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Response to referees

Manuscript ID AY-ART-04-2014-001011 entitled "A BODIPY based indicator for fluorogenic detection of salicylaldehyde with OFF-ON emission"

Dear Prof. Craig Banks:
Thank you for your hard work and appreciation on our manuscript! All of the authors appreciated the reviewers’ hard work and useful comments. The manuscript has been revised according to referees’ comments point by point.

Referee: 1

1. For salicylaldehyde, its activity is not as reactive dicarbonyl compounds such as methylglyoxal. The C=N reaction of salicylaldehyde might be gradual. Therefore, the reaction time should be examined in this study.

Authors’ response: The reaction time between Probe 1 and salicylaldehyde has been studied, and the Kinetic profiles of the reaction between Probe 1 and salicylaldehyde has been shown in Fig.2.

2. Methods need further be shown in details for repeat.

Authors’ response: In fact, the experiments have been repeated several times, and the repeated fluorescent titrations of salicylaldehyde to probe 1 was shown in fig. S3.

3. There are multiple grammatical and spelling errors in the manuscript. It should be rewritten in a concise manner, and checked by a native English language editor.

Authors’ response: The manuscript has been checked throughly, and grammatical and spelling errors has been revised accordingly.

4. The abstract section is too simple to summary the keynote of the study.

Authors’ response: The abstract has been enlarged.

5. The results and discussion part is disordered, it should be adjusted carefully.
Authors’ response: The results and discussion part has been revised.

6. Figure 4 should be enlarged.
Authors’ response: Figure 4 (figure 5 in the revised manuscript) has been enlarged

Referee: 2

1. Probe reacted with salicylaldehyde to form two major products, which should be isolated and well characterized. The percentage of each of them should also be calculated.
Authors’ response: The reaction of probe 1 with salicylaldehyde has been carried out again, and the two major products have been isolated and characterized by HR-MS and HNMR spectrum. In our experiment, we found the product P1 was not stable and not easy to isolated. Compound P2 was the main product, about 90% was obtained.

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A BODIPY based indicator for fluorogenic detection of salicylaldehyde with OFF-ON emission

Qian Li, Jian Xu, Ying Yue, Yuan Liao and Shijun Shao*

Abstract: A turn-on fluorescent indicator 1 based on BODIPY derivative for the detection of salicylaldehyde in aqueous solution by fluorescence spectroscopy has been developed. The formation of schiff base between probe 1 and salicylaldehyde suppressed the PET process and prohibited the C=N isomerization accounted for the fluorescence response of Probe 1. Two major reaction products of probe 1 with salicylaldehyde was obtained and characterized by 1H NMR and HR MS spectrum. Weakly fluorescent polymeric films have been obtained by embedding the indicator in the PMMA, which can be used for the fluorescent detection of the salicylaldehyde in polymer matrices.

Introduction

Aldehydes are important industrial chemicals which have been widely utilized in the manufacturing of various materials, ranging from pharmaceuticals to plastic additives. Among various aldehydes, salicylaldehyde is a common organic pollutant that builds up in the environment due to the inefficiency of traditional water purification processes, which finds their way into the water system through pharmaceutical medications, cosmetic products, and agricultural chemicals1; On the other hand, salicylaldehyde is neurotoxic and has effect on α-synuclein aggregation2. Despite of its vital roles in the environmental events and biological processes, the detection of salicylaldehyde has been less developed3.

The reliable and economic monitoring of aldehydes is crucial in the field of environmental pollution analysis & control and industrial chemistry. A number of methods have been reported in the literature, which can be classified into electrochemical4a, chromatographic methods4b and spectroscopic techniques4c. Among various methods, considerable efforts have been devoted to the optical chemosensors due to their low cost, simplicity, high degree of specificity and low detection limit5. Most optical aldehyde detection methods are based on nucleophilic addition of the amine to the aldehyde, forming an imine group (Schiff bases or Hydrazones). The imine formation usually generates a color or emission change, so great effort has gone into the synthesis of sophisticated optical indicators
with primary amine groups for aldehydes detection. In previous studies, much attention has been paid to the design of optical indicators for aliphatic aldehydes (Formaldehyde is the most favorable), indicators for aromatic aldehydes have been less developed. So the design of indicators for aromatic aldehydes is important and desirable.

