



Washington Red (WR) dyes and their imaging applications

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Abstract

Near-infrared (NIR) fluorescent dyes with optimum photophysical properties are highly useful for bioimaging in living systems, but such dyes are still rare. Recently, our laboratory developed a unique class of NIR fluorescent dyes with high quantum yields and large Stokes shifts. These dyes, named as Washington Red (WR), were found to be useful scaffolds to develop NIR fluorescent imaging probes upon easy modifications on their phenolic hydroxyl group. In this chapter, we provide a comprehensive summary of this work, including the design and synthesis of WR dyes, their photophysical properties studies, and applications in developing NIR probes to image H₂S in cells. The detailed experimental protocols are described.



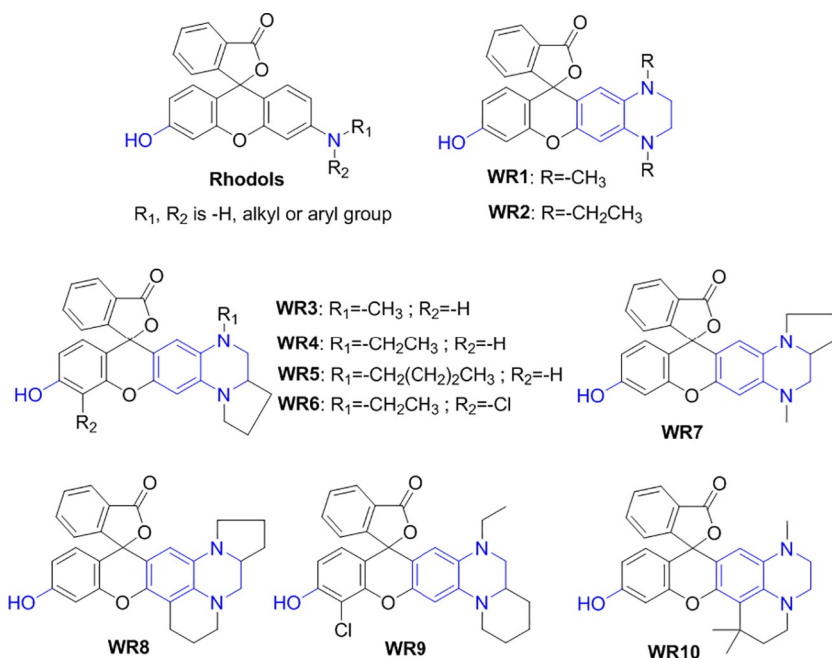
1. Introduction

Fluorescent probes with optimum photophysical properties are essential research tools for bioimaging due to their high sensitivity and fast response. So far, a great variety of fluorescent dyes have been used to construct various

types of fluorescent probes (Chan, Dodani, & Chang, 2012; Li, Gao, Shi, & Ma, 2014). In particular, xanthene-based dyes such as fluorescein, rhodamine, and rhodol are very popular fluorophore scaffolds because of their excellent photophysical properties (high quantum yields and extinction coefficients, excellent water solubility and photostability) (Beija, Afonso, & Martinho, 2009; Chen, Pradhan, Wang, Kim, & Yoon, 2012). While many xanthene-based fluorescent probes have been developed in the past several years, several challenging issues still need to be addressed: (1) Most xanthene dyes emit green to red fluorescence (500–600 nm). By contrast, NIR emission (650–900 nm) has distinct advantages including low photodamage to biological samples, deep tissue penetration, and minimum interference from background autofluorescence by biomolecules in living systems (Escobedo, Rusin, Lim, & Strongin, 2010; Hilderbrand & Weissleder, 2010). Therefore, more xanthene dyes with NIR emission need to be developed. (2) Considerable efforts have been devoted in recent years to modify xanthene core by the extension of π -conjugation or the replacement of the endocyclic oxygen atom with other elements (such as C and Si). These have yielded some novel red-emitting and NIR xanthene dyes (Butkevich et al., 2016; Grimm et al., 2016; Grimm et al., 2017; Sibrian-Vazquez, Escobedo, Lowry, Fronczek, & Strongin, 2012; Uno et al., 2014; Yuan, Lin, Yang, & Chen, 2012). However, like traditional xanthene dyes, they still suffer from the limitations of small Stokes shifts (typically less than 30 nm), which can result in serious self-quenching of fluorescence and measurement error from the excitation backscattering (Peng et al., 2005). (3) It is well-known that ideal NIR dyes suited for constructing sensitive imaging probes should not only have high fluorescence quantum yields and large Stokes shifts in aqueous solution, but also bear optically tunable groups which can be readily modified to obtain all sorts of reaction-based fluorescent probes for target analytes (Chan et al., 2012; Li et al., 2014). Unfortunately, current xanthene dyes that meet the aforementioned criteria are still quite limited. To address this problem, our laboratory recently developed a unique class of NIR xanthene-based dyes named Washington Red (WR). These dyes were found to be useful scaffolds to develop NIR fluorescent imaging probes through easy modifications on their phenolic hydroxyl groups (Chen, Xu, Day, Wang, & Xian, 2017). In this chapter, we provide a comprehensive summary of this work, including the design and synthesis of WR dyes, their photophysical properties studies, and applications in developing NIR probes to image H₂S in cells. The detailed experimental protocols are described.

