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Spirolactonized Si-rhodamine: a novel NIR fluorophore utilized as a platform to construct Si-rhodamine-based probes†

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Spirolactonized Si-rhodamine was prepared as a platform to construct Si-rhodamine-based probes by following the design strategy widely used in rhodamine systems. Among them, the reaction-based probe SiR-Hg was operated for NIR sensing and bioimaging of Hg^{2+} in living cells based on the similar irreversible spirolactam ring-opening process to traditional rhodamine derivatives.

The design of fluorescent probes for sensing and bioimaging of important species has emerged as a significant research area.[1] Among the probes developed, the rhodamine spirolactam based fluorescent probes have already attracted great interest due to the valuable characteristics of rhodamine derivatives, such as high molar extinction coefficient, large fluorescence quantum yield and a unique ring-opening process with turn-on signals.[2] However, the absorption and emission of most rhodamine derivatives are located in the visible range (<600 nm), which limits their further applications for bioimaging. In comparison to visible fluorescent probes, near-infrared (NIR, 650–900 nm)[3] fluorescent probes are expected to be superior for bioimaging because light in this wavelength region shows less overlap with the spectrum of background autofluorescence, lower light scattering, deeper tissue penetration and less phototoxicity.[3] Thus, it is highly desirable to develop novel NIR probes for bioimaging.[4] Very recently, a new class of NIR fluorescent dyes and probes, silicon-substituted rhodamines, has been developed.[5] Their excellent photophysical properties qualify them for NIR sensing and bioimaging. Among the limited examples, most Si-rhodamine-based probes are controlled by a photoinduced electron transfer (PeT) mechanism.[5c,5d] Besides PeT, the HOCl-induced ring-opening approach applied to sulphur-involved spirocycles[5b] is more attractive because of the chromogenic and fluorogenic turn-on signals with relatively low background. Unfortunately, modifying the specific spirocycle for other applications is of great difficulty. To address this problem, we focus on the close analogue of rhodamine B, spirolactonized Si-rhodamine (**SiR**). By following the design strategy widely used in rhodamine systems, spirolactone in **SiR** could be utilized as a platform to construct **SiR**-based probes. If such a design strategy can be experimentally demonstrated, it will extend the range of available NIR fluorescent turn-on probes utilizing the unique ring-opening process of the corresponding spirolactone or spirolactam (Fig. 1).

With this in mind, we reported the synthesis and photophysical properties of **SiR** and Si-rhodamine-hydrazine (**SiRH**). Subsequently, **SiR**-based probes **SiR-Cu** and **SiR-Hg** were prepared by introducing phenolic hydroxy and thiosemicarbazide moieties[6] for recognizing Cu^{2+} and Hg^{2+}, respectively. The recognition groups are selected for the following reasons: firstly, the ring-opening mechanisms represent two common ways: metal-coordination bonding and ion-triggered reaction. Secondly, it is interesting to make a comparison of properties between Si-rhodamines and rhodamine analogues. Lastly, there have been few reports on NIR fluorescent probes for sensing and bioimaging of these biologically relevant ions.[7] In particular, the ring-opening process accompanied by turn-on NIR signals was achieved in **SiR** and **SiR-Hg**. It clearly demonstrates that **SiR** has great potential for developing **SiR**-based NIR probes.

Although Qian and Nagano *et al.* have described the synthesis of several Si-rhodamines,[5] an efficient synthetic strategy to challenge spirolactonized **SiR** has not yet been reported. As presented in Scheme 1, a lithiated benzene moiety bearing a protected carbonyl group was employed to react with compound

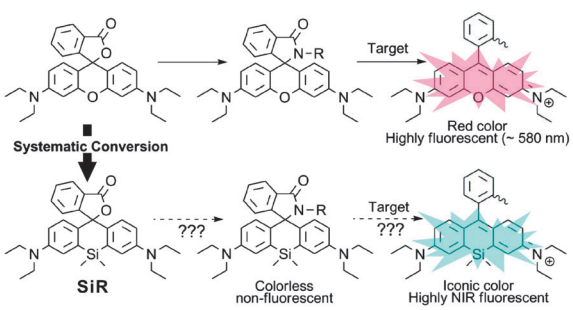

Fig. 1 Schematic representation of our strategy to develop **SiR**-based probes.

