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Two novel rhodamine-perylenediimide fluorescent probes: Synthesis, photophysical properties, and cell imaging

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ABSTRACT

In this study, two novel dual-switch fluorescent chemosensors based on rhodamine-peryleneiimide have been designed and synthesized. The dual-switching behaviors of the sensors were based on the structural transformations of rhodamine and an intramolecular photoinduced electron transfer (PET) process from rhodamine to perylenediimide. These probes exhibited excellent sensitivity to protons with enhanced fluorescence emission from 500 to 580 nm. The fluorescence changes of probes were reversible within a wide range of pH values from 2.0 to 11.0. Moreover, the sensors exhibited high selectivity, short response time, and long lifetime toward protons. The possible mechanism was investigated by the DFT calculation and ¹H NMR. According to the experiment of confocal laser scanning microscopy, these probes could be used to detect the acidic pH variations in living cells.

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1. Introduction

As common cations, protons play key roles in both of metabolism and cellular events, such as cell growth, calcium regulation chemotaxis, and cell adhesion [1]. Many detection methods in the determination of pH have resulted in the research of pH sensors [2–11]. Among these methods, fluorescent probes are regarded as outstanding detection systems due to their terms of high sensitivity, selectivity, and more convenient operation in many applications [12-14]. So far, strenuous efforts have been made to the development of efficient fluorescent chemosensors for the detection of pH [15-19]. Rhodamine derivatives have been widely used for detection of various metal ions due to their high photostability, high quantum yield, and special structure [20-27]. The sensing mechanism is based on fluorescence enhancement caused by spirolactam ring-opening of rhodamine derivatives [28]. The spirolactam structure is also sensitive to the pH value of the environment. Under neutral or basic conditions, the spirolactam remains closed and the rhodamine derivatives are non-fluorescent, while under acidic conditions, proton leads to spirolactam ring-opening and the rhodamine derivatives exhibit strong fluorescence emission [29-31]. Thus, rhodamine

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derivatives are suitable to monitor the pH value in acidic 28 environment with enhanced fluorescence signals. This type of 29 rhodamine-based pH probes have been prepared and attained 30 considerable effects [30-34], while pH-sensitive rhodamine 31 derivatives as pH fluorescence probes are less common. Among 32 these rhodamine-based probes, dual-switch pH sensors that emit 33 fluorescence with dual emission wavelengths and enable a built in 34 correction for the undesired environmental effects have received 35 more and more concerns. 36

Perylene tetracarboxylic diimides (PDI) have also generated 37 great interest in the fields of sensors because of their excellent 38 photo-stability, chemical stability, and high electron-accepting 39 ability [35–44]. Most perylenediimide fluorometric sensors were 40 designed to sense photophysical changes produced upon com-41 plexation, including photoinduced electron transfer (PET), intra-42 molecular charge transfer (ICT) [45], and fluorescence resonance 43 energy transfer (FRET) [46]. In these sensors, the PET-based PDI 44 fluorometric sensors [47,48] have been widely utilized in the 45 design of sensors due to the inherently higher sensitivity of PET in 46 comparison with the normal fluorescence guenching motif. 47 However, PET-based PDI fluorometric sensors with dual-switch 48 sensor for proton have not been reported before. 49

Herein, based on the unique structural transformations of 50 rhodamine in acid media and the high electron-accepting ability of 51 PDI, two novel dual-switch fluorescent probes **4** and **5** were 52 designed and synthesized as shown in Fig. 1. In these probes, 53 the perylene diimide chromophore plays as an electron acceptor; 54

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Fig. 1. Structure of the compounds 4 and 5.

55 the rhodamine units serve as electron donors, and the ethylene-56 diamine plays as spacer, which separates the two units. In order to 57 improve the solubility of PDI-core in probes, two PDI cores have 58 been prepared via modifying the bay region of perylene diimides 59 with 4-tert-butylphenol. In these cases, A PET process can occur 60 accompanying with the spirolactam ring-opening of rhodamine. 61 The probes represent off-state when the rhodamine units are in 62 spirocyclic, the protonation coordination of the amine fluorophore 63 would generate the rhodamine ring-open. At the same time, the 64 PET process should be blocked and the probes represented 65 "switched on" state.

