#### **RAPID COMMUNICATION**



# Preparation of a Novel pH-responsive Fluorescent Probe Based on an Imidazo[1,2-a]indole Fluorophore and its Application in Detecting Extremely Low pH in *Saccharomyces cerevisiae*

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#### Abstract

A novel pH-responsive probe based on an imidazo[1,2-a]indole fluorophore architecture is reported. The probe was highly selective to strongly acidic pH ( $pK_a = 3.56$ ) with high sensitivity and a fast response time (within 30 s). The probe did not demonstrate any fluorescence changes in the presence of interfering metal ions, and it featured excellent reversibility under strongly acidic conditions. The mechanism of detection of the probe was determined to be based on intramolecular charge transfer (ICT) at different pH. The probe was also able to be used for imaging for detecting acidic pH in *Saccharomyces cerevisiae*.

Keywords Imidazo[1,2-a]indole · pH fluorescent probe · Saccharomyces cerevisiae · ICT · Imaging

# Introduction

As an important parameter reflecting the acid-base strength of a solution, pH buffering allows cells and organisms to maintain homeostasis, thereby enabling normal structure and function [1]. The pH of a normal human body is maintained at 6.5–7.1, but the pH of different compartments within cells varies. For example, the pH within lysosomes and endosomes varies between 4.5 and 6.8 [2], the pH within mitochondria is approximately 8 [3], and the pH of the cytosol is maintained within 6.8– 7.4 in viable cells [4]. Changes in intracellular pH can affect the stability of the cellular environment as well as cellular function, such as proliferation, differentiation, apoptosis of cells, and ion transport; these cellular changes can also affect tissue function, such as muscle contraction [5]. Some diseases, such as cystic fibrosis, cancer, and

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neurodegenerative disorders, can provoke homoestatic pH balance in cells [6]. In addition, significant changes in the pH in the human body can cause physiological changes that can result in the development of cellular metabolic disorders.

Various techniques, such as absorption spectroscopy, electrochemistry, and nuclear magnetic resonance have been used to measure the pH within cells [7, 8]. Offering high sensitivity and excellent selectivity, as well as trivial and low-cost operation, fluorescent probes have been widely employed in molecular biology, biochemistry, medicine, and other fields [9]. To date, many small-molecule, pH-responsive fluorescent probes have been developed for measuring the pH in acidic organelles (lysosomes, pH 4.5–5.0,) and neutral organelles (mitochondria, pH 6.8-7.4) [10-16]. However, few pH fluorescent probes have been developed to measure extremely acidic pH(pH < 4). On the one hand, strong acidity is lethal to most organisms. Acidogenic bacteria and other bacterial species, such as Helicobacter pylori, can survive the acidic environment of the stomach of mammals and can cause infections, which can be lifethreatening [17]. On the other hand, the secretory and endocytic pathways of certain eukaryotic cell organelles can only be carried out under acidic conditions. Hence, it is necessary to design a highly pH-sensitive and photostable fluorescent probe that can effective measure very low pH.

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Scheme 1 Synthetic route of the probe YH-1

Indoles and other heterocyclic derivatives are often found in natural products, such as alkaloids, auxins, essential oils, and coal tar [18]. In addition, indoles are popular moieties in drug discovery and medicinal chemistry [19]. While many indole-containing compounds have



4.1
3.8
3.5
3.2
2.9
2.6
2.3
2.0
Experimental Section

### **Materials**

Fig. 1 The fluorescence spectrum of the probe YH-1 (1 $\mu$ M) dissolved in the B-R/DMSO (8/2, v/v) solution in the pH range of 2.0–7.0 ( $\lambda_{ex}$  = 350 nm)