2. Rational design and chemical structures of WR dyes

In the design of WR dyes, we were considering two main factors: (1) it is known that the free $-OH$ or $-NH_2$ groups in xanthene core are potential optically tunable groups, and their substitution is liable to cause the fluorescence changes (Beija et al., 2009; Chan et al., 2012; Chen et al., 2012; Li et al., 2014). Therefore, a free $-OH$ group was retained in xanthenes core so that it could function as a key fluorescence switch. (2) It is well-established that introducing rigid rings or increasing the electron-donating capability of the substituents on the nitrogen atom generally induced bathochromic shifts in rhodamine and rhodol dyes (Peng & Yang, 2010; Vogel, Rettig, Sens, & Drexhage, 1988). Herein we selected rhodols as our framework because they contain a free $-OH$ group and a substitutable $-NH_2$ group. We expected the incorporation of heteroatom containing fused rings would be favorable for improving the spectroscopic properties. As the first proof-of-concept, an electron-rich and rigid ring, tetrahydroquinoxaline, was used to study the role of substituent group on the changes of properties. Based on this idea, a series of WR dyes containing tetrahydroquinoxaline unit were synthesized (Scheme 1). Their structures were confirmed by NMR and HRMS.



Scheme 1 The structures of rhodols and WR dyes.



3. Photophysical properties of WR dyes

The photophysical properties of WR1–WR10 were tested in 50 mM PBS buffer solution (pH 7.4, containing 15% ethanol). The results are summarized in Table 1. As expected, compared with common rhodols, the absorption and emission maxima of WR dyes have distinct bathochromic shifts. Their aqueous solutions displayed pink color and NIR fluorescence emission with large Stokes shifts (>110 nm). Interestingly, when the *meta*-position of endocyclic oxygen atom has a pyrrolidine ring, it is helpful for enhancing fluorescence quantum yields (for WR3–6). The different alkylations on the *para*-nitrogen atom only had minor effects on their fluorescence quantum yields. However, when the pyrrolidine unit was at the *para*-position of the endocyclic oxygen atom (WR7 and WR8), or was replaced by piperidine ring (WR9), the quantum yields of resultant dyes were not improved but their emission wavelength showed obvious bathochromic shifts.

Table 1 Photophysical data of WR1–WR10 in 50 mM PBS buffer solution (pH 7.4 containing 15% ethanol).

Dye	$\lambda_{\text{abs}}/\text{nm}^{\text{a}}$	$\epsilon_{\text{max}}^{\text{b}}$	$\lambda_{\text{em}}/\text{nm}^{\text{c}}$	Stokes shift ^d	$\Phi_{\text{f}}^{\text{e}}$
WR1	539	31,000	668	129	0.06
WR2	545	30,000	668	123	0.07
WR3	540	35,500	656	116	0.20
WR4	543	44,500	656	113	0.21
WR5	545	32,500	662	117	0.19
WR6	551	42,000	662	111	0.20
WR7	539	29,500	676	137	0.06
WR8	545	30,000	676	131	0.05
WR9	557	39,000	706	149	0.04
WR10	542	30,500	666	124	0.06

^aThe maximal absorption of the dye.