66 2. Experimental

2.1. Materials

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68 Commercially available Rhodamine B, ethylenediamine and 69 perylene-3,4:9,10-tetraboxylic acid bisanhydride were used 70 without purification. The starting compounds 3 [49] and (1,7-71 bis(4-tert-butylphenyloxy)perylene-3,4:9,10-tetracarboxylic acid 72 bisanhydride) **2** [50,51] were prepared according to the reported 73 procedures. All solvents used in spectroscopic measurements 74 were of analytical grade. Reactions were monitored by thin layer 75 chromatography using Merck TLC Silica gel 60 F254. Silica gel 76 column chromatography was performed over Merck Silica 77 gel 60. Dilute hydrochloric acid or sodium hydroxide was used 78 for tuning pH values.

79 2.2. Methods

80 ¹H NMR and ¹³C NMR spectra were recorded on a Bruker DMX 300 NMR spectrometer and a Bruker ADVANCE 500 NMR 81 82 spectrometer in CDCl₃ with tetramethylsilane (TMS) as internal 83 standard. Mass spectra were recorded on Agilent Technologies 84 6530 Accurate-Mass Q-TOF LC/MS. HRMS were recorded on an 85 Ultraflex II MALDI-TOF mass spectrometer. UV-visible absorption spectra were determined on a Shimadu UV-3600 spectrophotom-86 87 eter. Fluorescence spectra were measured on a HORIBA FL-4 Max 88 spectrometer. FT-IR spectra were recorded on a Nicolet 750 series in the region of 4000–400 cm⁻¹ using KBr pellets. DFT calculations 89 90 of compounds were performed using the Gaussian 03 program 91 package. The calculations were optimized at the B3LYP/6-31G (d) 92 level of theory. The molecular orbitals were visualized using 93 GaussView.

94 2.3. Synthesis of amino-functional rhodamine B (3)

Rhodamine B 5.0 g (11.2 mmol) and ethylenediamine 9.0 mL
(134.8 mmol) were dissolved in ethanol (50 mL) in a 250 mL flask,
then the mixture was heated at 80 °C for 7 h. After ethanol was
removed under vacuum, the residue was purified by column

chromatography on silica gel (CH2Cl2/MeOH, 10:1) to give **3** as a
pale yellow powder 4.7 g, yield: 85%. M.p. 216~218 °C (the
reference value 217~219 °C), yield: 75%. ¹H NMR (CDCl3, ppm): δ
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7.89 (m, 1H), 7.42 (m, 2H), 7.07 (m, 1H), 6.40 (dd, J = 8.9, 2.6 Hz,
4H), 6.26 (dd, 2H, J = 8.8, 2.7 Hz), 3.32 (m, 8H), 3.18 (t, 2H,
J = 6.6 Hz), 2.40 (t, 2H, J = 6.6 Hz), 1.15 (t, 12H, J = 6.9 Hz).99
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2.4. Synthesis of 1,7-Bis(4-tert-butylphenyloxy)perylene-3,4:9,10tetracarboxylic Acid Bisanhydride (**2**)

- (1) A mixture of 5.0 g (13 mmol) of perylene-3,4:9,10-tetraboxylic 109 acid bisanhydride (1) and sulfuric acid (100 mL) was stirred at 111 50 °C for 12 h, and subsequently I_2 (0.56 g) was added. The 112 reaction mixture was heated to 80 °C, and bromine 2.2 g 113 (15 mmol) was added dropwise over a time period of 2 h. 114 After bromine addition, the reaction mixture was heated for 115 an additional 48 h, the excess bromine was removed by 116 saturated aqueous solution of K₂CO₃, and water (100 mL) was 117 added carefully. The precipitate was separated by filtration, 118 washed with water (100 mL), and dried in a vacuum to give a 119 red powder 6.0 g. Without further purification it was used to 120 the next reaction. 121
- (2) A mixture of compound 1,7-dibromoperylene-3,4:9,10-tetra-123 carboxylic acid bisanhydride 2.0 g (3.6 mmol), 4-tert-butyl-124 phenol 1.8 g (12.0 mmol), and K_2CO_3 2.4 g (6.8 mmol) in dry 125 DMF (120 mL) was heated at the refluxing temperature for 4 h 126 under an N₂ atmosphere. The reaction mixture was poured into 127 water (100 mL) and neutralized with aqueous 1.2 N HCl 128 solutions. The formed precipitate was collected by filtration 129 and washed with water and methanol to give crude product 2 130 3.1 g, yield: 92%. As this product showed poor solubility in 131 common organic solvents, it was used for the next reaction 132 without further purification. 133

