Cite this: Chem. Commun., 2011, 47, 7734-7736

## COMMUNICATION

## 9-Aryl-1,2-dihydropyrrolo[3,4-b]indolizin-3-one (Seoul-Fluor) as a smart platform for colorful ratiometric fluorescent pH sensors<sup>†</sup>

Eunha Kim,<sup>a</sup> Sanghee Lee<sup>a</sup> and Seung Bum Park\*<sup>ab</sup>

*Received 4th May 2011, Accepted 23rd May 2011* DOI: 10.1039/c1cc12618k

In this communication, we report that 9-aryl-1,2-dihydropyrrolo-[3,4-*b*]indolizin-3-one (Seoul-Fluor) can serve as a potential platform for colorful ratiometric fluorescent pH sensors by simple incorporation of pH responsive elements on Seoul-Fluor. Seoul-Fluor-based fluorescent pH sensors allow the emission- and pH-tuning ability upon protonation by varying their  $pK_a$  values and electronic characteristics of substituents by a rational design.

The investigation of pivotal events in a much smaller world than the one to which we are adapted has been of prime interest to scientific communities, therefore various molecular devices have been invented to convert invisible molecular events into visible signals.<sup>1</sup> Owing to their advantageous features, fluorescent molecules have been widely used in various biomedical applications, including cellular imaging,<sup>2,3</sup> biological assays,<sup>4</sup> medical diagnostics,<sup>5</sup> and environmental monitoring.<sup>6</sup> However, despite the high demands for these molecules, only a limited number of fluorescent core skeletons are used.<sup>7,8</sup> Moreover, most of the reported fluorescent sensors use a turn-on/off sensing mechanism via photoinduced electron transfer (PET) events of the traditional fluorophores.9,10 However, the signal of turn-on/off sensors, which show a single increase or decrease in the emission intensity, can be complicated by external influence. Owing to increasing attention on multiplexing capability in image-based biological applications, it is important to develop a series of colorful ratiometric fluorescent sensors for the better understanding of biological systems at the molecular level.<sup>11–14</sup> Therefore, the development of a new molecular framework for fluorophores with a tunable emission wavelength and the subsequent use of the fluorophores in the design of ratiometric sensors for the study of individual events in a complex environment can help satisfy current demands of the biomedical community.

Recently, we reported Seoul-Fluor (SF), a novel fluorescent core skeleton with emission wavelength tunability covering the full color range and simple changes in the electronic state at the  $R^1$  and  $R^2$  positions of Seoul-Fluor result in dramatic emission changes from blue to red (Fig. 1).<sup>15,16</sup> On the basis of our previous studies, we envisioned that the unique features of Seoul-Fluor could lead to new applications for the Seoul-Fluor core skeleton, as a colorful ratiometric fluorescent sensor platform. In other words, in biological systems, target events associated with changes in the electronic states of specific functional groups at the molecular level can induce changes in the photophysical properties of Seoul-Fluor, especially the emission wavelength.

For a proof-of-concept study, we chose pH changes as the target molecular level event and designed novel ratiometric fluorescent pH sensors through the simple introduction of pH responsive elements at specific positions of the Seoul-Fluor core skeleton. The protonation of these pH responsive elements leads to changes in their electronic characteristics in accordance with their  $pK_a$  values and subsequent dramatic shifts in the emission wavelength of Seoul-Fluor. We first introduced an amine moiety as a pH responsive element at the R<sup>1</sup> position of Seoul-Fluor, because we envisioned that the protonation of the emission wavelength



**Fig. 1** (a) Chemical structure of Seoul-Fluor and schematic illustration of systematic changes in the emission wavelength with the protonation of pH responsive moieties at the  $R^1$  and  $R^2$  positions of Seoul-Fluor. (b) Structures of Seoul-Fluor derivatives and corresponding photography images taken under irradiation at 365 nm.

