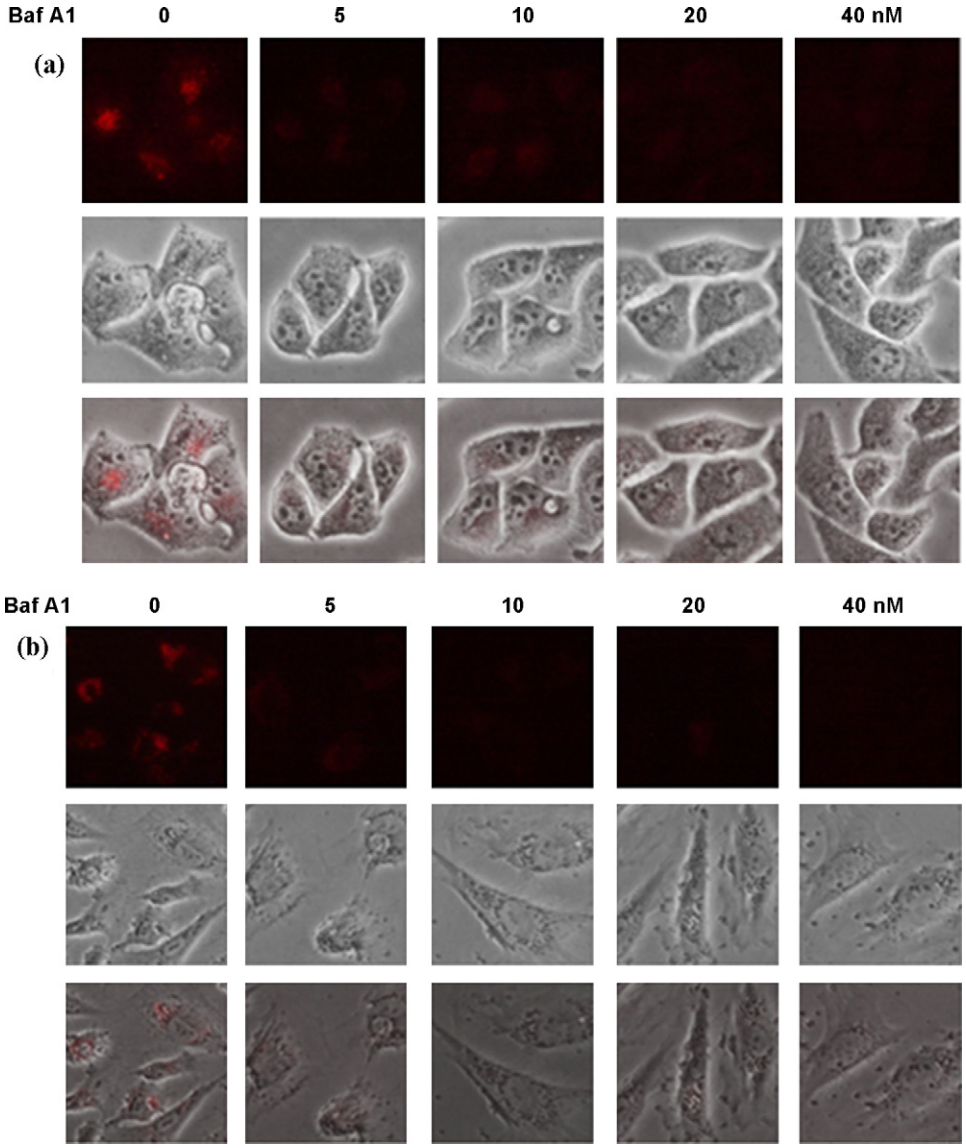


Fig. 7. (a) Fluorescence microscope images of living Hela cells with 3 μM probe **5** and 0–40 nM bafilomycin A1 for 12 h at 37 °C; (b) fluorescence microscope images of HUVECs with 3 μM probe **5** and 0–40 nM bafilomycin A1 for 12 h at 37 °C.

