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New strategy for detection of hydrogen peroxide based on bi-nucleophilic reaction

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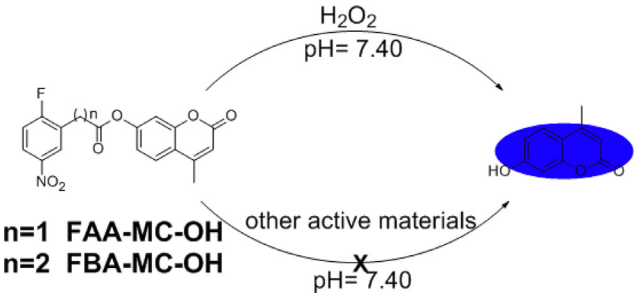
HIGHLIGHTS

- Two fluorescent probes **FAA-MC-OH** and **FBA-MC-OH** were synthesized.
- Both the fluorescent probes could detect H_2O_2 in PBS buffer (pH = 7.40).
- Fluorescent probe **FBA-MC-OH** was more sensitive and selective to detect H_2O_2 than the fluorescent probe **FAA-MC-OH**.

GRAPHICAL ABSTRACT

Two 7-hydroxy-4-methyl-coumarin based fluorescent probes **FAA-MC-OH** and **FBA-MC-OH** could detect H_2O_2 in PBS buffer (pH= 7.40). Of these two fluorescent probes, **FBA-MC-OH** has a better selectivity and sensitivity to H_2O_2 than the fluorescent probe **FAA-MC-OH**.



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ABSTRACT

Two novel fluorescent probes based on 7-hydroxy-4-methyl-coumarin, **FAA-MC-OH** (2-fluoro-4-nitro-phenylacetyl hydroxyl coumarin) and **FBA-MC-OH** (2-fluoro-4-nitro-benzoyl hydroxyl coumarin) are first synthesized, and spectral studies confirm that both the probes display highly selective and sensitive to H_2O_2, especially **FBA-MC-OH** has a shorter response time. Moreover, it is worth noting that the reaction mechanism is based on bi-nucleophilic substitution instead of oxidation or hydrolysis, which is different from previous reported probes'.

1. Introduction

As one of the reactive oxygen species (ROS) in the cells, hydrogen peroxide (H_2O_2) plays an important role not only in the physiology, but also in the pathology.[1–9] In the abnormal cells, it's recognized as a second messenger to transfer signal and indicate the intracellular oxidant levels.[3,10,11]However, when the level of H_2O_2 in cells is over normal capacity, it would lead to diseases, such as cancers,[12] cardiovascular disease,[13] Alzheimer's disease,[14] genetic instability and so on.[15] Therefore, it's very urgent for us to develop efficient methods for detection of H_2O_2 in the cells.

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Recently, there are many different tools that have been applied into detection of H_2O_2 in the cells, such as mass probes,[16] proteomics probes,[17]and fluorescent probes.[15] Especially, fluorescent probes have been widely used for the features, because of high sensitivity and low destructivity. In the previous reported work, fluorescent probes have been used to detect H_2O_2 base on these reaction mechanisms, such as borate hydrolysis,[18,19] H_2O_2 oxidation,[20–22] and sulfonate hydrolysis.[23] However, other species in the cells may have some interference to the H_2O_2, such as NO,[24–27] O_2^-,[28–31] and so on. So, it's necessary to have a better fluorescent probe to detect H_2O_2 in the cells which will also suppress interference of other species.

In this work, the compounds **FAA-MC-OH** and **FBA-MC-OH** were synthesized as Scheme 1, their structures were confirmed by [1]H NMR, [13]C NMR and Mass spectrum respectively (Figure S1-S12), and both probes were used to detect H_2O_2 based on the bi-nucleophilic substitution instead of oxidation or hydrolysis. When in the basic system (pH = 7.40), H_2O_2 may knock off one of two protons, and become a negative monovalent anion HO_2^-, a very good nucleophilic group.[32–35] Then, a leaving group, such as the fluorine atom of compound **FAA-MC-OH** or **FBA-MC-OH**, is easy replaced by HO_2^- when *para*-position of the fluorine is nitro, which has a good character of electrical drawing. After the HO_2^- hold together with benzene ring through α-oxygen, the other proton is also easy to be splited in the basic system, and α'-oxygen will replace the electrophilic group if it lies in the *ortho*-position of the oxygen, and if the leaving group is a fluorescent molecule, it will also accompany with the changes of the fluorescence intensity in the system (Scheme 2). Meanwhile, the proposed mechanism was evidenced by detection of the corresponding HR-ESI-MS as well (Fig S14). Upon the addition of 3 equiv H_2O_2 in the solution of **FBA-MC-OH**, the main peak was observed at *m*/*z* 199.0368, which represented complex 5-nitro-3*H*-benzo[*c*][1,2]dioxol-3-one.