^bUnit: $\text{M}^{-1} \text{cm}^{-1}$

^cThe maximal emission of the dye.

^dUnit: nm.

^e Φ_{f} is the relative fluorescence quantum yield estimated by using seminaphthofluorescein-10 ($\Phi_{\text{f}} = 0.35$ in pH 11 Glycine-NaOH buffer) as a reference standard (Azuma et al., 2012).

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Adding another condensed ring (WR10) or introducing a chloride to the xanthene core (WR6, WR9) also did not enhance fluorescence quantum yields. Below, we describe the detailed experimental protocols that were used to evaluate these WR dyes.

3.1 Materials and experimental methods

- (1) All WR dyes are stable in solid form and can be stored for at least 1 year at 4°C. They can dissolve easily in dimethyl sulfoxide (DMSO), methanol, and ethanol. The dyes may be sensitive to light. So both solid and stock solutions should be kept away from light by storing in dark. The stock solutions of WR1–WR10 (1 mM) were prepared in ethanol, respectively. The stock solutions need to be freshly prepared before use.
- (2) PBS buffer (50 mM, pH 7.4) was prepared by dissolving NaCl (20 g), KCl (0.5 g), Na₂HPO₄ (3.6 g), and KH₂PO₄ (0.6 g) in 500 mL double deionized water. Then pH was adjusted to 7.4 by 1 M HCl or 1 M NaOH. PBS buffers with different pH can also be obtained by adding 1 M HCl or 1 M NaOH. All aqueous solutions were prepared using double deionized water from a Nano pure ultrapure water system.
- (3) The photophysical properties of WR1–WR10 were tested in 50 mM PBS buffer solution (pH 7.4 containing 15% ethanol). All the measurements were carried out at room temperature according to the following procedure. In a test tube, 3.4 mL of PBS buffer and 400 µL of ethanol were mixed, followed by the addition of 8 µL of testing WR dyes stock solution. The final volume of the mixture solution was adjusted to 4 mL with ethanol. After mixing and then standing for 1 min at room temperature, a 3.5-mL portion of the mixture solution was transferred to measure absorbance and fluorescence intensity. The testing solutions should be optically clear and without particulates.
- (4) Absorption spectra were recorded on a Thermo 300 UV/VIS spectrophotometer. Fluorescence excitation and emission spectra were measured on Cary Eclipse fluorescence spectrophotometer.

3.2 Safety precautions

Because ethanol is always used in the photophysical properties studies of WR1–WR10. Experiments should be kept away from any ignition sources, and be performed with sufficient ventilation. The preparation of all stock solutions and testing solutions should be performed in a fume hood whenever possible.