Among various fluorophores, BODIPY dyes have been widely employed to develop optical chemosensors for various species due to the remarkable photophysical properties, such as sharp absorption and fluorescence peaks, high stability, and high fluorescence quantum yield. In spired by previous work on aldehydes detection and our recent work on BODIPY based fluorescent sensors, we reported a new BODIPY based indicator with two amino groups for the OFF-ON fluorescent detection of Salicylaldehyde herein. The fluorescence response of 1 to formaldehyde is much smaller compared to that of salicylaldehyde. The Salicylaldehyde induced OFF-ON fluorescence could be attributed to the formation of C=N unit and the geometrically restricted six-membered ring resulted from the intramolecular H-bonding interaction, which suppressed the photo-induced electron transfer (PET) process and prohibited the C=N isomerization.

Results and discussion

The structure and synthesis of Probe 1 were shown in Scheme 1, which was prepared through the condensation reaction of 3,5-dinitrobenzoyl chloride with 2,4-dimethylpyrrole and reduced with Pd/C-N₂H₄ according to a published procedure. The purity and structure of 1 was confirmed by the ¹H NMR and HRMS spectra (see ESI†, Fig. S1 and Fig. S2), which was consistent with the reported data.

![Scheme 1 Synthesis of probe 1](image)

The sensing properties of probe 1 toward various aldehydes were studied in EtOH-H₂O (v/v=4:1, with AcOH as catalyst) solution. Probe 1 showed quite weak fluorescence with a
fluorescent emission intensity at 522 nm due to an efficient PET process from the lone pairs of electrons on amino groups, which has inherent electron donating property, to the excited BODIPY fluorophore. Various aldehydes analyte (the formaldehyde, benzaldehyde, p-nitrobenzaldehyde, p-methoxybenzaldehyde, 4-diethylaminobenzaldehyde, salicylaldehyde, 3-hydroxybenzaldehyde, 4-(diethylamino)salicylaldehyde, 4-hydroxybenzaldehyde and 5-nitrosalicylaldehyde) were added to a freshly prepared solution of 1. The resulting solution was subsequently tempered for 1 h at room temperature and then analyzed by fluorescence spectroscopy. The emission data of Probe 1 for the 10 selected aldehydes are depicted in Fig. 1. Probe 1 exhibited strong OFF-ON fluorescence responses toward salicylaldehyde and the solution gave off visible green fluorescent emission. In the presence of benzaldehyde, the fluorescence was also turned on to some extent, but it was much smaller compared to that of salicylaldehyde. Under the same condition, the fluorescence responses of 1 toward other aldehydes were slim. So probe 1 could act as an efficient fluorescent indicators for salicylaldehyde.

![Fig. 1 Changes of the fluorescent emission in EtOH-H$_2$O solutions containing molecular probe 1 (5 µM) after addition of different aldehydes (5 mM). Prior to the measurement, the solutions were tempered for 1 h at room temperature.](image)

The interaction of probe 1 with salicylaldehyde was further investigated in detail through emission spectroscopic titrations. Time course study revealed that the reaction between Probe 1 and salicylaldehyde was completed at about 50-60 minutes (Fig. 2) in EtOH-H$_2$O solution.
Fig. 2 Kinetic profiles of the reaction between Probe 1 and salicylaldehyde. The reaction process was monitored by fluorescence emission intensity at 522 nm ($\lambda_{ex}=503$ nm) in EtOH-H$_2$O containing Probe 1 (5 µM) and salicylaldehyde (10 mM).