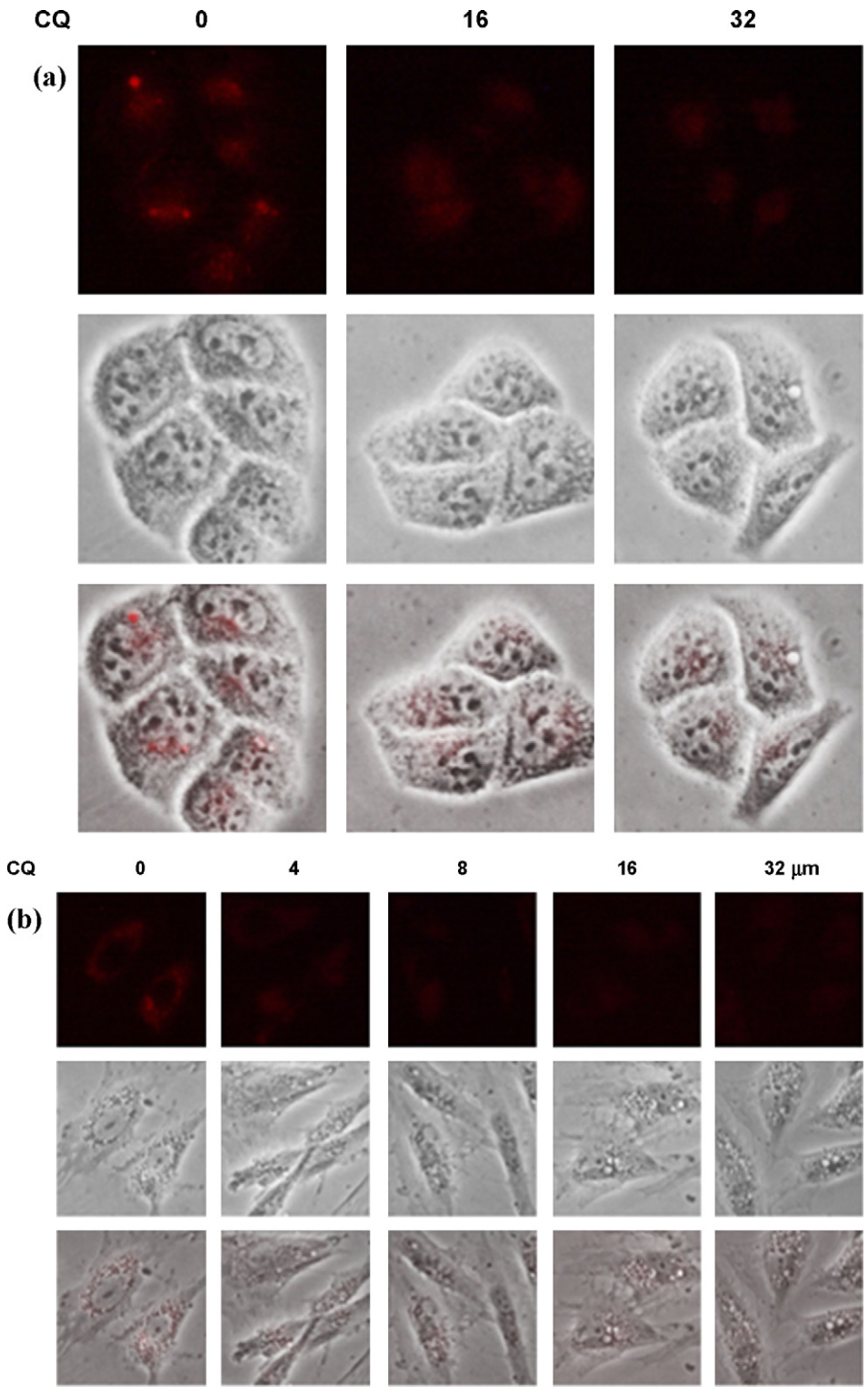


Fig. 8. (a) Fluorescence microscope images of living Hela cells with 3 μM probe **5** and 0–32 μM chloroquine for 12 h at 37 °C; (b) fluorescence microscope images of HUVECs with 3 μM probe **5** and 0–32 μM chloroquine for 12 h at 37 °C.

