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A new fluorescent pH probe for imaging lysosomes in living cells

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ABSTRACT

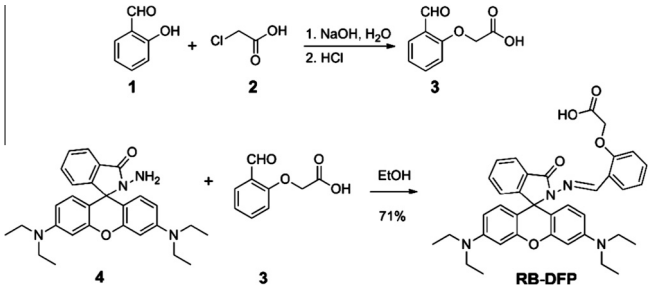
A new rhodamine B-based pH fluorescent probe has been synthesized and characterized. The probe responds to acidic pH with short response time, high selectivity and sensitivity, and exhibits a more than 20-fold increase in fluorescence intensity within the pH range of 7.5–4.1 with the pK_a value of 5.72, which is valuable to study acidic organelles in living cells. Also, it has been successfully applied to HeLa cells, for its low cytotoxicity, brilliant photostability, good membrane permeability and no 'alkalizing effect' on lysosomes. The results demonstrate that this probe is a lysosome-specific probe, which can selectively stain lysosomes and monitor lysosomal pH changes in living cells.

Intracellular pH plays a critical role in many cellular events, such as cell growth,[1,2] apoptosis,[3] autophagy,[4] signal transduction[5] and proliferation.[6] Abnormal intracellular pH values indicate abnormal cell events and are observed in some diseases including cancer[7] and Alzheimer's disease.[8] The pH value of the cytoplasma is about 7.2, while some organelles, such as endosomes and lysosomes, have acidic microenvironment 4.0–6.0[9,10] which can facilitate the degradation of proteins in cellular metabolism. Thus critical information of physiological and pathological processes can be provided by accurately measuring intracellular pH values.

Fluorescence detection has been widely used because of its high sensitivity, simple operation and real-time monitoring. The binding of analytes to probe causes fluorescence enhancement ('turn on') or fluorescence quenching ('turn off'). Fluorescent quenching probes may provide incorrect results because quenching can be caused by other quenchers in real samples.[11,12] Therefore, it is desirable to develop fluorescence-enhanced probes. Some small molecular fluorescent probe for pH have been reported.[13–18] Rhodamine derivatives with a spirocyclic structure are non-fluorescent and colorless, while the ring-opening of spirocyclic structure gives a strong fluorescence emission and a pink color. This makes rhodamine derivatives serve as excellent 'turn on' fluorescent probes. In recent years, a large number of rhodamine-based fluorescent probes for metal ions and biologically relevant species have been developed.[19,20] The spirocyclic structure is also 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. Although some rhodamine-based fluorescent pH probes have been reported, only few are applied for cell imaging.[21–23] In order to take advantage of the excellent photophysical and structural properties of rhodamine derivatives, it is necessary to design new pH probes which are suitable for cell imaging.

As a continuation of our work on the fluorescent probes for metal ions and pH,[24–27] here, we designed and synthesized a new rhodamine B-based probe (**RB-DFP**) for detecting H^+. The probe with a pK_a value of 5.72 is suitable for studying acidic organelles in living cells.

Chemistry: Probe **RB-DFP** was readily synthesized from rhodamine B hydrazide (**4**) and 2-(2-formylphenoxy) acetic acid (**3**) in EtOH under reflux (Scheme 1). The structure of **RB-DFP** was confirmed by ^{1}H NMR, ^{13}C NMR, IR and HRMS. A solution of **RB-DFP** in EtOH/HEPES (pH 7.2) is colorless and non-fluorescent, indicating



Scheme 1. Synthesis of compound **RB-DFP**.

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that **RB-DFP** has the spirolactam form only. The characteristic peak of the spiro carbon in the ^{13}C NMR spectrum at 66.6 ppm also supports this consideration.[28]

As Figure 1a shows, **RB-DFP** is nearly non-fluorescent at pH 7.5. However, a strong fluorescence enhanced signal appeared at 589 nm and increased drastically caused by the H^+-induced ring opening of spirolactam (Scheme 2) when the pH decreased to 4.1 from 7.5. Meanwhile, the fluorescence quantum yield (Φ_F) of **RB-DFP** at pH = 4.2 reached 0.39, using rhodamine B (Φ_F = 0.69 in ethanol) as a standard.[29] Moreover, the fluorescence titration data yielded a pK_a of 5.72 (Fig. 1b), indicating that the detection range of **RB-DFP** can cover both normal and abnormal lysosome pH. Also, the trends of absorption spectra depending on pH are in agreement with emission spectra (Fig. S1).

The time course study showed that the fluorescence intensity of **RB-DFP** can reach the maximum in about 1 min in acidic solution (Fig. S2a). Furthermore, **RB-DFP** exhibits a good reversibility between pH 4.2 and pH 7.5 (Fig. S2b). Thus, the probe can monitor real-time pH changes with very short response time, which is one of the merits of it.