Deringer

Except for special instructions, all reagents were purchased from commercial sources and were used directly

demonstrated a myriad of essential biological activities,

few reports on their optical properties have been pub-

lished due to limitation in the synthetic methods of in-

doles [20, 21]. In this article, we report the development

of a new derivative of imidazo[1,2-a]indole, denoted **YH-1**, which is a simple, yet novel, small-molecule fluorescent probe for measuring the pH in highly acidic environ-

ments using intramolecular charge transfer (ICT) as its

response mechanism. Compared to other pH-responsive

fluorescent probes [22–24], YH-1 could measure the pH

of a solution very quickly (within 30 s) with high sensi-



In neutral solution

In acidic solution

without further processing. To avoid any interferences from impurities, deionized water was used to prepare all solutions throughout the experiments. The chloride salt was dissolved in deionized water to prepare the metal ion solution to avoid any interferences from contamination by other metal ions. The sample solutions used in the experiment were all prepared under natural conditions, shaken for 15 s, and then allowed to stand for 10 min. Then, UV-Vis and fluorescence spectroscopy experiments were performed. Solutions of Britton-Robinson buffer (B-R) that were used in the following experiments were prepared by mixing 40 mM acetic acid, phosphoric acid, and boric acid in deionized water. The pH of the solution was adjusted with dilute aqueous NaOH or HCl.

### Instruments

All absorption spectra were acquired on a UV-2600 spectrophotometer (Shimadzu), and all fluorescence spectra were recorded on a FS5 fluorescence spectrophotometer (Edinburgh Instruments). <sup>1</sup>H NMR and <sup>13</sup> C NMR spectra were acquired on a Bruker Avance 400 (400 MHz) spectrometer using DMSO- $d_6$  as the solvent and tetramethylsilane (TMS) as the internal standard material.

An FE28-standard pH meter (Shanghai Mettler) was used to measure pH. All cell imaging experiments were performed on a microscope Ti 2 (Nikon, ECLIPSE) with an excitation wavelength of 350 nm.

#### **Fungus Imaging**

Saccharomyces cerevisiae (abbrev. S. cerevisiae), a species of fungus used in breadmaking (including steamed bread) and brewing, was extracted from yeast at 30 °C in peptone glucose (YPD) medium (containing 2 % tryptophan, 1 % yeast extract, and 2 % glucose (w/v)) and then stirred in a table concentrator (ZHI) at 200 rpm for 12 h. The cultured S. cerevisiae solution was placed in a 2 mL Eppendorf tube and centrifuged at  $4500 \times g$  for 2 min to collect the S. cerevisiae cells. The resulting pellet was resuspended in 1 mL of Britton-Robinson buffer at different pH (3.0, 5.0, and 7.0). The centrifuge tube was then placed in a bench top concentrator. The YH-1 probe was dissolved in DMSO. After 2 h, the probe solution was diluted into each tube containing buffer solution to a final probe concentration of 5 µM, and the tubes were incubated for 30 min. Finally, a glass microscope slide was coated with the probe solution,



Fig. 2 a The fluorescence titration pH value of 2.0 to 7.0 at 450 nm fluorescence intensity. b The linear relationship between the fluorescence intensity of the probe YH-1 at 450 nm and the pH value (pH 3.2–4.4) (R<sup>2</sup> =-0.9975) ( $\lambda_{ex} = 350$  nm,  $\lambda_{em} = 450$  nm)



Fig. 3 The linear regression relationship between the pH value and "(log  $[(F_{max}\text{-}F_X)/(F_X\text{-}F_{min})]$ "

which was observed with a microscope Ti 2 (Nikon, ECLIPSE) at a wavelength of 350 nm.

# Synthesis and Characterization of Probe 9-((benzyloxy)carbonyl)-1-methyl-1 H- imidazo[1,2-a] indole-3-carboxylic Acid (YH-1)

From commercially available 1-fluoro-2-nitrobenzene (1), compounds 2–7 were synthesized according to previously published literature procedures [25]. Compound 7 (0.96 g, 2.56 mmol) and NaOH (0.12 g, 3 mmol) were added to a solution of ethanol (20 mL) and water (10 mL), and the resulting mixture was stirred for 4 h at 80 °C. The crude reaction mixture was decanted in 40 mL of water, and the pH of the solution was adjusted to 2.0 with aqueous hydrochloric acid, after which the product precipitated from



Fig. 4 The reversibility of the fluorescence emission intensity of the probe YH-1 between pH 2.6 and pH 5.0 ( $\lambda_{ex}$  = 350 nm,  $\lambda_{em}$  = 450 nm)