To access the sensitivity of Probe 1-based assay, various amounts of salicylaldehyde were spiked into the solution of Probe 1. The solutions were incubated at room temperature for 1 h and then analyzed by fluorometry. Fig. 3 revealed that fluorescence emission centered at 522 nm increased as a function of salicylaldehyde concentrations in aqueous solution with a concentrations between 0.1 and 10 mM were observed, indicating fluorogenic detection of salicylaldehyde. Probe 1 showed off-on emission toward salicylaldehyde and this method could be repeated (see ESI†, Fig. S3).

Fig. 3 Fluorescence emission spectra of Probe 1 (5 µM) in EtOH-H$_2$O solution in the presence of salicylaldehyde (0-15 mM). The inset shows the titration curve of salicylaldehyde with Probe 1 by fluorescence emission intensity at 522 nm.
To access the identity of the possible adduct in the assay conditions, the reaction product of probe 1 with salicylaldehyde was isolated and analyzed by HR-MS and $^1$HNMR spectrometry. As shown in fig. 4, the major peaks located at 459.2155 and 563.2403 were identified, which were consistent with the theoretical molecular weight of the proposed products P1 ($\text{C}_{26}\text{H}_{26}\text{BF}_2\text{N}_4\text{O}^+$, M+H: 459.2167) and P2 ($\text{C}_{33}\text{H}_{29}\text{BF}_2\text{N}_4\text{O}^2$; M+H: 563.2430), confirming formation of schiff base in the assay system, as shown in scheme 2. Compound P2 was proposed the main product, and about 90% was obtained.

![Fig. 4](image.png)

**Fig. 4** HR MS spectra of reaction products of probe 1 with salicylaldehyde

From the $^1$HNMR spectrum (see ESI†, Fig. S4 and S5), the chemical shifts of phenolic proton (OH) of the products P1 and P2 were 12.86 and 12.85 ppm, respectively, which indicated that OH proton of the adduct formed intramolecular H-bonding with the imine nitrogen. The intramolecular H-bonding resulted in a geometrically restricted six-membered
ring and enhanced the reactive activity. The formation of Schiff base suppressed the PET process and the intramolecular H-bonding inhibited the C=N isomerization\textsuperscript{11} accounted for the turn on fluorescence response of probe 1 (Scheme 2).

Scheme 2 Proposed mechanism of the turn on fluorescence response of Probe 1 to salicylaldehyde.

Over the passed years, much attention has been focu s on the sensors functioning in solution by means of spectroscopic instrumentation, which restricted the practical applications of the sensors. For simplicity, convenient and low cost test kits is highly demanding. We also examined whether Probe 1 could be employed for the sensing of aldehydes in the polymer metrics. For this purpose, a sensor film F1 with a poly(methyl methacrylate) (PMMA) layer containing 0.5 wt% of Probe 1 were prepared. Sensor film F1 was initially nonfluorescent, when in contact with aqueous solutions containing salicylaldehyde, a bight fluorescent emission appeared in two minutes (Fig. 5). Again, probe 1 could function in polymer metric for the efficient detection of salicylaldehyde with off-on fluorescence response, which could be advantageous in application.

![Fig. 5 Changes in fluorescene images (excitation at 365 nm with a UV lamp) of sensor film F1 containing probe 1 upon contaction with salicylaldehyde solution. ([salicylaldehyde]:b~f, 0.1, 0.2, 0.5, 1, and 5mM)
Conclusions

In conclusion, using a BODIPY fluorophore, we developed a PET and C=N isomerization-based OFF-ON probe 1 that can selectively detect tracing salicylaldehyde in aqueous solution under mild condition. The probe exhibits a clear salicylaldehyde induced changes in the intensity of emission spectra. When embedded in a polymer film, Probe 1 can also be employed for the fluorescent detection of salicylaldehyde in polymer metrics. The new PET-based probe 1 is expected to be utilized in a variety of chemical applications.