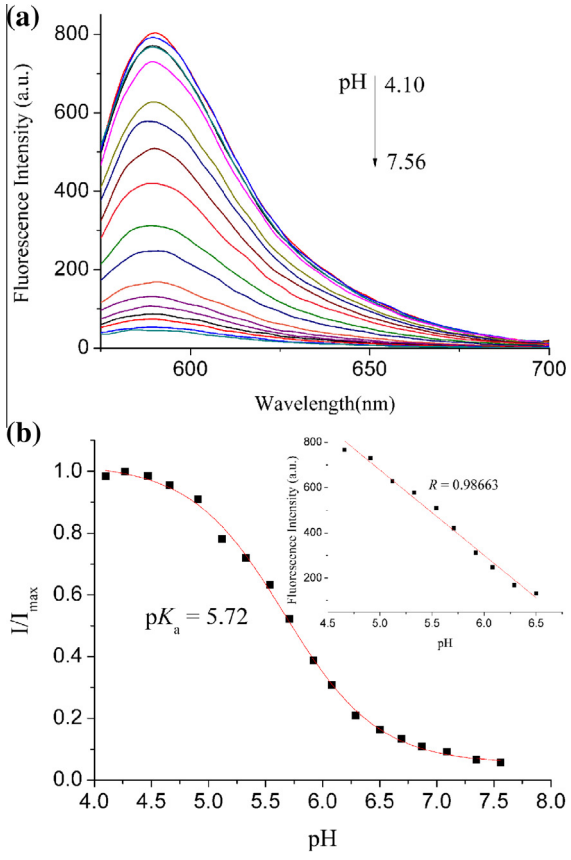
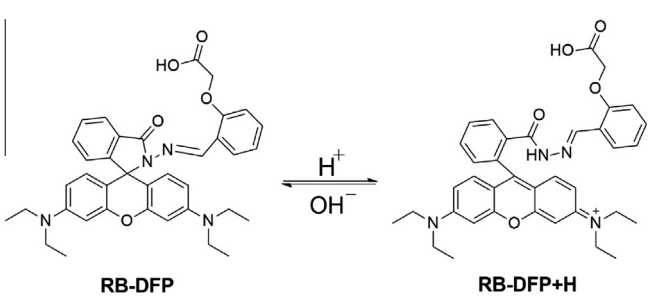


Figure 1. (a) Fluorescence spectra of **RB-DFP** (10 μM) in aqueous solution (Britton–Robinson buffer–EtOH, 9:1, v/v) with different pH; (b) pH titration curve of **RB-DFP**, λ_{ex} = 563 nm, λ_{em} = 589 nm. The inset shows the linear relationship of fluorescence intensity at 589 nm and varying pH values from 4.6 to 6.5.



Scheme 2. Detection mechanism of **RB-DFP** for pH.

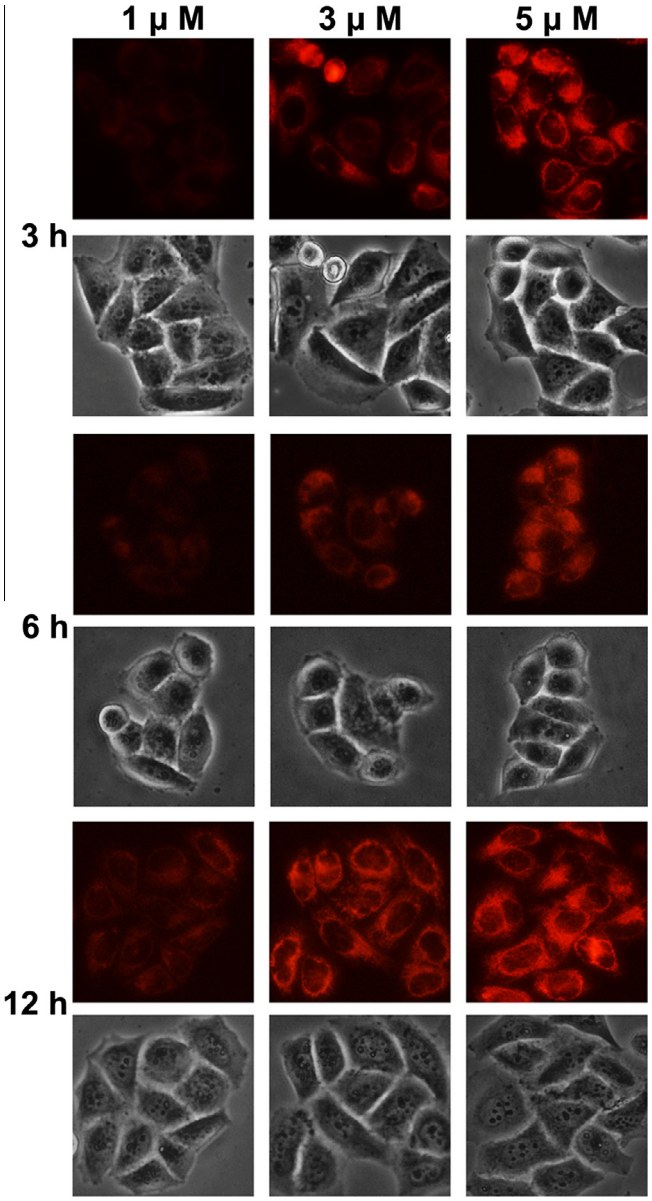


Figure 2. Fluorescence microscope images of living HeLa cells with different concentrations of **RB-DFP** for 3–12 h at 37 °C.

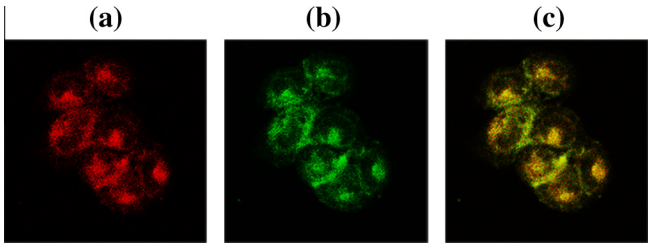


Figure 3. Fluorescence microscope images of living HeLa cells co-stained with 1 μM **RB-DFP** and 0.25 μM LysoSensor green. (a) Red emission from **RB-DFP**; (b) green emission from LysoSensor green; (c) overlay of (a) and (b), areas of co-localization appear in yellow.