Fig. 5 The fluorescence intensity of the probe YH-1 changes with time in  $0-10 \text{ min} (\lambda_{ex} = 350 \text{ nm}, \lambda_{em} = 450 \text{ nm})$ 

solution. The product was isolated by suction filtration and dried in an oven to afford **YH-1** as a yellow solid with a yield of 82 % (0.78 g). mp: 216–218 °C. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  13.27 (s, 1 H), 8.65 (d, J=8.2 Hz, 1 H), 8.15 (s, 1 H), 8.07 (d, J=8.0 Hz, 1 H), 7.49 (d, J=7.3 Hz, 2 H), 7.41 (t, J=7.3 Hz, 2 H), 7.35 (d, J=7.1 Hz, 1 H), 7.25 (t, J=7.5 Hz, 1 H), 7.11 (t, J= 7.6 Hz, 1 H), 5.34 (s, 2 H), 4.06 (s, 3 H).<sup>13</sup> C NMR (100 MHz, DMSO- $d_6$ )  $\delta$  163.66, 160.92, 144.88, 137.77, 133.56, 131.18, 128.96, 128.50, 128.30, 126.81, 123.61, 120.71, 119.60, 115.22, 114.96, 80.89, 64.78, 37.15. HRMS ([M+H]<sup>+</sup>): Calcd for C<sub>20</sub>H<sub>17</sub>N<sub>2</sub>O<sub>4</sub>: 349.1188; found: 349.1185.

# **Results and Discussion**

#### Synthesis of the Probe YH-1

Scheme 1 shows the general synthetic route of the probe. The structure of the final probe was characterized by HRMS and  $^{1}$  H/ $^{13}$  C NMR.

# Spectral Characteristics of Probe N-1 and its Optical Response to pH

For the fluorescence experiments, all samples were dissolved in a mixture of Britton-Robinson buffer (B-R) and DMSO (8/2, v/v) and measured after 10 min. As shown in Fig. 1, probe **YH-1** was highly fluorescent, and the fluorescence intensity at 450 nm remained unchanged at pH 4.4 and above. Over the pH range of 2.0–4.4, the fluorescence intensity decreased significantly as the pH decreased. The fluorescence intensity increased significantly from 29346.2 at pH 2.0 to 100201.8 at pH 4.4. The quantum yield at pH 4.4 was calculated to be 0.115 (Quinine sulphate dehydrate in 0.1 N H<sub>2</sub>SO<sub>4</sub> was used as the main standard,  $\varphi = 0.546$ ,  $\lambda_{ex} = 350$  nm).



**Fig. 6** Changes in the fluorescence intensity of probe **YH-1** in the solution (8/2, B-R/DMSO, v/v) under the influence of different metal ions and amino acids at (a) pH 2.6 and (b) pH 5.0 (probe  $(1\mu M)$ , Zn<sup>2+</sup>

(5 $\mu$ M), Fe<sup>3+</sup> (5 $\mu$ M), Cu<sup>2+</sup>(5 $\mu$ M), Mg<sup>2+</sup> (5 $\mu$ M), Ca<sup>2+</sup> (10 $\mu$ M), Na<sup>+</sup> (10 $\mu$ M), K<sup>+</sup> (10 $\mu$ M), H<sub>2</sub>O<sub>2</sub> (5 $\mu$ M), GSH (5 $\mu$ M), Cys (5 $\mu$ M), Hcy (10 $\mu$ M),  $\lambda_{ex}$  = 350 nm,  $\lambda_{em}$  = 450 nm)

As shown in Fig. 2, the x-axis and y-axis represented the pH and fluorescence intensity, respectively, and the data are arranged in a "Z" arrangement (at an emission wavelength of 450 nm). In Fig. 2, over the pH range of 2.3–4.4, the fluorescence intensity and pH demonstrated a linear relationship ( $R^2 = 0.9975$ ). The p $K_a$  of the probe was then measured in a mixture of Britton-Robison buffer and DMSO (8:2 v/v).