Rhodamine-based probes have a similar mechanism based on the change between spirolactam/lactone and ring-opening forms [40,41]. There are two possible open-ring structural forms, *i.e.* amide form and enol form. ^{1}H NMR spectra of probe **5** under neutral and acidic conditions have been determined in CD_3OD/D_2O (10:1, v/v) as shown in Fig. 4(a). It can be seen that chemical shifts of all protons changed under acidic condition. We can see clear down-field shift for the signals of the xanthene protons and the methylene protons connected with the quaternary ammonium ion under acidic condition. In addition, the ring-opening of spirolactam causes down-field shift of the methylene protons of ethylenediamine. Although accurate ownership of the protons is difficult because some signals are overlapped, the integration of the signals indicates that the two open-ring structural forms exist in the

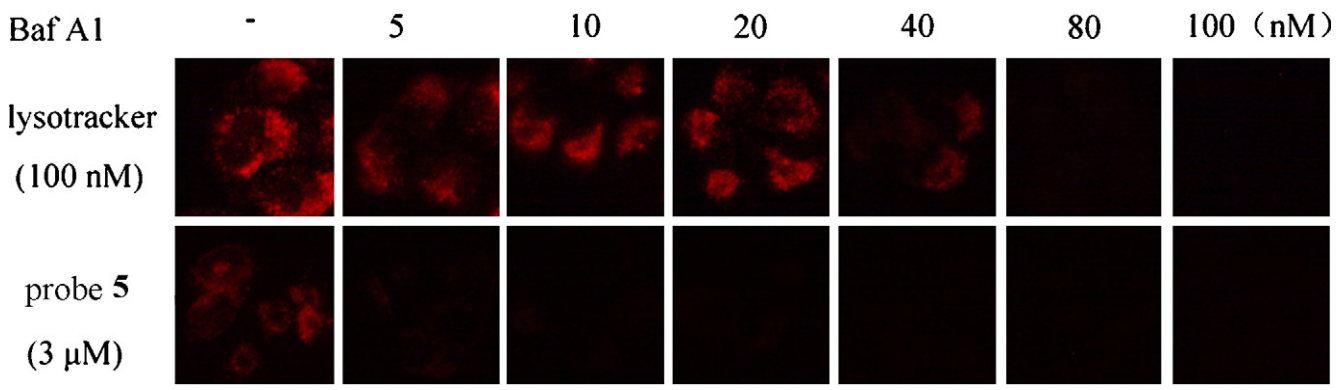


Fig. 9. Fluorescence microscope images of living Hela cells with 3 μM probe **5** or 100 nM lysotracker and 0–40 nM bafilomycin A1 at 37 °C.

acidic solution simultaneously. The stronger signals belong to the more stable amide form, while the weaker signals belong to the enol form. The proportion of the two forms is about 7:3. Our observations are consistent with reported mechanism [42]. The H^+-induced spirolactam ring opening process of the probe is shown in Fig. 4(b).

3.3. Fluorescence imaging in living cells

We applied probe **5** to Hela cells to investigate its imaging in cells. Incubation of Hela cells with different concentrations of probe **5** for 3–12 h gave intracellular fluorescence as monitored by fluorescence microscopy (Fig. S6). The results demonstrated that the probe was cell-permeable and the fluorescence intensity increased in concentration and time dependent manner. From Fig. 5, it can be seen that fluorescence inside the cells is mainly concentrated near the nucleus, displaying the preferential affinity of probe **5** for lysosomes.

To further confirm the subcellular distribution of probe **5**, Hela cells were co-stained with probe **5** and a commercially available lysosome-specific staining probe, LysoSensor® Green DND-189. Fig. 6 shows that the probe **5** can co-localize with LysoSensor® Green, suggesting that the probe **5** can be used to selectively mark lysosomes.