2.5. Rhodamine-perylenediimide (4)

Compound **3** 1.1 g (2.3 mmol), compound **2** 0.42 g (0.6 mmol), 135 and N $(C_3H_7)_3$ (0.5 mL) were dissolved in DMF (25 mL), the mixture 136 was heated at 120 °C for 12 h under nitrogen and the reaction was 137 followed by TLC (DCM: Methanol = 10:1). On completion of the 138 reaction the solvent was removed under reduced pressure, a red-139 black solid got, washed with waters (100 mL), dry in 60 °C, a more 140 rigorous purification was then carried out via column chromatog-141 raphy (DCM/Methanol = 20:1) to give a purple solid **4** 0.79 g, yield: 142 80%. m.p. > 300 °C. FT-IR (KBr, cm^{-1}): 3422 (ν_{NH}), 2950–2863 143 (ν_{CH}) , 1696 $(\nu^{as}_{N-C=O})$, 1665 $(\nu^{s}_{N-C=O})$, and 1578 $(\nu_{N-C=O})$. ¹H NMR 144 (CDCl₃, ppm): δ 8.53(s, 2H), 8.33 (d, 4H, J = 8.1 Hz), 7.79~7.71 (m, 145 2H), 7.43~7.41 (m, 8H), 7.14 (s, 2H), 7.05 (d, 2H, J = 6.0 Hz), 6.96 (d, 146 4H, J = 8.4 Hz), 6.62~6.60 (m, 4H), 6.43~6.40 (m, 4H), 6.29~6.26 147 (m, 4H), 3.74 (t, 4H, J = 4.5 Hz), 3.52 (t, 4H, J = 4.5 Hz), 3.28 (m, 148 16H), 1.65 (s, 18H), 1.13 (t, 24H, J = 6.3 Hz); ¹³C-NMR (CDCl₃): δ 149 168.4 163.2, 155.7, 153.8, 152.8, 147.0, 132.8, 131.1, 128.8, 127.8, 150 126.5, 123.7, 122.9, 122.7, 120.1, 119.5, 119.2, 44.4, 44.3, 44.2, 39.4, 151 34.3, 31.6, 31.4, 29.6, and 12.5. MALDI-TOF-MS: *m*/*z*. Calculated: 152 $[M + H]^+ = 1621.7635$, found: 1621.7639. 153

2.6. Compound **5** was prepared according the same method of compound **4**

A oxblood red solid **5** was got 1.1 g, yield: 84%. 156 m.p. = $292 \sim 294 \,^{\circ}$ C. FT-IR (KBr, cm⁻¹): 3423 (ν_{NH}), 2969–2863 157 (ν_{CH}), 1695($\nu^{as}_{N-C=0}$), 1616($\nu^{s}_{N-C=0}$), 1592($\nu_{N-C=0}$). ¹H NMR 158 (300 MHz): δ 8.45 (m, 8H, J = 8.1 Hz), 7.94 \sim 7.85 (m, 2H), 159 7.45 \sim 7.36 (m, 4H), 7.02 (dd, 2H, J = 5.1, 4.2 Hz), 6.5 (d, 4H, 160 J = 8.7 Hz), 6.31 (d, 4H, J = 2.1 Hz), 6.03 (dd, 4H, J = 8.7, 1.8 Hz), 4.25 161 (t, 4H, J = 3.2 Hz), 3.57 (t, 4H, J = 3.2 Hz), 3.12 (m, 16H), 1.04 (t, 24H, 162