Based on the Henderson-Hasselbach equation, the pKa of the probe was calculated using the fluorescence emission intensity of the probe at 450 nm fluorescence according to Eq. 1:

$$N = 2n (n - 1) + m \tag{1}$$

where F represented the probe's emission wavelength at 450 nm. The pKa of **YH-1** was determined to be 3.56 (Fig. 3),



Fig. 7 The <sup>1</sup>H NMR spectrum of probe YH-1 in DMSO-*d*<sub>6</sub> under neutral conditions and acid conditions (CF<sub>3</sub>COOH)

Fig. 8 Fluorescence images of  $H^+$ in S. cerevisiae with probe YH-1 (5  $\mu$ M, 30 min). Excitation: 350 nm, Emission: 450–480 nm



which made the probe suitable for measuring the pH of strongly acidic solutions. Over the pH range of 2.3–4.4, and a linear relationship between the pH and log[ $(F_{max}-F_x)/(F_x-F_{min})$ ] was obtained, with a linear regression equation of pH = – 0.8497x + 3.5605, where x was equivalent to log [(Fmax-Fx)/(Fx-Fmin)]. This equation was used to calculate the pH of the samples over the range of 2.3–4.4.

As shown in Fig. 4, the fluorescence emission of the probe at 450 nm was reversible between pH 2.6 and 5.0, which meant the probe was applicable in various acidic environments with different pH values. In addition, the pH response time of the probe under different conditions did not exceed 30 s (Fig. 5). There was also no change in fluorescence intensity of the probe in the presence of different metal ions and amino acids, which indicated that the probe responded selectively to protons (Fig. 6). These results substantiated that the probe was capable of measuring the intracellular pH of *S. cerevisiae*.

#### **Mechanism of pH Detection**

<sup>1</sup>H NMR was used to compared the electronics of the probe under neutral and acidic conditions (trifluoroacetic acid,  $CF_3CO_2H$ ) (Fig. 7). No significant shifts in the proton resonances were observed in the spectra of two species, which indicated that the nitrogen bridgehead was not protonated; therefore, the indole nitrogen did not undergo protonation. Under neutral conditions, the 1-N atom was very electron-rich and able to be protonated by the carboxylic acid, which indicated that the ring system was a push-pull system. However, under acidic conditions, the carboxylic acid groups functioned as Lewis bases. Compared to protons under neutral conditions, the imidazo[1,2-a]indole protons absorbed under a higher electric field in acidic conditions. Therefore, the rate of ICT should change under acidic conditions. Scheme 2 shows the process of protonation.

To verify whether the probe can be applied in biological systems, we tested it in *S. cerevisiae* under strongly acidic conditions. After incubation of LB medium at different pH's (3.0, 5.0, and 7.0) with the probe for 30 min, the medium was incubated with *S. cerevisiae* for 30 min. Based on the confocal

fluorescence images (Fig. 8), no fluorescence was observed in the yeast cells at a pH of 3.0. As the intracellular pH increased, the fluorescence intensity of the probe increased significantly. These results indicated that the probe was applicable in biological systems with low pH. Compared to other probes (Table S1), this probe not only detected strong acidic pH but was useful for detecting intracellular acidic pH's in *S. cerevisiae*.

# Conclusions

In short, the simple and novel pH-responsive fluorescent probe **YH-1** based on an imidazo[1,2-a]indole fluorophore was synthesized and evaluated for pH-responsiveness under strongly acidic conditions. This was the first report of an imidazo[1,2-a]indole derivative that was used as fluorophore for pH detection. <sup>1</sup>H NMR analysis of the fluorophore under neutral and acidic conditions indicated that the response mechanism of the probe to pH was consistent with ICT. In addition, the probe responded quickly to protons (within 30 s), had a high selectivity to protons over other interfering metal ions and amino acids, was sensitive to pH, and was reversible. More importantly, the *S. cerevisiae* experiments corroborated the utility of the probe in fluorescence imagine of yeast because it demonstrated robust imaging capabilities under strongly acidic conditions in *S. cerevisiae*.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10895-021-02739-8.

Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Yanhao Xu, and Ruikang Duan. Data analysis were performed by Hao Liu, and Chengcai Xia. The first draft of the manuscript was written by Guiyun Duan and Yanqing Ge. All authors read and approved the final manuscript.

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**Data Availability** The authors declare that the data supporting the findings of this study are available in the article and the supplementary materials.

# Declarations

**Competing Interests** The authors declare that there are no conflicts of interest.

**Ethics Approval** For this type of study, the ethical approval was not required, because this study does not involve cell or animal manipulation.

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