3.4. Lysosomal pH changes in Hela cells and vascular endothelial cells induced by bafilomycin A1

In order to further assess the potential applications of the probe as an acid pH indicator with an optimum effect at pH 4–6, we examined the fluorescence of the probe in Hela cells or HUVECs that were treated with Bafilomycin A1. Bafilomycin A1 is a selective inhibitor of the vacuolar-type H^+-ATPase (V-ATPase) and thereby could inhibit lysosomal acidification. We incubated Hela cells or HUVECs with bafilomycin A1 (0–40 nM) and the probe (3 μM) for 12 h, then fluorescent imaging inside the cells was monitored by fluorescence microscopy (Fig. 7). Compared with relatively strong fluorescence in control groups, fluorescence in bafilomycin A1 treated groups was obviously weaker, in both cell types. The results suggested the probe can monitor the changes of lysosomal pH.

3.5. Lysosomal pH changes in Hela cells and vascular endothelial cells induced by chloroquine

We further confirmed the above findings by treating Hela cells and HUVECs with chloroquine (0–32 μM) for 12 h. Chloroquine is believed to be another agent capable of impairing lysosomal acidification and inducing cytosolic vacuolation. As predicted, chloroquine weakened significantly the fluorescence of the probe, consisting with the changes induced by bafilomycin A1 (Fig. 8).

3.6. Comparison of probe *5* and lysotracker Red DND-99

In order to investigate the ability of probe **5** to detect pH changes, we compared this probe with commercial lysotracker Red DND-99. As shown in Fig. 9, the fluorescence intensity in Hela cells incubated with probe **5** and 5 nM bafilomycin A1 significantly weakened. While after treated with 40 nM bafilomycin A1, the fluorescence intensity of lysotracker Red DND-99 began to weaken. This demonstrated that probe **5** can more sensitively detect the pH changes in lysosomes caused by bafilomycin A1 than lysotracker Red DND-99.

4. Conclusion

In summary, we have described the synthesis, characterization and cellular applications of a novel rhodamine B-based pH probe. This probe has specific fluorescent response to acidic pH and high quantum yield in acidic condition. The fluorescence intensity of the probe increases about 46-fold from pH 6.7 to 4.4 with a pK_a value of 5.05, which is very suitable for studying acidic organelles in living cells. Furthermore, this probe shows a good liner relationship between fluorescence intensity and pH in the range of 4.7–5.7. We applied the probe in Hela cells and vascular endothelial cells to monitor pH changes in lysosomes. The probe can be used to selectively mark lysosomes. The fluorescence of the probe in cells weakened with the increase of Baf A1 or CQ which induce pH value increase in lysosomes. We believe that this probe will have significant application in cell imaging and monitoring of lysosomes pH changes.

Acknowledgments

This study was supported by 973 Program (2010CB933504) and National Natural Science Foundation of China (90813022 and 20972088).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2012.12.014.

References

[1] S.C. Burleigh, T. van de Laar, C.J. Stroop, W.M. van Grunsven, N. O'Donoghue, P.M. Rudd, G.P. Davey, Synergizing metabolic flux analysis and nucleotide sugar metabolism to understand the control of glycosylation of recombinant protein in CHO cells, BMC Biotechnology 11 (2011) 95–111.
[2] T. Otomo, K. Higaki, E. Nanba, K. Ozono, N. Sakai, Lysosomal storage causes cellular dysfunction in mucolipidosis II skin fibroblasts, Journal of Biological Chemistry 286 (2011) 35283–35290.