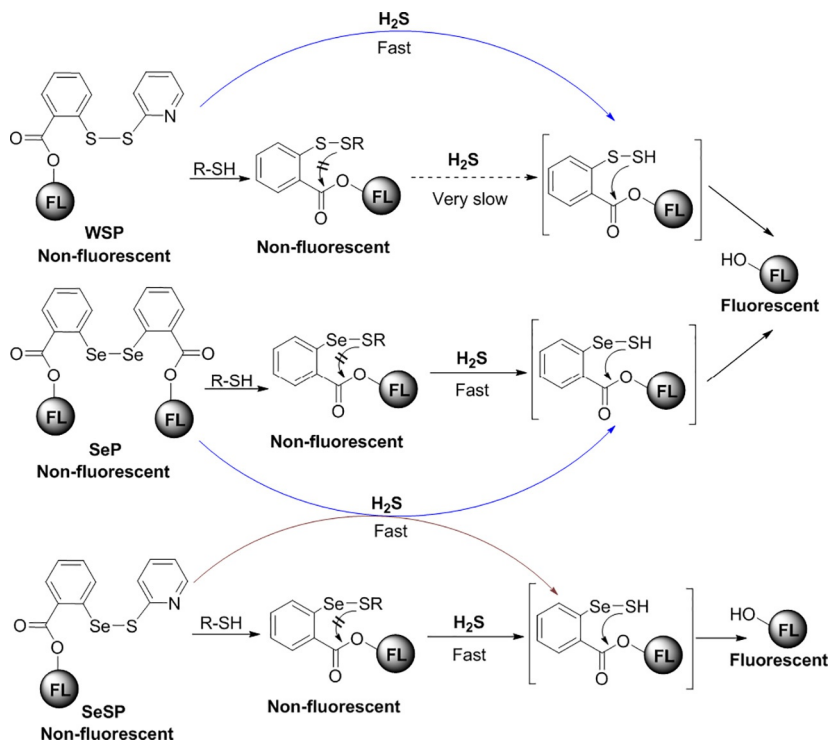


4. Applications of WR dyes in developing NIR probes to image H₂S in cells

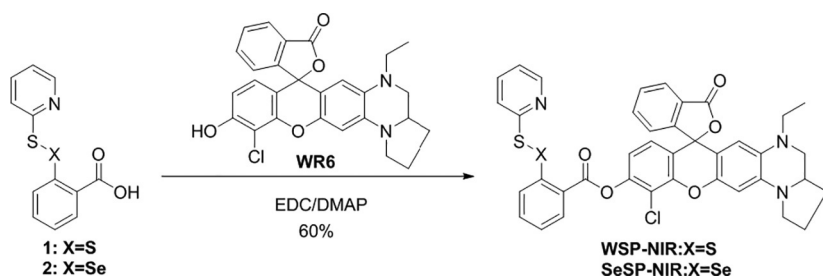
As a proof-of-concept, we verified the usefulness of WR dyes as effective fluorophore templates by developing new “turn-on” NIR fluorescent probes. H₂S is a gasotransmitter that modulates diverse cellular function. In the past several years the development of fluorescence probes for H₂S has attracted considerable attention. Although many H₂S probes have been reported for bioimaging, the maximum emission of most probes are below 600 nm with small Stokes shifts. Only a few can be applied to real biological detection (Hartle & Pluth, 2016; Lin, Chen, Xian, & Chang, 2015; Xuan, Sheng, Cao, He, & Wang, 2012; Zhou, Lee, Xu, & Yoon, 2015). These problems could be addressed by new NIR fluorescent probes based on WR dyes.

4.1 Rational design and synthesis of NIR H₂S probe based on WR dyes

In 2011 our laboratory developed the first nucleophilic reaction-based H₂S probes (WSP, Scheme 2). 2-(Pyridin-2-yl)disulfaneylbenzoate was used as the recognition unit for H₂S (Liu et al., 2011; Peng et al., 2014). In this design, pyridyl disulfide reacts with H₂S to form a persulfide intermediate, which then undergoes an intramolecular cyclization to release a fluorophore containing free -OH group. However, these probes can be consumed by thiols (RSH), which could lead to decreased sensitivity for H₂S in the presence of high concentrations of RSH. To address this problem we then developed diselenide-based SeP probes in 2015 (Peng et al., 2015). The fast reaction between SeP and H₂S (Scheme 2) made the presence of biothiol not a problem. However, two identical fluorophores are needed in SeP. That does not allow much flexibility to tune their reactivity toward H₂S, and also results in the poor water solubility of probes. To further optimize the SeP template, and inspired by the intermediate of SeP reacting with RSH, we reasoned that the selenenyl sulfide (Se—S) linkage should have a similar reactivity to that of SeP toward H₂S. It should react with H₂S rapidly to produce -Se—SH. The following cyclization should release the fluorophore and turn-on fluorescence. While the Se—S bond could also react with RSH, the product should still have the -Se—S- linkage and therefore be easily turned on by H₂S. This new SeSP probe should have high selectivity to



Scheme 2 Design strategies of WSP/SeP/SeSP probes.



Scheme 3 Synthesis and structures of NIR H_2S probes.

H_2S , and not be consumed by thiols. This new strategy allowed the probes more structurally tunable and synthetically feasible.

Based on our design, WR6 was used as the fluorophore to construct two NIR H_2S probes (WSP-NIR; SeSP-NIR) for comparison. WR6 was separately conjugated with compound **1** and **2** to provide WSP-NIR and SeSP-NIR under mild conditions (Scheme 3).