2. Experimental section

In order to prove the validity of hypothesis above, spectral study experiments were carried out in PBS buffer (pH = 7.40). In all the tests, the concentrations of the fluorescent probes are 10 μM.

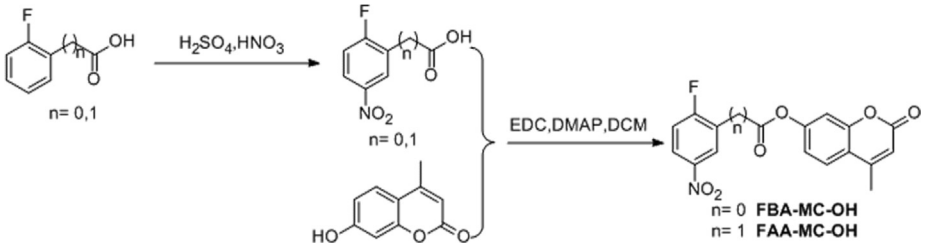
Firstly, the responses of probes **FAA-MC-OH** and **FBA-MC-OH** toward H_2O_2 were evaluated by UV–vis absorption (Fig. 1). The free probes **FAA-MC-OH** and **FBA-MC-OH** displayed a peak at 275 nm with molar absorption coefficients of 16496 $cm^{-1}M^{-1}$ and 14148 $cm^{-1}M^{-1}$, respectively. Both bands were red shifted with addition of H_2O_2 (200 μM) in the spectra, which was consistent with the absorption of 7-hydroxy-4-methyl-coumarin, the results indicated that the probes **FAA-MC-OH** and **FBA-MC-OH** could react with H_2O_2 and may undergo the mechanism as described Scheme 2, which was similar to other reported bi-nucleophilic reaction.[32] We studied the fluorescent changes of 10 μM **FAA-MC-OH** (Fig. 2a) and **FBA-MC-OH** (Fig. 2b) by titration with various concentrations of H_2O_2 in air. As shown in Fig. 2, the fluorescence emission intensity at 440 nm gradually increased with increasing concentration. Remarkably, 10 μM **FAA-MC-OH** and **FBA-MC-OH** showed excellent linear correlation between fluorescence intensity and the concentrations of H_2O_2 (0–100 eq) with R^2 = 0.9946 and 0.9993 (Fig. S13).

In order to obtain the optimal time for probes **FAA-MC-OH** and **FBA-MC-OH** to respond to hydrogen peroxide, time-dependent fluorescence emission experiments were performed on the solution of probes in the presence of H_2O_2. As shown in Fig. 3, the fluorescence emission intensity of **FAA-MC-OH** gradually increased with the extension of time, reached stability around 40 min (Fig. 3a Insert). While **FBA-MC-OH** had a shorter response time, reached stability around 20 min (Fig. 3b Insert). Additionally, upon reaction with H_2O_2, two probes display color change from colorless to blue-fluorescence within 10 min, we saw that clearly with the naked eye (Fig. 3 Insert).