Acknowledgement

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Notes and references

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Statement of Societal Impact

Salicylaldehyde is a common organic pollutant that builds up in the environment and neurotoxic having effect on α-synuclein aggregation, however the detection of salicylaldehyde has less been developed. In this work, we developed a new PET and C=N isomerization-based OFF-ON fluorescent probe 1 based on BODIPY fluorophore that can selectively detect tracing salicylaldehyde in aqueous solution under mild condition, which is expected to be utilized in a variety of chemical applications.
A BODIPY based indicator for Fluorogenic detection of Salicylaldehyde with OFF-ON emission

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A turn-on fluorescent indicator 1 based on BODIPY derivative for the detection of salicylaldehyde in aqueous solution by fluorescence spectroscopy has been developed. The formation of Schiff base between probe 1 and salicylaldehyde suppressed the PET process and prohibited the C=N isomerization accounted for the fluorescence response of Probe 1.
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Experimental

Apparatus and materials

¹H NMR spectra were recorded in DMSO-d6 solution on the Inova 400 MHz instruments, and spectral data were reported in ppm relative to tetramethylsilane (TMS) as internal standard. HR MS were carried out on QToF-Micro YA 263 instruments. And fluorescent spectra were recorded on a Perkin Elmer LS55 Fluorescence Spectrophotometer.

Synthesis

Synthesis of compound 2

To a solution of 2,4-dimethylpyrrole (2 equiv.) in absolute CH₂Cl₂ solution was added the 3,5-dinitrobenzoyl chloride (1 equiv.). The solution was stirred at room temperature for 24 hours, the color changing slowly from pale brown to deep red. Et₃N (6 equiv.) was then added, followed by a subsequent addition of BF₃-Et₂O (8 equiv.). The mixture was stirred for another 6 hours at room temperature. The reaction was stopped by the addition of saturated aqueous NaHCO₃ (100ml), and then was washed 3 times with saturated aqueous NaHCO₃. The organic layer was then dried over MgSO₄, filtered and the solvent removed. Chromatography on silica gel (CH₂Cl₂/cyclohexane, 1:1 as eluent) gave the pure compound 2.

Synthesis of compound 1

Compound 2 (0.7 g, 1.9 mmol) was dissolved in 50 mL of absolute THF and 50 mL of absolute MeOH. After purged with N₂, 5%Pd/C (1.0g) and 2.0 ml hydrazine were added. The
solution was stirred at reflux under N$_2$ for 5 hours. When TLC monitoring (silica gel; CH$_2$Cl$_2$) showed complete consumption of 2, the reaction mixture was filtered, and evaporated. The compound was purified by silica gel column chromatography (CH$_2$Cl$_2$ and CH$_2$Cl$_2$/MeOH as eluent) to give a red solid (83%). Recrystallized from CH$_2$Cl$_2$/n-hexane to afford orange crystals 1.

$^1$H NMR (DMSO-d$_6$, 400 MHz): 6.13-6.18 (m, 2H), 5.89 (s, 1H), 5.67-5.68 (m, 2H), 4.95 (s, 4H), 2.41-2.48 (m, 6H), 1.68 (s, 6H).


Which was consistent with the reported data.

Fig. S1 $^1$HN MR Spectra of probe 1.
Fig. S2 HR-MS Spectra of probe 1.
**Fig. S3** Fluorescence emission spectra of Probe 1 (5 µM) in EtOH-H$_2$O solution in the presence of salicylaldehyde (0-15 mM). The inset shows the titration curve of salicylaldehyde with Probe 1 by fluorescence emission intensity at 522 nm.

**Fig. S4** $^1$HN NMR Spectra of reaction product P1 of probe 1 with salicylaldehyde
Fig. S5 $^1$HN MR Spectra of reaction product P2 of probe 1 with salicylaldehyde