4.2 Chemistry and properties of NIR H₂S probes

4.2.1 Time-course assays

With WSP-NIR and SeSP-NIR in hand, we first tested their fluorescence responses to H₂S. As expected, WSP-NIR and SeSP-NIR showed non-fluorescent (quantum yields <0.01) themselves while gave obvious fluorescence enhancements in the presence of H₂S. The maximum emission intensities of WSP-NIR and SeSP-NIR were reached within 2 and 8 min respectively, indicating their fast and sensitive response to H₂S. Here, we describe the detailed experimental protocols:

- (1) WSP-NIR and SeSP-NIR are stable in solid form and can be stored for at least 1 year at 4 °C. They can dissolve easily in DMSO. The probes may be sensitive to light, so both solid and stock solutions should be kept away from light. The stock solutions (0.4 mM) of WSP-NIR and SeSP-NIR were prepared in DMSO. The stock solutions need to be freshly prepared before use.
- (2) Na₂S stock solution was prepared freshly by dissolving 9.61 mg sodium sulfide nonahydrate (ACROS ORGANICS, 98 +%, ACS reagent) in 4 mL PBS buffer (50 mM, pH 7.4), concentration: 10 mM. This operation should be performed in a fume hood whenever possible, due to potential toxic issue of H₂S.
- (3) CTAB (hexadecyltrimethylammonium bromide/cetyltrimethylammonium bromide, ACROS, 99 +%) stock solution was prepared freshly by dissolving 7.29 mg CTAB in 4 mL ethanol (200 proof), concentration: 5 mM.
- (4) The fluorescence responses of the probes to H₂S were tested in 50 mM PBS buffer solution (pH 7.4) containing 100 μM CTAB. All the measurements were carried out at room temperature according to the following procedure: in a test tube, 3 mL of 50 mM PBS buffer (pH 7.4) and 80 μL of the stock solution of CTAB were mixed, followed by the addition of a requisite volume of Na₂S stock solution. The final volume of the reaction solution was adjusted to 3.98 mL with 50 mM PBS buffer. Then 20 μL of the stock solution of WSP-NIR or SeSP-NIR was added into this mixture. After mixing at room temperature, a 3.5-mL portion of the mixture solution was transferred to measure fluorescence intensity changes at different time ($\lambda_{\text{ex/em}} = 571/656 \text{ nm}$). The testing solutions should be optically clear and without particulates. Results are shown in Fig. 1.

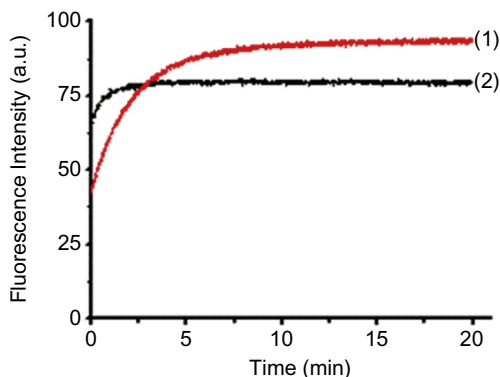


Fig. 1 Time-dependent fluorescence intensity changes of the probes in the presence of Na_2S : (1) $2\ \mu\text{M}$ SeSP-NIR + $20\ \mu\text{M}$ Na_2S ; (2) $2\ \mu\text{M}$ WSP-NIR + $5\ \mu\text{M}$ Na_2S .

4.2.2 Selectivity experiments

A series of reactive sulfur species including glutathione (GSH), cysteine (Cys), homocysteine (Hcy), glutathione disulfide (GSSG), Na_2S , Na_2SO_3 , and $\text{Na}_2\text{S}_2\text{O}_3$ were used to investigate the selectivity of WSP-NIR and SeSP-NIR for H_2S . As shown in Fig. 2, no significant fluorescence enhancement was observed except for Na_2S . Other common amino acids also showed no response to the probes. We also tested the responses of the probes to H_2S in the presence of high concentrations of RSH. The results showed that SeSP-NIR still produced strong fluorescence, but WSP-NIR displayed dramatically decreased fluorescence. These results clearly demonstrated the advantage of the SeSP template in the development of H_2S probes. Experimental procedure is as following:

- (1) The stock solutions ($100\ \text{mM}$) of GSH, Cys and Hcy were prepared in PBS buffer. The stock solutions ($10\ \text{mM}$) of GSSG, Na_2SO_3 , $\text{Na}_2\text{S}_2\text{O}_3$, alanine, arginine, glutamine, glycine, proline, serine were prepared in PBS buffer. All the stock solutions need to be freshly prepared before use.
- (2) 18 empty vials ($4\ \text{mL}$) were numbered #1 to #18. To each vial was added $3\ \text{mL}$ PBS buffer.
- (3) $80\ \mu\text{L}$ CTAB stock solution was added into each vial (#1 to #18). Final concentration of CTAB was $100\ \mu\text{M}$.
- (4) $900\ \mu\text{L}$ PBS buffer was added to vial #1. This was the control (blank sample).

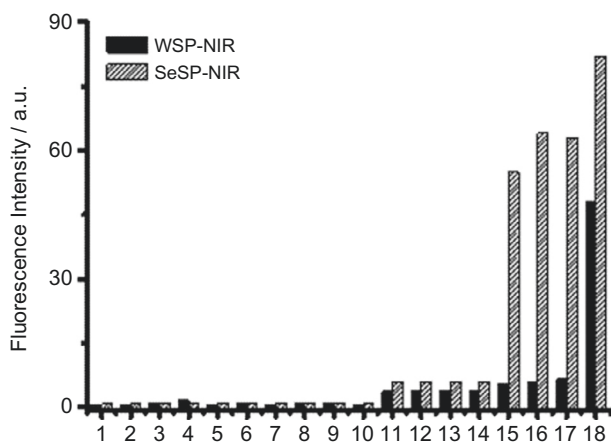


Fig. 2 Fluorescence response of WSP-NIR (2 μ M) or SeSP-NIR (2 μ M) in the presence of various reactive sulfur species and amino acids: (1) probe alone; (2) 100 μ M GSSG; (3) 100 μ M $\text{Na}_2\text{S}_2\text{O}_3$; (4) 100 μ M Na_2SO_3 ; (5) 100 μ M alanine; (6) 100 μ M arginine; (7) 100 μ M glutamine; (8) 100 μ M glycine; (9) 100 μ M proline; (10) 100 μ M serine; (11) 2 mM GSH; (12) 10 mM GSH; (13) 1 mM Cys; (14) 1 mM Hcy; (15) 2 mM GSH+50 μ M Na_2S ; (16) 1 mM Cys+50 μ M Na_2S ; (17) 1 mM Hcy+50 μ M Na_2S ; (18) 50 μ M Na_2S . Reprinted with permission from Chen, W., Xu, S., Day, J. J., Wang, D., & Xian, M. (2017). A general strategy for development of near-infrared fluorescent probes for bioimaging. *Angewandte Chemie International Edition*, 56, 16611–16615. Copyright 2017 John Wiley & Sons, Inc.

- (5) 40 μ L stock solution of GSSG, $\text{Na}_2\text{S}_2\text{O}_3$, Na_2SO_3 , alanine, arginine, glutamine, glycine, proline, serine was added to vials #2 to #10, respectively. Final concentration of them was 100 μ M.
- (6) 80 μ L and 400 μ L stock solution of GSH was added to vial #11 and #12, respectively. The corresponding final concentration of GSH was 2 mM and 10 mM.
- (7) 40 μ L stock solutions of Cys and Hcy were added to vial #13 and #14, respectively. Final concentration was 1 mM.
- (8) 80 μ L stock solution of GSH and 20 μ L stock solution of Na_2S were added to vial #15. The final concentrations of GSH and Na_2S were 2 mM and 50 μ M, respectively.
- (9) 40 μ L stock solution of Cys and 20 μ L stock solution of Na_2S were added to vial #16. The final concentrations of Cys and Na_2S were 1 mM and 50 μ M, respectively.
- (10) 40 μ L stock solution of Hcy and 20 μ L stock solution of Na_2S were added to vial #17. The final concentrations of Hcy and Na_2S were 1 mM and 50 μ M, respectively.