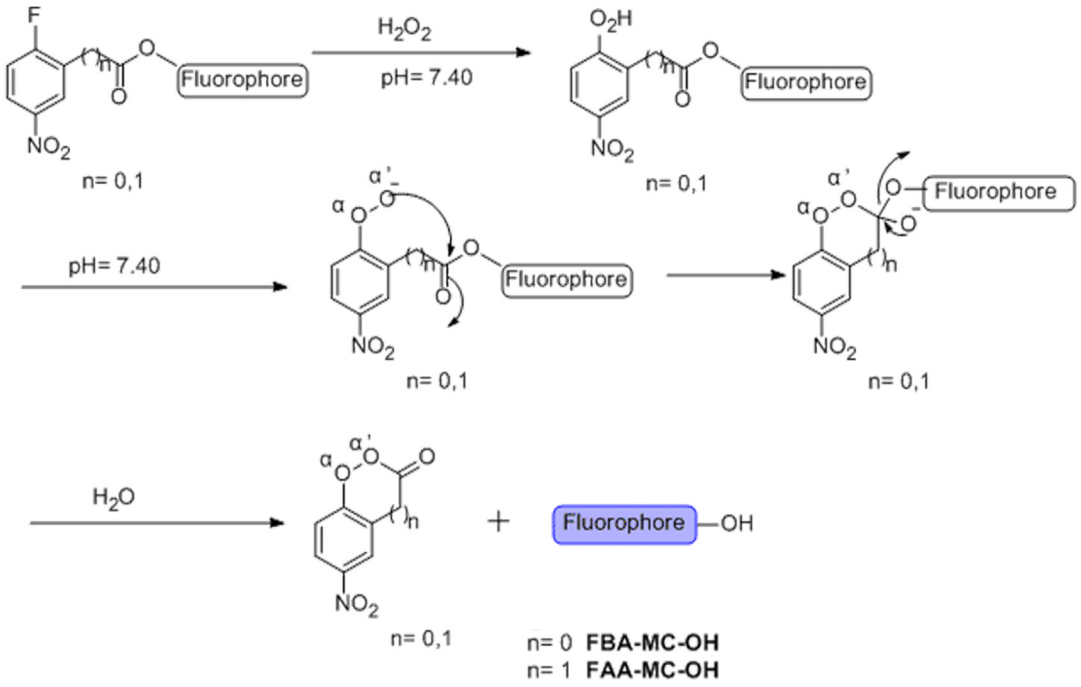
To further understand the optical behavior between the probes and H_2O_2, the response of various concentration of H_2O_2 to probes were conducted. We can see from Fig. 4, as time went on, the corresponding florescence intensity increased with increasing concentration of H_2O_2, which make sure that the probe can react with H_2O_2. Meanwhile, the higher concentration of H_2O_2, the faster reaction rate.

The results of comparison between two probes could tell us that the performance of **FBA-MC-OH** was better than that of **FAA-MC-OH**, because **FBA-MC-OH** could response to the equilibrium in a short time. The reason could be speculated as follows. When H_2O_2 reacted with these probes through bi-nucleophilic substitution route, the corresponding intermediates were shown in Fig. 5, that is, α-O atom of H_2O_2 attacked **FBA-MC-OH**, and substituted F atom to form Fig. 5a, then another O atom, α'-O, subsequently acted on carbon labeled as C2 of carbonyl group, which linked benzene ring directly, to split ester bond. Thus, the fluorescent probe 7-hydroxy-4-methyl-coumarin was set free. This finding could be also found in the formation of Fig. 5b. But unlike Fig. 5a, α'-O atom would connect to C4, which was apart from benzene ring by a $-CH_2-$ spacer. It was a tiny structural distinction that led to the results. In other words, when α'-O atom attacked C2, sp^2 hybrid C2 bonded benzene ring provided a exactly right position to meet the α'-O atom, and a five-member conjugated ring was produced. In this sense, the structure of **FBA-MC-OH** was benefit to the occurrence of recognition. On the other hand, if C4 contacted α'-O atom, it is necessarily for it to overturn to an appropriate position to meet the requirement of the substitution. Further studies indicate that a non-conjugated six member ring would be emerged. Both of the inversion of configuration and the creation of non-conjugated ring were disadvantageous and energy-consuming. So, the reaction rate and recognition property of **FBA-MC-OH** were better than that of **FAA-MC-OH**.

As we all known, there are many other species in the cells, so it is very vital for us to know if other species have an effect on our experiments. In order to prove that, the selective experiments were completed, as shown in Fig. 6. The other relevant species were Na_2S_2, Na_2S, KO_2, NaClO, t-BuOOH, •OH, $ONOO^-$, Na_3PO_4, Na_2SO_4, $KBrO_3$, KF, NaCl, KBr, Na_2CO_3, KNO_3 and Na_2SO_4, the concentrations



Scheme 1. Synthesis of **FAA-MC-OH** and **FBA-MC-OH**.



Scheme 2. Reaction process of H_2O_2 with fluorescent probes.