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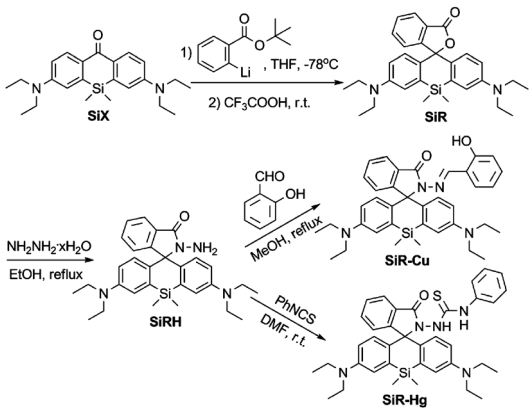

Scheme 1 Synthesis of **SiR**, **SiRH**, **SiR-Cu** and **SiR-Hg**.

SiX, finally affording **SiR** in medium yield after deprotection and cyclization. Subsequent treatment of **SiR** with hydrazine hydrate gave **SiRH**, which served as a key intermediate to create probes **SiR-Cu** and **SiR-Hg** through convenient chemical modification. X-Ray crystallographic investigation further confirmed the existence of the silicon-substituted ring and unique spirolactam structure in **SiR-Hg**. To the best of our knowledge, this is the first example of the X-ray structure of the Si-rhodamine bearing the spirocyclic moiety (ESI†, Fig. S1).

It is known that the ring-opening of spirolactone or spirolactam in rhodamines gives rise to a pink color and strong fluorescence emission. Similarly, **SiR** formed colorless and nonfluorescent solution in most aprotic organic solvents, but underwent a reversible blue color change after adding AcOH or Et_3N (ESI†, Fig. S2). This phenomenon implied that a similar reversible ring-opening process of the corresponding spirolactone may occur in **SiR**. We examined the spectral profile of **SiR** at different pH values in HEPES buffer solution. As shown in Fig. 2a and b, the solution of **SiR** kept a bright blue color (λ_{abs} = 650 nm, ε = 50 000 M^{-1} cm^{-1}) and high NIR fluorescence emission (λ_{em} = 666 nm, ϕ = 0.19) over a wide pH range from pH 5 to 10, exiting in its zwitterionic form. However, under more acidic conditions (pH < 5), the blue color solution turned colorless and the fluorescence quenched significantly. On the other hand, the fluorescence intensity also decreased at higher pH values (pH > 10). The fluorescence on–off switching of **SiR** is very similar to that of the classic rhodamines.[3b,8] Additionally, **SiR** has sufficient photostability for potential biological applications (ESI†, Fig S3). The cell fluorescence imaging as shown in Fig. S4 (ESI†) demonstrated that **SiR** with desirable features was

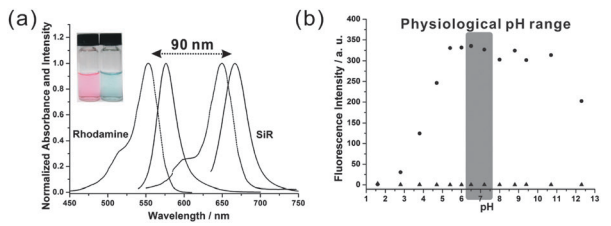

Fig. 2 (a) The absorption and emission spectra of respective rhodamine B and **SiR** in HEPES buffer solution at pH 7.4. Inset: color pictures of rhodamine B and **SiR**. (b) pH-dependence of the fluorescence intensity of **SiR** (circles) and **SiRH** (triangles).

suitable for bioimaging at physiological pH. In sharp contrast, **SiRH** showed no obvious characteristic color or fluorescence change between pH 1.6 to 12.3 (Fig. 2b), suggesting that **SiRH** preferred the nonfluorescent spirocyclic form over a wide pH range. This characteristic implied that Si-rhodamine amide derivatives with low background fluorescence satisfied the criteria for turn-on sensing and bioimaging applications.