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163 $J = 6.9 \, \text{Hz}$); ¹³C-NMR (CDCl₃): δ 168.8, 162.5, 153.4, 148.6, 135.4,164135.4, 134.1, 132.6, 132.3, 132.1, 131.2, 130.8, 131.3, 128.0, 127.8,165126.4, 124.4, 123.7, 123.3, 122.7, 129.8, 118.3, 108.0, 97.8, 77.4,16677.0, 76.5, 73.6, 61.6, 58.8, 45.8, 41.5, 38.8, and 12.5. MALDI-TOF-167MS: m/z. Calculated: $[M + H]^+ = 1325.5859$, found: 1325.5854.

168 **3. Results and discussion**

169 3.1. Synthesis of probe molecules **5** and **4**

170 Compounds 2, 4, and 5 were synthesized via simple steps using 171 standard organic reactions, as depicted in Schemes 1 and 2. Details 172 of the synthesis of compounds 4 and 5 have been discussed above. 173 Compound 2 was synthesized by refluxing 1,7-dibromoperylene-174 3,4:9,10-tetracarboxylic acid bisanhydride and 4-tert-butylphenol 175 in DMF, as its poor solubility in common organic solvents, it was 176 used for the next reaction without further purification. Compounds 177 4 and compound 5 were prepared by refluxing compounds 3 and 2 178 in imidazole in the presence of NEt₃, their yields were 80% and 82%, 179 respectively. All of the new compounds were fully characterized by FT-IR, ¹H NMR, ¹³C NMR and high-resolution mass spectrometry 180 181 (HRMS-MALDI-TOF).

182 3.2. The pH dependence of absorption and fluorescence spectra of 183 compounds 4 and 5

184 Fig. 2 presents the absorption spectra of compounds 4 and 5 in 185 DMSO/H₂O at different pH values. The maximum absorption peaks of probe **4** are at 480 and 530 nm, which are corresponding to the 186 187 absorption of rhodamine and PDI units in probes, respectively. As shown in Fig. 2-left the absorption spectra of probe 4 showed 188 nearly no changes when the pH value > 7.0, which is ascribed to its 189 190 spirolactam form of probe in solution; while the pH decreases to 191 pH value < 7.0 the absorption intensity of probe 4 at 530 nm and 192 480 nm increases, respectively. Meanwhile, an obvious absorption 193 blue shifts generates, indicating the formation of the ring-opened 194 amide form of rhodamine units in probe 4. The nearly same results 195 can be obtained in the probe **5** (Fig. 2 right). The synchronous 196 changes of absorption spectra at 480 nm and 530 nm maybe can 197 prove the dual-switch process happened in the probes.

198The pH dependence of fluorescence spectra of compounds 4 and1995, obtained after excitation within the spectral region of maximal200absorption of the peripheral fluorophore (λ_{ex} = 480 or 530 nm),201show two emission bands at 550 nm and 580 nm, corresponding to202the emission bands of rhodamine units and the PDI core in203compounds 4 and 5 as shown in Fig. 3. The corresponding data is204showed in Table 1. From the insets photons, we can see that the



Scheme 1. Synthesis of 1,7-bis(4-*tert*-butylphenyloxy)perylenediimide 2.



Scheme 2. Synthesis of the probes 4 and 5.

probe **4** is non-fluorescent when the pH value > 7.0, when the 205 value of the pH falls to less than 7.0, the fluorescence intensity 206 gradually increases to about 170-fold from pH 7.8 to 5.4. The 207 quantum yield of the probe **4** is determined to be 0.46 in acidic 208 condition (pH 5.1) and < 0.01 in neutral condition with rhodamine 209 6G as a standard [52]. The similar results can be obtained for 210 compound 5, as shown in Fig. 3 right. Compared with to the 211 fluorescent spectra of the two probes, the nearly same results can 212 be obtained in probes 4 and 5, which can be attributed to the nearly 213 same molecular structure of probes. As shown in Fig. 3, the 214 synchronous enhanced fluorescence intensity of probes to 215 the various pH values at 550 nm and 580 nm further prove that 216 the dual-switch processes exactly happened in the probes. 217

$$\log\left(\frac{I_{Fmax}-I_F}{I_F-I_{Fmin}}\right) = pH-pKa$$
(1)



Fig. 2. The pH dependence of absorption spectra of compounds **4** and **5** ($c = 10 \,\mu$ mol/L, in DMSO/H₂O, 1:9).