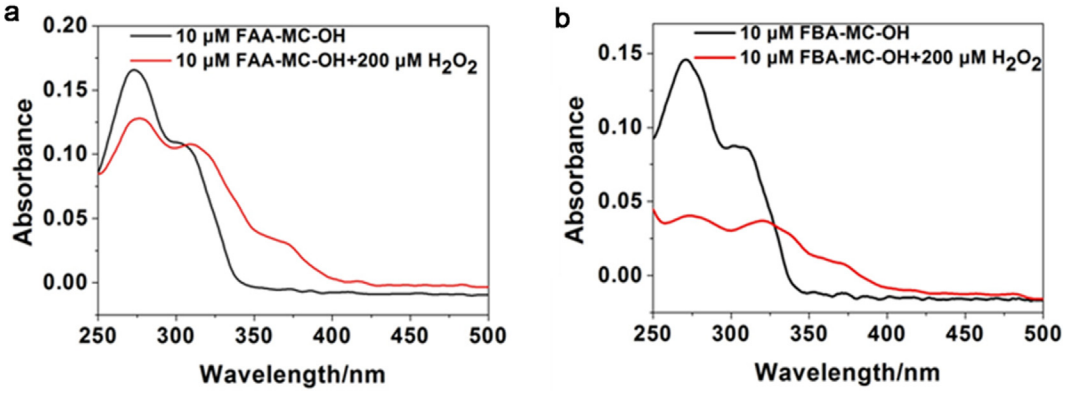


Fig. 1. UV–vis spectra changes of the probes **FAA-MC-OH** (a) and **FBA-MC-OH** (b) upon the addition of H_2O_2 in PBS buffer (pH = 7.40, 10 mM) in 2 h.

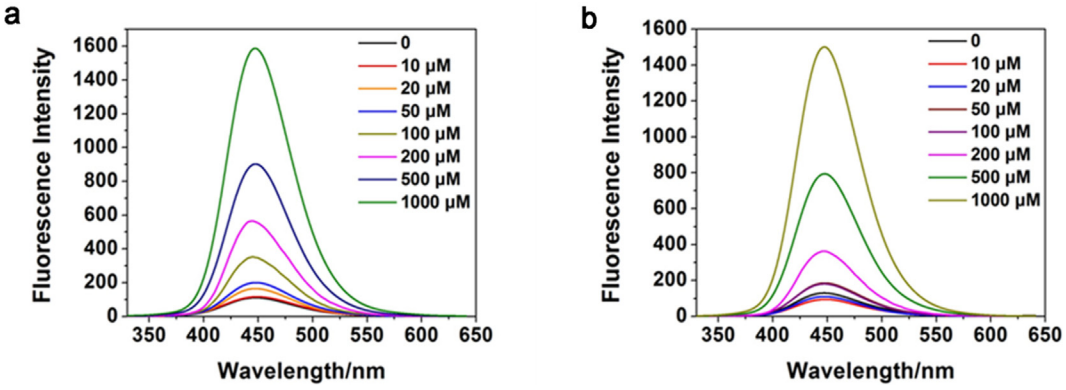


Fig. 2. The fluorescence increment (440 nm) of **FAA-MC-OH** (10 μM, a) and **FBA-MC-OH** (10 μM, b) with various concentrations of H_2O_2 in PBS buffer after 30 min. (10 mM, pH = 7.40). (λ_{ex} = 325 nm).