[3] S.H. Yoo, Y.S. Hur, Enrichment of the inositol 1,4,5-trisphosphate receptor/Ca^{2+} channels in secretory granules and essential roles of chromogranins, Cell Calcium 51 (2012) 342–350.
[4] R. Martínez-Zaguilán, B.F. Chinnock, S. Wald-Hopkins, M. Bernas, D. Way, M. Weinand, W.H. Witte, R.J. Gillies, $[Ca^{2+}]_i$ and pH_{in} homeostasis in kaposi sarcoma cells, Cellular Physiology and Biochemistry 6 (1996) 169–184.
[5] S. Humez, M. Monet, F. van Coppenolle, P. Delcourt, N. Prevarskaya, The role of intracellular pH in cell growth arrest induced by ATP, American Journal of Physiology – Cell Physiology 287 (2004) C1733–C1746.
[6] M. Czepán, Z. Rakonczay, A. Varró, I. Steele, R. Dimaline, N. Lertkowit, J. Lonovics, A. Schnúr, G. Biczó, A. Geisz, G. Lázár, Z. Simonka, V. Venglovecz, T. Wittmann, P. Hegyi, NHE1 activity contributes to migration and is necessary for proliferation of human gastric myofibroblasts, Pflügers Archiv European Journal of Physiology 463 (2012) 459–475.
[7] J.K. Laihia, J.P. Kallio, P. Taimen, H. Kujari, V.M. Kähäri, L. Leino, Protodynamic intracellular acidification by cis-urocanic acid promotes apoptosis of melanoma cells in vitro and in vivo, Journal of Investigative Dermatology 130 (2010) 2431–2439.
[8] J. Suk, S.S. Kwak, J.H. Lee, J.H. Choi, S.H. Lee, D.H. Lee, B. Byun, G.H. Lee, C.O. Joe, Alkaline stress-induced autophagy is mediated by mTORC1 inactivation, Journal of Cellular Biochemistry 112 (2011) 2566–2573.
[9] H. Izumi, T. Torigoe, H. Ishiguchi, H. Uramoto, Y. Yoshida, M. Tanabe, T. Ise, T. Murakami, T. Yoshida, M. Nomoto, K. Kohno, Cellular pH regulators: potentially promising molecular targets for cancer chemotherapy, Cancer Treatment Reviews 29 (2003) 541–549.
[10] V. Vingtdeux, M. Hamdane, S. Bégard, A. Loyens, A. Delacourte, J.C. Beauvillain, L. Buée, P. Marambaud, N. Sergeant, Intracellular pH regulates amyloid precursor protein intracellular domain accumulation, Neurobiology of Disease 25 (2007) 686–696.
[11] M. Stubbs, P.M. McSheehy, J.R. Griffiths, C.L. Bashford, Causes and consequences of tumor acidity and implications for treatment, Molecular Medicine Today 6 (2000) 15–19.
[12] E.J. Blott, G.M. Griffiths, Secretory lysosomes, Nature Reviews Molecular Cell Biology 3 (2002) 122–131.
[13] J. Stinchcombe, G. Bossi, G.M. Griffiths, Linking albinism and immunity: the secrets of secretory lysosomes, Science 305 (2004) 55–59.
[14] J. Han, K. Burgess, Fluorescent indicators for intracellular pH, Chemical Reviews 110 (2010) 2709–2728.
[15] L.Q. Ying, B.P. Branchaud, Selective labeling and monitoring pH changes of lysosomes in living cells with fluorogenic pH sensors, Bioorganic & Medicinal Chemistry Letters 21 (2011) 3546–3549.
[16] T. Hasegawa, Y. Kondo, Y. Koizumi, T. Sugiyama, A. Takeda, S. Ito, F. Hamada, A highly sensitive probe detecting low pH area of Hela cells based on rhodamine B modified beta-cyclodextrins, Bioorganic & Medicinal Chemistry 17 (2009) 6015–6019.
[17] H. Zhu, J. Fan, Q. Xu, H. Li, J. Wang, P. Gao, X. Peng, Imaging of lysosomal pH changes with a fluorescent sensor containing a novel lysosome-locating group, Chemical Communications 48 (2012) 11766–11768.
[18] F. Galindo, M.I. Burguete, L. Vigara, S.V. Luis, N. Kabir, J. Gavrilovic, D.A. Russell, Synthetic macrocyclic peptidomimetics as tunable pH probes for the fluorescence imaging of acidic organelles in live cells, Angewandte Chemie International Edition 44 (2005) 6504–6508.
[19] H.M. Kim, M.J. An, J.H. Hong, B.H. Jeong, O. Kwon, J.Y. Hyon, S.C. Hong, K.J. Lee, B.R. Cho, Two-photon fluorescent probes for acidic vesicles in live cells and tissue, Angewandte Chemie International Edition 47 (2008) 2231–2234.
[20] X. Zhou, F. Su, H. Lu, P. Senechal-Willis, Y. Tian, R.H. Johnson, D.R. Meldrum, An FRET-based ratiometric chemosensor for in vitro cellular fluorescence analyses of pH, Biomaterials 33 (2012) 171–180.
[21] H. Sun, K. Almdal, T.L. Andresen, Expanding the dynamic measurement range for polymeric nanoparticle pH sensors, Chemistry Communications 47 (2011) 5268–5270.