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Fig. 3. The pH dependence of fluorescence spectra of compounds **4** and **5** (*c* = 10 μmol/L, λ_{ex} = 480 nm, in DMSO/H₂O, 1:9). Inset: fluorescence intensity of probes at various pH values.

Table 1

Fluorescent properties of compounds ${\bf 4}$ and ${\bf 5}$ in DMSO/H₂O, 1/9.

| Compounds | pH | $\lambda_{1abs}^{max}(\epsilon_1)/^a\lambda_{2abs}^{max}(\epsilon_2)$ | $\lambda_{1 \ flu}/\lambda_{2 \ flu} (nm)^{b}$ | $\Delta v_1 / \Delta v_2 (\text{nm})^{c}$ | $\Phi_1^{ m ex}/\Phi_2^{ m ex \ d}$ |
|--------------------------|-----|---|--|---|-------------------------------------|
| 4 | 7.8 | 496/531 | 554/587 | 58/56 | - |
| 4 -H ⁺ | 5.1 | 486/521 | 554/587 | 68/66 | 0.46/0.38 |
| 5 | 7.8 | 491/526 | 544/582 | 53/56 | |
| 5 -H⁺ | 5.1 | 491/526 | 544/582 | 53/56 | 0.42/0.36 |

The fluorescence quantum yields were determined using rhodamine 6G (Φ =0.95 in ethanol) as a standard.

The max absorption was used as the excited wavelength.

^a Absorption maximum.

^b Emission maximum.

^c Stoke's shift.

^d Fluorescence quantum yield. λ_{ex} = 480 nm, $(\lambda, \Delta v, \Phi, E, \tau)_1$ = Rh units in probe, $(\lambda, \Delta v, \Phi, E, \tau)_2$ = PDI unit in probe.

229 The abilities of the probes to recognize proton were also 221 investigated. From Fig. 3, it can be seen that the linear response 222 range covers the acidic pH range from 5.1 to 7.0. The acidity 223 constants apparent pKa of probes 4 and 5 were determined by 224 fluorimetric titration as a function of pH, taking the part of the inset graph in Fig. 3 located between pH 5.1 and 7.0, the pKa values of 225 226 the light harvesting probes 4 and 5 have been calculated by Eq. (1)[53]. The calculated pKa values are 6.12 and 6.30 for probes 4 and 5, 227 respectively. These pKa values prove that the probes are valuable 228 229 for the study of acidic environment.

3.3. Sensing mechanism and DFT calculations

In these probes, there are two important factors that lead to the 231 fluorescence quenching of compounds **4** and **5**. The first factor is 232 that the PET process from the rhodamine units to the perylene-233 diimide core in probes. The other is the closed spirolactam of the 234 rhodamine units in the probes as shown in Fig. 4. Therefore, while 235 the pH decreased to pH value < 7.0 the PET process in probes was 236 pressed and the fluorescence of PDI core in probe recovered. 237 Meanwhile, the closed spirolactam of the rhodamine units in the 238



Fig. 4. Photo-induced electron transfer (PET) process for the proposed sensors.

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Fig. 5. Structure of the model compounds 6, 7, and 8.

probes also opened, the probes show strong fluorescence with dual
emission wavelength at 550 nm and 580 nm. The fluorescence
response processes of probes proved that the design of the dualswitch state is favorable for the probe to work as a pH indicator.
Moreover, the ring-open of rhodamine can be confirmed by ¹H
NMR, a new multiplet around 9.6 ppm is assigned to ring-open of
probe 4 (Fig. S5 in supporting information).