<sup>&</sup>lt;sup>a</sup> Department of Chemistry, Seoul National University, Seoul 151-747, Korea. E-mail: sbpark@snu.ac.kr; Fax: +82 2 884 4025;

Tel: +82 2 880 9090

<sup>&</sup>lt;sup>b</sup> Department of Biophysics and Chemical Biology,

Seoul National University, Seoul 151-747, Korea

<sup>†</sup> Electronic supplementary information (ESI) available: Detailed synthetic procedures, photophysical property data of all compounds, and spectroscopic data of all new compounds. See DOI: 10.1039/c1cc12618k

through the reduction of electron donating characters (EDG) at the  $R^1$  position (Fig. 1a). Seoul-Fluor with a dipropylamino group at the  $R^1$  position and an acetyl group at the  $R^2$  position, which we refer to as SF24, is a candidate for a ratiometric fluorescent pH biosensor. As shown in Fig. 2a, SF24 showed a clear hypsochromic shift of the emission wavelength from 619 nm to 532 nm with a pH change from 7.0 to 3.0 (Fig. S1, ESI<sup>†</sup>). This confirmed that the protonation of the dipropylamino group on SF24 perturbs the electronic states of Seoul-Fluor, leading to the drastic changes in the emission properties. We also examined the ratiometric response of SF24 in the pH range 3.0-6.0 and observed a gradual reduction in the emission intensity at 619 nm as the acidity increased. Concurrently, a gradual increase in the emission intensity at a wavelength of 532 nm was observed as the pH decreased. The sigmoid curves obtained from the emission spectra of SF24 at variable pH clearly confirmed the potential of Seoul-Fluor as a ratiometric sensor platform (Fig. S2, ESI<sup>†</sup>). The inflection point of the two sigmoid curves for SF24 is pH 3.7.

Introduction of a carboxylic acid moiety as a pH responsive element at the R<sup>2</sup> position of Seoul-Fluor (CF<sub>3</sub> moiety at the  $R^1$  position, hereafter referred to as SF45) resulted in a bathochromic shift of the emission wavelength from 473 nm to 503 nm for a change in the pH from 7.0 to 3.0 (Fig. 2b and Fig. S1, ESI<sup>†</sup>). The protonation of the carboxylic acid reduces the electron-donating character, which perturbs the electronic state of Seoul-Fluor. However, in this case, an electronic change occurs at the  $R^2$  position, causing a bathochromic shift rather than a hypsochromic shift. We observed a 30 nm bathochromic shift of the emission wavelength upon protonation of the carboxylic acid at the R<sup>2</sup> position (SF45), while protonation of the amine moiety at the  $R^1$  position (SF24) caused an 87 nm hypsochromic shift of the emission wavelength. This observation can be explained by the pronounced change in the electronic state of dialkylamine (SF24) upon protonation and the subsequent



**Fig. 2** Schematic representation of structural changes in Seoul-Fluor derivatives with a change in the pH from 7.0 to 3.0. The pH responsive elements are either introduced at the  $R^1$  (a: SF24) or  $R^2$  (b: SF45) position or not introduced at all (c: SF46). Photography images of Seoul-Fluor derivatives in buffer solutions with different pH values were taken under irradiation at 365 nm.

larger shift in the emission wavelength compared to that in the case of carboxylate (SF45). However, no shift was observed in the emission wavelength if Seoul-Fluor was not modified to contain a pH responsive element, as in the case of the compound SF46 (Fig. 2c and Fig. S1, ESI†). This result confirmed that the Seoul-Fluor skeleton could serve as an appealing molecular framework for ratiometric fluorescent sensors through the introduction of electronic state perturbing elements such as pH responsive moieties.