[22] L. Yuan, W. Lin, B. Chen, Y. Xie, Development of FRET-based ratiometric fluorescent Cu^{2+} chemodosimeters and the applications for living cell imaging, Organic Letters 14 (2012) 432–435.
[23] Z. Yang, M. She, B. Yin, J. Cui, Y. Zhang, W. Sun, J. Li, Z. Shi, Three rhodamine-based "off-on" chemosensors with high selectivity and sensitivity for Fe^{3+} imaging in living cells, Journal of Organic Chemistry 77 (2012) 1143–1147.
[24] V. Bhalla, Roopa, M. Kumar, P.R. Sharma, T. Kaur, New fluorogenic sensors for Hg^{2+} ions: through-bond energy transfer from pentaquinone to rhodamine, Inorganic Chemistry 51 (2012) 2150–2156.
[25] S. Kang, S. Kim, Y.-K. Yang, S. Bae, J. Tae, Fluorescent and colorimetric detection of acid vapors by using solid-supported rhodamine hydrazides, Tetrahedron Letters 50 (2009) 2010–2012.
[26] W. Zhang, B. Tang, X. Liu, Y. Liu, K. Xu, J. Ma, L. Tong, G. Yang, A highly sensitive acidic pH fluorescent probe and its application to HepG2 cells, Analyst 134 (2009) 367–371.
[27] Q.A. Best, M.E. McCarroll, L. Wang, D.J. Dyer, Design and investigation of a series of rhodamine-based fluorescent probes for optical measurements of pH, Organic Letters 12 (2010) 3219–3221.
[28] N.B. Yapici, S.R. Mandalapu, T.L. Chew, S. Khuon, L. Bi, Determination of intracellular pH using sensitive, clickable fluorescent probes, Bioorganic & Medicinal Chemistry Letters 22 (2012) 2440–2443.
[29] L. Yuan, W. Lin, Y. Feng, A rational approach to tuning the pK_a values of rhodamines for living cell fluorescence imaging, Organic & Biomolecular Chemistry 9 (2011) 1723–1726.
[30] V.B. Bojinov, A.I. Venkova, N.I. Georgiev, Synthesis and energy-transfer properties of fluorescence sensing bichromophoric system based on Rhodamine 6G and 1,8-naphthalimide, Sensors and Actuators B 143 (2009) 42–49.
[31] Q.J. Ma, H.P. Li, F. Yang, J. Zhang, X.F. Wu, Y. Bai, X.F. Li, A fluorescent sensor for low pH values based on a covalently immobilized rhodamine–napthalimide conjugate, Sensors and Actuators B 166–167 (2012) 68–74.
[32] Z. Li, S. Wu, J. Han, S. Han, Imaging of intracellular acidic compartments with a sensitive rhodamine based fluorogenic pH sensor, Analyst 136 (2011) 3698–3706.
[33] A. Chatterjee, M. Santra, N. Won, S. Kim, J.K. Kim, S.B. Kim, K.H. Ahn, Selective fluorogenic and chromogenic probe for detection of silver ions and silver nanoparticles in aqueous media, Journal of the American Chemical Society 131 (2009) 2040–2041.
[34] S.K. Kashaw, V. Gupta, V. Kashaw, P. Mishra, J.P. Stables, N.K. Jain, Anticonvulsant and sedative-hypnotic activity of some novel 3-[5-(4-substituted)phenyl-1,3,4-oxadiazole-2yl]-2-styrylquinazoline-4(3H)-ones, Medicinal Chemistry Research 19 (2010) 250–261.
[35] B.M. Jaffe, H.R. Behrman, C.W. Parker, Radioimmunoassay measurement of prostaglandins E, A, and F in human plasma, The Journal of Clinical Investigation 52 (1973) 398–405.
[36] C.A. Parker, W.T. Rees, Correction of fluorescence spectra and measurement of fluorescence quantum efficiency, Analyst 85 (1960) 587–600.
[37] A.W. Czarnik, Chemical communication in water using fluorescent chemosensors, Accounts of Chemical Research 27 (1994) 302–308.
[38] S.J. Pond, O. Tsutsumi, M. Rumi, O. Kwon, E. Zojer, J.L. Brédas, S.R. Marder, J.W. Perry, Metal-ion sensing fluorophores with large two-photon absorption cross sections: aza-crown ether substituted donor–acceptor–donor distyrylbenzenes, Journal of the American Chemical Society 126 (2004) 9291–9306.
[39] Z. Xu, J. Yoon, D.R. Spring, Fluorescent chemosensors for Zn^{2+}, Chemical Society Reviews 39 (2010) 1996–2006.
[40] H.N. Kim, M.H. Lee, H.J. Kim, J.S. Kim, J. Yoon, A new trend in rhodamine-based chemosensors: application of spirolactam ring-opening to sensing ions, Chemical Society Reviews 37 (2008) 1465–1472.
[41] X. Chen, T. Pradhan, F. Wang, J.S. Kim, J. Yoon, Fluorescent chemosensors based on spiroring-opening of xanthenes and related derivatives, Chemical Reviews 112 (2012) 1910–1956.
[42] J. Wang, Q. Yang, H. Song, W. Zhang, A fluorescent probe of N′-formylrhodamine B hydrazide: structure and spectral properties of protonation behaviour, Organic & Biomolecular Chemistry 10 (2012) 7677–7680.