DFT calculations were performed to understand the PET 246 247 progress of 4 at the molecular level. The HOMOs and LUMOs of 248 model compounds **4** and $\mathbf{4}$ -H⁺ are showed in Fig. S10. The LUMOs 249 (-5.44 eV) of model compound 4 are evenly delocalized over the 250 rhodamine units and its HOMOs (-10.9072 eV) are mainly 251 delocalized over perylenediimide units. On the other hand, The 252 HOMOs (-8.3504 eV) of model compound **4**-H⁺ are mainly 253 delocalized over rhodamine units and its LUMOs (-2.2848 eV) 254 are mainly delocalized over perylenediimide unit. Meanwhile, in 255 order to understand the PET process, three model compounds 6.7. and **8** (Fig. 5) are used for DFT calculations. The Optimized 256 257 geometries and calculated HOMO and LUMO density maps are showed in Fig. 6. The calculated HOMOs of compounds 7 and 8 are 258 law compared with to that of compound **6**. These results reflect 259 that the energy level of PDI units in probe 4 in acid environment 260 261 changes higher than the HOMOs of PDI units in probe 4 in neutral

environment. So, it can be concluded that the PET of probes can be262repressed in acid environment.263

3.4. Interference of metal cations in the fluorescence intensity of dyes 264

Fig. 7 shows the selectivity of probes **4** and **5** to various ions. 265 respectively. Upon addition of Hg^{2+} , Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Mg^{2+} , Pd^{2+} , Pb^{2+} , Fe^{2+} , Mn^{2+} , and Al^{3+} ions to the 266 267 10 µmol/L probe solutions, no significant fluorescence intensity 268 changes were observed. However, the same amount of H⁺ led to a 269 remarkable enhancement in fluorescence intensity, and the 270 fluorescence intensity changes caused by H⁺ are not obviously 271 influenced by the coexisting metal ions such as Hg²⁺, Na⁺, K⁺, Ca²⁺ 272 Mg²⁺, Co²⁺, Ni²⁺, Cu²⁺, Zn²⁺, Mg²⁺, Pd²⁺, Pb²⁺, Fe²⁺, Mn²⁺, and Al³⁺ 273 ions. Meanwhile, the fluorescence spectra of probes 4 and 5 to the 274 mixture of various metal salts were showed in Fig. 8. The results 275 indicate that probes **4** and **5** have high selectivity to protons in the 276 presence of other metal ions. 277

3.5. Reproducibility, reversibility, and response time

In order to study the response time, and reversibility of the 279 sensors, the reversible nature of the sensors were examined by 280 recording the ratio of fluorescence intensity at 550 nm with 281 respect to the change of pH from acidic (pH 2.0) to alkaline (pH 7.8) 282 range and vice versa up to 7 cycles. Fig. 9 shows the fluorescence 283 intensity change with time upon switching from one solution to 284 the other. The relative standard deviations from seven measure-285 ments for blank solution of pH 7.0 was found to be 0.1% and the 286 relative standard deviations in fluorescence intensities recorded 287 from five replicates of pH 2.0 was estimated as 1.1%. The response 288 time were 5-8 s for probes of pH 2.0, meanwhile, it was found that 289 the recovering time was independent of the H⁺ concentration 290 change. Results indicate the reversibility between the protonated 291



Q2

Fig. 6. Optimized geometries and calculated HOMO and LUMO density maps for model compounds according to DFT calculations at B3LYP/6-31* level.





Fig. 7. Fluorescence responses of compounds 4 and 5 to various metal salts of metal cations, such as K⁺, Fe³⁺, Ca²⁺, Mg²⁺, Cn³⁺, Al³⁺, and Co²⁺ (4 eq.).

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Fig. 8. Fluorescence responses of probes to the mixture of various metal salts, such as K⁺, Fe³⁺, Ca²⁺, Mg²⁺, Mn²⁺, Cr³⁺, Al³⁺, and Co²⁺ (4 eq.).