Except for the similarities to rhodamine B, some different properties are observed. It is worth noting that the pH-dependent spectral profile of **SiR** changed when the content of MeCN increased in buffer solution (ESI†, Fig. S5). We also notice that **SiR** amide derivatives showed considerable tolerance to acid (ESI†, Fig. S6 and S7). These facts indicate that spirocyclic structure in Si-rhodamines is more stable than that in rhodamine analogues.

As we know, rhodamine-hydrazine (**RH**) has been proved to be an efficient chemodosimeter for sensing Cu^{2+} based on a Cu^{2+}-induced ring-opening process.[9] Thus we reason that the analogue **SiRH** owns the same ability to recognize Cu^{2+} *via* the similar mechanism. Surprisingly, the addition of Cu^{2+} resulted in slight fluorescence enhancement and a yellow color change with the formation of two absorption bands, the dominating one centered at 420 nm and the weak one appeared at 654 nm (ESI†, Fig. S8 and S9). In order to better understand the interaction, the ESI mass spectra of the complex were recorded and they displayed two unique peaks at m/z 497.5 and 1055.5, which may be assigned to the dehydrogenation product of **SiRH** (calc 497.3) and the 2 : 1 (**SiRH**–Cu^{2+}) chelating complex (calc 1055.5), respectively (ESI†, Fig. S10). In addition, the 2 : 1 binding stoichiometry was further confirmed by the Job's plot analysis (ESI†, Fig. S11). Based on the above findings, we infer that the binding between **SiRH** and Cu^{2+} produces the metal-chelating complex (ESI†, Fig. S12) with a small amount of ring-opened form, which are responsible for the observation of short and long absorption bands, respectively. Furthermore, other metal ions exerted a little or slow effect on the color change of **SiRH** under identical conditions (ESI†, Fig. S13), confirming the selectivity to Cu^{2+}. Similar results were obtained when probe **SiR-Cu** interacted with Cu^{2+}, suggesting the similar Cu^{2+} binding behavior to **SiRH** (ESI†, Fig. S14 and S15).

The distinct recognition behavior should be attributed to the stability of spirolactam in Si-rhodamine, which favors the spirocyclic form rather than the ring-opened form when interacting with Cu^{2+} *via* metal-coordination bonding. Although probes **SiRH** and **SiR-Cu** could perform as colorimetric probes for turn-on sensing of Cu^{2+}, the unique ring-opening process with characteristic turn-on NIR fluorescence emission was not achieved. Thus, we turn our attention to the reaction-based probe **SiR-Hg**, the spirolactam in which could be liberated by the breaking of covalent bonds.[10]

In HEPES buffer solution at pH 7.4, the free **SiR-Hg** formed a colorless solution, indicating that it predominantly existed in the spirocyclic form. As expected, the addition of Hg^{2+} caused an iconic blue color change instantaneously at room temperature (ESI†, Fig. S16 and S17). Concomitantly, an intense absorption band appeared at 664 nm (ε = 160 000 M^{-1} cm^{-1}), suggesting the formation of ring-opened structure from **SiR-Hg** (ESI†, Fig. S18). Further evidence was

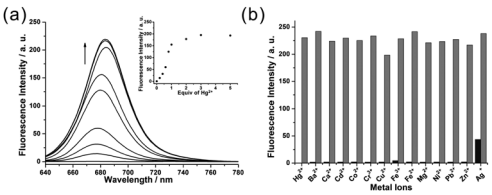

Fig. 3 (a) Emission spectra changes of **SiR-Hg** in the presence of increasing concentration of Hg^{2+} in HEPES buffer solution at pH 7.4. Inset: plot of fluorescence intensity depending on the number of equivalents of Hg^{2+}. (b) Fluorescence intensities of **SiR-Hg** upon the addition of various metal ions. Black bars represent the fluorescence response of **SiR-Hg** to the excess metal ion (50 μM) of interest. Gray bars represent the subsequent addition of 50 μM Hg^{2+} to the solution.