Biographies

Hong-Shui Lv received his B.Sc. in chemistry from Shandong University in 2008. At present, he is a PhD student in Institute of Organic Chemistry, School of Chemistry and Chemical Engineering, Shandong University. His current research interest involves the synthesis of the fluorescence materials and the application of fluorescent sensors.

Jing Liu received her B.Sc. in Cell Biology from Shandong Normal University in 2011. At present, she is a graduate student in Cell Biology, School of Life Science, Shandong University. Her current research interest involves the research on differentiation, autophagy, or apoptosis and modulation of cell fate by small molecules with structural diversity.

Jing Zhao received her PhD in Cell Biology from School of Life Science, Shandong University in 2006. After postdoctoral fellowship with Professor Bao-Xiang Zhao at Shandong University, she spent her career at the Shandong University where she has been associate professor of Cell Biology since 2008. Her main scientific interests are the research on differentiation, autophagy, or apoptosis and modulation of cell fate by small molecules with structural diversity.

Bao-Xiang Zhao received his PhD (1998) in chemistry from the Nagoya University, Japan. Then, he became associate professor at Shandong University. After postdoctoral fellowship with Professor S. Blechert at Technology University Berlin, he spent his career at the Shandong University where he has been Professor of Organic Chemistry since 2000. His main scientific interests are the design and synthesis of small molecule with structural diversity for chemical biology research. One of his current research interests is synthesis of fluorescent probe for detecting ions in water and in living cells.

Jun-Ying Miao received her PhD (1997) in Cell Biology from the Nagoya University, Japan. She spent her career at the Shandong University where she has been Professor of Cell Biology since 1999. Her main scientific interests are the research on differentiation, autophagy, or apoptosis and modulation of cell fate by small molecules with structural diversity.