The first interesting feature of Seoul-Fluor is that its pH response is directly correlated with the  $pK_a$  of the pH responsive element used for its modification. This allows the fine-tuning of the pH sensitivity of Seoul-Fluor through the introduction of substituents having different  $pK_a$  values. To validate this statement, we incorporated several amine-based, pH responsive elements with different  $pK_a$  values at the R<sup>1</sup> position of Seoul-Fluor by simple modifications to the alkyl groups of the aniline moiety, from propyl (SF24) to ethyl (SF44) to methyl (SF47), leading to fine changes in the  $pK_a$  value of each fluorescent compound.<sup>17</sup> As expected, the emission wavelengths of SF24, SF44, and SF47 are almost identical (Fig. S3, ESI<sup>+</sup>) and all three sigmoid curves in the pH-response scatter plot of the emission intensity exhibited the same pattern, but the inflection points of each curve were shifted from pH 3.7 to pH 4.0 and pH 4.8 (Fig. 3). This result confirmed that Seoul-Fluor-based pH sensors can be designed to have a unique pH response with identical photophysical properties through the incorporation of desired pH responsive elements with appropriate  $pK_a$  values at the R<sup>1</sup> or R<sup>2</sup> position of Seoul-Fluor.

The second interesting feature of Seoul-Fluor is that the electronic characters of substituents at the R<sup>1</sup> and R<sup>2</sup> positions display opposite trends with regard to changes in their emission wavelengths. While the enhancement of the EWG character at the R<sup>2</sup> position triggers a bathochromic shift of the emission wavelength, the same change at the R<sup>1</sup> position causes a hypsochromic shift (Fig. 1a). Therefore, we can control the direction of color change in the pH, simply by choosing the location of pH responsive elements between the R<sup>1</sup> and R<sup>2</sup> positions of the Seoul-Fluor skeleton (Fig. 2). Considering that the current ratiometric fluorescent pH sensors have quite limited flexibility with regard to the range in which their emission can vary<sup>18</sup> and considering that it has now become



**Fig. 3** Scatter plot of the emission intensity about SF24 (red), SF44 (black), and SF47 (blue) for a change in the pH from 3.0 to 6.0 with the excitation/emission wavelength at 417/532 nm.



**Fig. 4** Normalized emission spectra and photography images of fluorescent pH sensors in a pH 7.0 buffer solution. The images of each compound in the buffer solution were taken under irradiation at 365 nm.

possible to control both the hypsochromic or bathochromic shift of the emission wavelength, our results are of specific interest and clearly show that the incorporation of electronicstate-perturbing elements at specific locations in Seoul-Fluor makes Seoul-Fluor a remarkable ratiometric and emissiontunable fluorescent sensor platform.

We then directed our attention to color-tuning of Seoul-Fluor-based fluorescent pH sensors. As discussed, the Seoul-Fluor system has two different sites ( $\mathbf{R}^1$  and  $\mathbf{R}^2$ ) that can be modified to control the emission wavelength. Therefore, we can consider one site as a pH responsive position and the other site as a color tuning position. As shown in Fig. 4a, substituent changes at the  $R^2$  position from hydrogen (SF48) to phenyl (SF49) and acetyl (SF24) for a fixed pH-responsive dipropylamino group at the R<sup>1</sup> position triggered a bathochromic shift of the emission maximum from 502 nm (SF48) to 550 nm (SF49) and 619 nm (SF24) at pH 7.0. This significant bathochromic shift might be caused by strengthening the intramolecular charge transfer (ICT) process involving the enrichment of electron-withdrawing characters at the R<sup>2</sup> position of Seoul-Fluor. All three compounds exhibited the hypsochromic shift of the emission wavelength for a change in the pH from 7.0 to 3.0 (Fig. S4, ESI<sup>†</sup>). Likewise, with a fixed pH-responsive carboxylic acid group at the R<sup>2</sup> position, substituent changes at the  $R^1$  position from CN (SF50) to Me (SF51) and to OMe (SF52) triggered the bathochromic shift of the emission maximum from 474 nm (SF50) to 496 nm (SF51) and 506 nm (SF52) at pH 7.0 (Fig. 4b). All three compounds also exhibited the bathochromic shift of the emission wavelength for a change in the pH from 7.0 to 3.0 (Fig. S4, ESI<sup>+</sup>). Thus, we have clearly demonstrated that Seoul-Fluor-based fluorescent pH sensors can be tailored through a rational design to display

the desired emission wavelength and identical pH response by carrying out simple substituent modifications on the Seoul-Fluor skeleton.