Fig. 9. The ratio of fluorescence intensity of probes at 550 nm (*c* = 10 μmol/L, λ_{ex} = 480 nm, in DMSO/H₂O, 1:9) upon consecutive addition of HCl (1 mol/L) and NaOH (1 mol/L) solution up to seven cycles.

and deprotonated forms of the sensors. Thus the sensors might beapplicable for real time pH monitoring.

294 3.6. Long-time stability and lifetime

295 To investigate the short-term stability of the sensors, the 296 fluorescence intensity of the sensors exposed to solution of pH 297 2.0 and 7.8 were tested over a period of seven days. The 298 fluorescence emission intensities were recorded every 24 h. As 299 shown in Fig. 10, the relative standard error of < 1% was obtained 300 for the solution. The solution exhibits good stability and has a 301 lifetime of at least one month. Thus, the probes have remarkably 302 long lifetime.

303 3.7. Confocal fluorescence images

HeLa cell was cultured in media (RPMI 1640 supplemented 304 305 with 10% PBS, 100 units/mL of penicillin and 100 units/mL of 306 streptomycin) at 37 °C in a humidied incubator, and culture 307 media were replaced with fresh media every day. The utilities of 308 probes **4** and **5** in living cells were studied. The HeLa cell lines 309 were incubated with receptor probes [1.0 µmol/L in DMSO/H₂O 310 (1:9, v/v) buffered with HEPES, pH 7.0] in a RPMI-1640 medium 311 for 1 h at 37 °C and washed with a phosphate-buffered saline 312 (PBS) buffer (pH 7.2) to remove excess receptor 4 or 5. The cells 313 were then treated with pH 5.8 in the RPMI-1640 medium, incubated again for 30 min at 37 °C, and washed with a PBS buffer. After treatment with pH 5.8 in the RPMI-1640 medium, confocal fluorescence images were studied, the cells show enhanced red fluorescence emission as shown in Fig. 11-B and D. These results suggested that probes **4** and **5** are effective dualswitch fluorescent pH probes intracellular imaging. 319



Fig. 10. The time courses of fluorescence intensity of probes in buffers of various pH values (7.8, 2.2, and 2.0, respectively) λ_{ex} = 480 nm, λ_{em} = 550 nm.

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Fig. 11. Confocal fluorescence images of Hela cells incubated with 1 μ .mol/L probes 4 (Fig. A and B) and 5 (Fig. C and D) for 30 min (pH 5.8, Fig. B and D: fluorescence image; pH 7.2, Fig. A and C: photos in bright field).

320 4. Conclusion

321 In summary, two novel pH fluorescent chemosensors based on 322 rhodamine-perylenediimide have been designed, synthesized, and fully characterized by ¹H NMR, ¹³C NMR, and HRMS-MALDI-TOF. 323 The dual-switches of the sensors were based on the structural 324 325 transformation of rhodamine units and an intramolecular photo-326 induced electron transfer (PET) process that occurred with the 327 switching effects of rhodamine units in the probes. These probes 328 showed excellent fluorescent sensitivity for protons with en-329 hanced emission from 550 to 580 nm. The fluorescence changes of 330 probes **4** and **5** were reversible within a wide range of pH values 331 from 2.0 to 11.0. Furthermore, the sensors exhibited short response 332 time, long lifetime, and high selectivity toward protons in the presence of various other metal cations, such as Hg²⁺, Fe³⁺, Cu²⁺, 333 Mg²⁺, Ca²⁺, Mn²⁺, Co²⁺, Ni²⁺, Zn²⁺, Cd²⁺, Ba²⁺, and Pb²⁺. The possible 334 mechanism was investigated by the DFT calculation and ¹H NMR. 335 According to the experiments of confocal laser scanning micros-336 337 copy, compounds **4** and **5** could be used to detect the acidic pH 338 variations in living cells with an effective dual-switches fluores-339 cent signal. Thus, we are convinced that the design strategy will 340 help to develop a new platform for the design of new dual-switches 341 fluorescent probes for other target analytes.

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compounds were in the Supplementary information.

349 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.cclet.2016.01.039.

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