obtained by comparing the ESI mass spectra (ESI†, Fig. S19). The unique peak at m/z 600.4 (calcd 600.3) corresponding to the desulfurized and cyclized product from **SiR-Hg** was clearly observed when 1.5 equiv. of Hg^{2+} was added. Fluorescence titration of Hg^{2+} was further conducted (ESI†, Fig. S20). As depicted in Fig. 3a, with the gradual addition of Hg^{2+}, a simultaneous enhancement in fluorescence intensity at 680 nm (ϕ = 0.12) was noticed. The final intensity of **SiR-Hg** upon addition of 10 equiv. of Hg^{2+} was found to enhance by 500 fold, partly due to the extremely low background fluorescence of **SiR-Hg**. The selectivity of **SiR-Hg** observed for Hg^{2+} over other ions is very similar to its rhodamine analogue. Various metal ions did not cause any significant fluorescence intensity changes. In addition, the enhancement in fluorescence intensity resulting from the addition of Hg^{2+} was not influenced by the presence of other metal ions (Fig. 3b).

Owing to the favourable properties of probe **SiR-Hg**, it should be ideally suited to monitor Hg^{2+} in living cells. To test this proposal, imaging of Hg^{2+} in SH-SY5Y cells was evaluated. Fluorescence microscopic images illustrated in Fig. 4 showed that **SiR-Hg** existed in nonfluorescent spirocyclic form at physiological pH and gave no background fluorescence. After reacting with Hg^{2+}, the fluorescent product formed through an irreversible spirolactam ring-opening process and displayed a strong NIR fluorescence emission. Taken together, these results show that probe **SiR-Hg** is cell membrane permeable and capable of turn-on bioimaging Hg^{2+} in living cells.

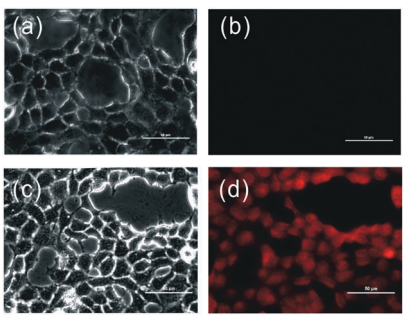

Fig. 4 Cells incubated with **SiR-Hg** (50 μM) and Hg^{2+} (50 μM). (a) Microscopic image of SH-SY5Y cells treated with **SiR-Hg** in the absence of external Hg^{2+}. (b) Fluorescence microscopic image of SH-SY5Y cells treated with **SiR-Hg** in the absence of external Hg^{2+}. (c) Microscopic image of SH-SY5Y cells treated with both **SiR-Hg** and Hg^{2+}. (d) Fluorescence microscopic image of SH-SY5Y cells treated with both **SiR-Hg** and Hg^{2+}. Scale bar = 50 μm.

In conclusion, we have described the first synthesis of the spirolactonized Si-rhodamine, which undergoes the similar pH-dependent ring-opening process to its rhodamine analogue accompanied by chromogenic and fluorogenic turn-on NIR signals. Taking advantage of this feature, **SiR** could be utilized as a platform to construct **SiR**-based probes by following the design strategy widely used in rhodamine systems. Among them, probes **SiRH** and **SiR-Cu** preferred the spirocyclic form when interacting with Cu^{2+} *via* the metal-coordination bonding due to the stability of spirolactam. In contrast, the reaction-based probe **SiR-Hg** underwent the irreversible ring-opening process *via* efficient desulfurization and cyclization promoted by Hg^{2+}, giving rise to a blue color and strong NIR fluorescence emission. Importantly, a similar design strategy is expected to be applicable to other **SiR**-based NIR probes for sensing and bioimaging important species.

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