In this study, we showed that Seoul-Fluor derivatives can function as a novel molecular framework for developing ratiometric fluorescent pH sensors when they are incorporated with pH-responsive elements at specific positions of Seoul-Fluor. Each pH sensor exhibited dramatic changes in the emission wavelength with changes in the solution pH. Moreover, the direction of emission shift (bathochromic or hypsochromic shift) and the desired emission color can be controlled by inducing simple changes in the electronic state or the position of substituents. In addition, a unique pH response can be achieved by fine-tuning the  $pK_a$  values of the substituents on Seoul-Fluor. Therefore, Seoul-Fluor can serve as a colorful palette for ratiometric fluorescent sensors, not only for pH, but also for various biological events that can change the electronic state of substituents on Seoul-Fluor. We envision that the tunable photophysical properties of Seoul-Fluor with a rational design will lead to the diverse application of Seoul-Fluor for the development of unique ratiometric fluorescent sensor platforms, which can be quite useful in high-content screening and multiplex monitoring applications.

This study was supported by the National Research Foundation of Korea (NRF) and the WCU program of the NRF, funded by the Korean Ministry of Education, Science, and Technology (MEST). E. Kim and S. Lee are grateful for the fellowships awarded by the BK21 Program and the Seoul Science Fellowship.

## Notes and references

- 1 S. M. Borisov and O. S. Wolfbeis, Chem. Rev., 2008, 108, 423-461.
- 2 X. Chen, K.-A. Lee, E.-M. Ha, K. M. Lee, Y. Y. Seo, H. K. Choi, H. N. Kim, M. J. Kim, C.-S. Cho, S. Y. Lee, W.-J. Lee and J. Yoon, *Chem. Commun.*, 2011, **47**, 4373–4375.
- 3 B. C. Dickinson, J. Peltier, D. Stone, D. V. Schaffer and C. J. Chang, *Nat. Chem. Biol.*, 2011, 7, 106–112.
- 4 K. K. Ghosh, E. Yap, H. Kim, J.-S. Lee and Y. T. Chang, *Chem. Commun.*, 2011, 47, 4001–4003.
- 5 R. Adhikary, P. Mukherjee, G. Krishnamoorthy, R. A. Kunkle, T. A. Casey, M. A. Rasmussen and J. W. Petrich, *Anal. Chem.*, 2010, 82, 4097–4101.
- 6 D. T. Quang and J. S. Kim, Chem. Rev., 2010, 110, 6280-6301.
- 7 M. S. T. Gonçalves, Chem. Rev., 2009, 109, 190-212.
- 8 E. Kim and S. B. Park, Chem.-Asian J., 2009, 4, 1646-1658.
- 9 A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515–1566.
- 10 Y. Urano, M. Kamiya, K. Kanda, T. Ueno, K. Hirose and T. Nagano, J. Am. Chem. Soc., 2005, **127**, 4888–4894.
- 11 B. Liu and H. Tian, Chem. Commun., 2005, 3156-3158
- 12 M. Taki, J. L. Wolford and T. V. O'Halloran, J. Am. Chem. Soc., 2004, 126, 712–713.
- 13 Z. Xu, Y. Xiao, X. Qian, J. Cui and D. Cui, Org. Lett., 2005, 7, 889–892.
- 14 C. J. Chang, J. Jaworski, E. M. Nolan, M. Sheng and S. J. Lippard, Proc. Natl. Acad. Sci. U. S. A., 2004, 101, 1129–1134.
- 15 E. Kim, M. Koh, B. J. Lim and S. B. Park, J. Am. Chem. Soc., 2011, 133, 6642–6649.
- 16 E. Kim, M. Koh, J. Ryu and S. B. Park, J. Am. Chem. Soc., 2008, 130, 12206–12207.
- 17 B. B. P. Tice, I. Lee and F. H. Kendall, J. Am. Chem. Soc., 1963, 85, 329–337.
- 18 J. Han and K. Burgess, Chem. Rev., 2010, 110, 2709-2728.