- (11) 20 μL stock solution of Na_2S was added to vial #18. The final concentration of Na_2S was 50 μM .
- (12) The final volume of the reaction solution was adjusted to 3.98 mL with 50 mM PBS buffer (pH 7.4) in each vial. Then 20 μL stock solution of WSP-NIR or SeSP-NIR was added. The final concentration of the probe was 2 μM . After mixing and standing for 10 min at room temperature, a 3.5-mL portion of the reaction solution was transferred into a 1-cm quartz cuvette to measure fluorescence with $\lambda_{\text{ex/em}} = 571/656 \text{ nm}$. Results are shown in Fig. 2.

4.2.3 The model reaction of SeSP with H_2S

To validate the fluorescence turn-on mechanism of SeSP-NIR by H_2S , a model reaction using phenyl 2-((pyridin-2-ylthio)selenenyl)benzoate was carried out in a mixed solution of THF/PBS (Scheme 4). As expected, the products 2,1-benzothiaselenol-3-one and phenol were obtained with high yields, which supported the reaction mechanism we proposed.

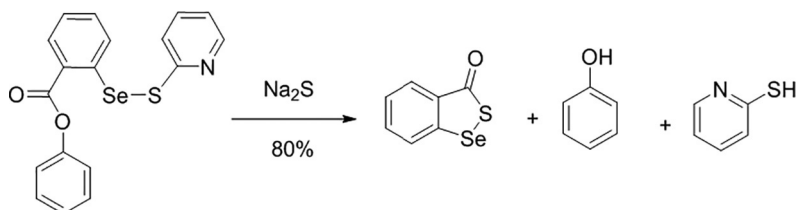
The following are the detailed experimental procedures:

- (1) Phenyl 2-((pyridin-2-ylthio)selenenyl)benzoate (38 mg) was dissolved in 2 mL THF. Na_2S (120 mg) was dissolved in 2 mL PBS buffer.
- (2) These two solutions were mixed and stirred at room temperature for 3 h.
- (3) The reaction mixture was extracted by CH_2Cl_2 (10 mL $\times$ 3).
- (4) The combined organic layers were dried by MgSO_4 , filtered, and concentrated. The crude products were purified by flash chromatography (20% ethyl acetate in hexane) to afford the products shown in Scheme 4.

4.3 Applications of SeSP-NIR in H_2S imaging in cell-based experiments

4.3.1 Materials

The stock solutions of SeSP-NIR and CTAB were prepared using the same protocols described in Section 4.2.1.



Scheme 4 The reaction between Phenyl 2-((pyridin-2-ylthio)selenenyl)benzoate and H_2S .

- (1) Growth media: Dulbecco's modified Eagle's Medium (DMEM, Cellgro) supplemented with 10% fetal bovine serum, 4 mM glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin.
- (2) Washing media: Dulbecco's modified Eagle's Medium (DMEM, Cellgro) supplemented without glutamine and phenol red, Dulbecco's phosphate-buffered saline (DPBS, Hyclone).
- (3) HeLa cells were grown on glass-bottom culture dishes (Corning Inc.) in DMEM supplemented with 10% (v/v) FBS, penicillin (100 U/mL) and streptomycin (100 μ g/mL) at 37 °C under a humidified atmosphere containing 5% CO₂. Before use, the adherent cells were washed one time with FBS-free DMEM.
- (4) H₂S donor N-(benzoylthio)benzamide (Zhao, Wang, & Xian, 2011) stock solution was freshly prepared by dissolving 2.57 mg the donor in 1 mL DMSO, concentration: 10 mM.
- (5) H₂S biosynthesis inhibitor DL-propargylglycine (PAG) stock solution was freshly prepared by dissolving 1.13 mg inhibitor in 1 mL DMEM, concentration: 10 mM.
- (6) A solution of 4 μ M SeSP-NIR was prepared by adding 10 μ L probe stock solution to 990 μ L DMEM and mixing thoroughly; a solution of 60 μ M PAG was prepared by adding 6 μ L PAG stock solution to 994 μ L DMEM and mixing thoroughly; a solution of 100 μ M H₂S donor N-(benzoylthio)benzamide was prepared by adding 10 μ L N-(benzoylthio)benzamide stock solution to 990 μ L DMEM and mixing thoroughly.

4.3.2 Fluorescence imaging of H₂S in HeLa cells

To investigate the capability of SeSP-NIR in imaging H₂S in cells, the following three different group experiments were carried out:

- (1) Group a: All cell media was removed and exchanged with 500 μ L 4 μ M SeSP-NIR solution. Then cells were incubated for 20 min at 37 °C. After removal of excess probe and washed with PBS buffer (pH 7.4), cells were incubated with PBS buffer (pH 7.4, containing 100 μ M CTAB) at room temperature for 15 min. Cell imaging was carried out after removal of the culture solution.
- (2) Group b: All cell media was removed and exchanged with 500 μ L 60 μ M PAG solution. Then cells were incubated for 40 min at 37 °C. All cell media was next removed and exchanged with 500 μ L 4 μ M SeSP-NIR solution, then cells were incubated for 20 min at 37 °C.

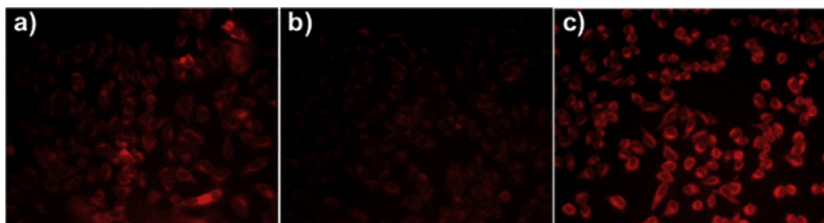


Fig. 3 Fluorescence imaging of H_2S in HeLa cells. Cells were imaged after incubation with $4\text{ }\mu\text{M}$ SeSP-NIR for 20 min after pretreatment with (A) no pretreatment, (B) $60\text{ }\mu\text{M}$ PAG for 40 min, or (C) $100\text{ }\mu\text{M}$ H_2S donor for 60 min. Reprinted with permission from Chen, W., Xu, S., Day, J. J., Wang, D., & Xian, M. (2017). A general strategy for development of near-infrared fluorescent probes for bioimaging. *Angewandte Chemie International Edition*, 56, 16611–16615. Copyright 2017 John Wiley & Sons, Inc.

After removal of excess probe and washed with PBS buffer (pH 7.4), cells were incubated with PBS buffer (pH 7.4, containing $100\text{ }\mu\text{M}$ CTAB) at room temperature for 15 min. Cell imaging was carried out after removal of the culture solution.

- (3) Group c: All cell media was removed and exchanged with $500\text{ }\mu\text{L}$ $100\text{ }\mu\text{M}$ H_2S donor N-(benzoylthio)benzamide solution. Then cells were incubated for 60 min at 37°C . All cell media was next removed and exchanged with $500\text{ }\mu\text{L}$ $4\text{ }\mu\text{M}$ SeSP-NIR solution, then cells were incubated for 20 min at 37°C . After removal of excess probe and washed with PBS buffer (pH 7.4), cells were incubated with PBS buffer (pH 7.4, containing $100\text{ }\mu\text{M}$ CTAB) at room temperature for 15 min. Cell imaging was carried out after removal of the culture solution. As can be seen in Fig. 3, compared to group a (control cells), a significant reduced fluorescence was observed in cells pretreated with PAG. However, cells pretreated with a H_2S donor showed obvious fluorescence enhancement. These results showed SeSP-NIR had good cell permeability and was sensitive to image H_2S in cells.



5. Conclusions

In this chapter we described the design and evaluation of a unique class of NIR dyes (named as Washington Red-WR) with large Stokes shifts and high quantum yields. Among them, WR3–WR6 displayed excellent photophysical properties in aqueous solutions. Moreover, their fluorescence properties were found to be tunable by easy modifications on the

phenolic –OH group. Based on WR6, two probes (WSP-NIR and SeSP-NIR) were developed for the detection of H₂S. SeSP-NIR overcame a major limitation of our previously reported WSP and SeP probes, and showed much improved sensitivity to H₂S. SeSP-NIR was also used for imaging intracellular H₂S.

Acknowledgments

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