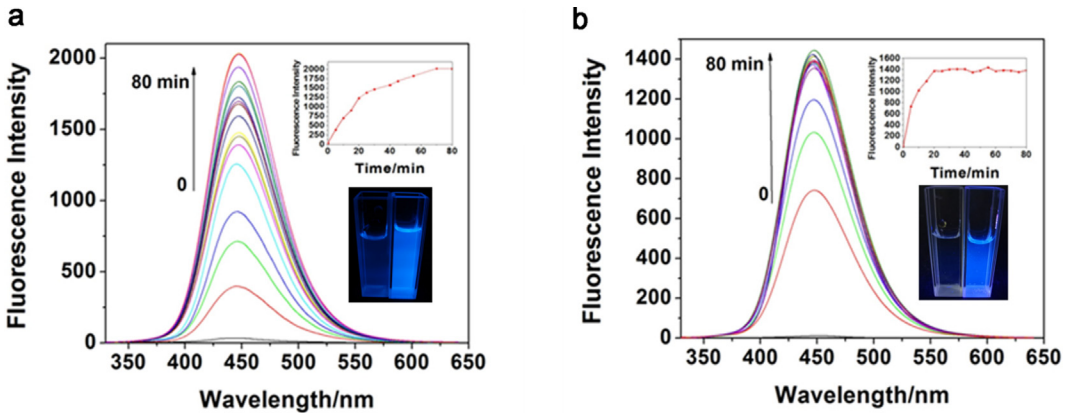


Fig. 3. Time course of fluorescence changes of **FAA-MC-OH** (10 μ M, a) and **FBA-MC-OH** (10 μM, b) in the presence of H_2O_2 (1 mM) in PBS buffer (10 mM, pH = 7.40). (λ_{ex} = 325 nm).

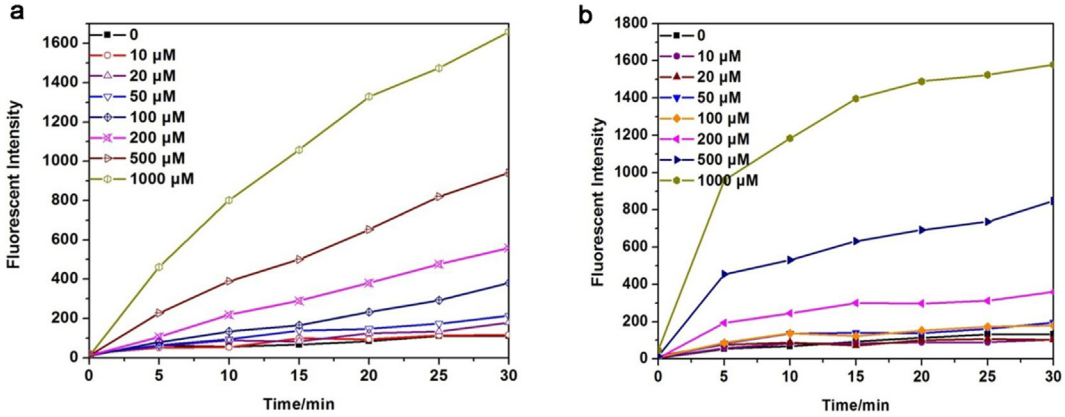


Fig. 4. Time course of the fold of fluorescence increment (440 nm) of **FAA-MC-OH** (10 μ M, a) and **FBA-MC-OH** (10 μM, b) with various concentrations of H_2O_2 in PBS buffer (10 mM, pH = 7.40). (λ_{ex} = 325 nm).

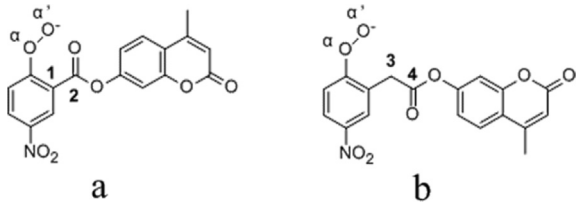


Fig. 5. The structure of intermediates of **FBA-MC-OH** (a) and **FAA-MC-OH** (b) after reacted with H_2O_2.

of all the species including H_2O_2 are 100 μM. We found that only H_2O_2 could cause dramatic fluorescence intensity increasing for both probes, while Na_2S_2 led to slight fluorescence changes as their structures are similar. The other relevant species didn't cause any fluorescence response. Therefore, both of these probes showed higher selectivity to the H_2O_2, especially the probe **FBA-MC-OH**.

In conclusion, we have successfully designed probes **FAA-MC-OH** and **FBA-MC-OH**, and applied them to detect H_2O_2. It is worth noting that the mechanism is based on bi-nucleophilic substitution which is different from other reported probes. The spectral studies confirmed that both the probes displayed highly selective and sensitive to the H_2O_2, especially the **FBA-MC-OH**.

CRediT authorship contribution statement

Chenxin Mao: Validation, Investigation, Resources, Data curation. **Yafei Tian:** Visualization, Investigation, Software. **Shuoshuo Wang:** Formal analysis, Resources. **Bei Wang:** Data curation, Writing - original draft, Writing - review & editing. **Xiang Liu:** Conceptualization, Methodology, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The authors have no competing interests to declare.

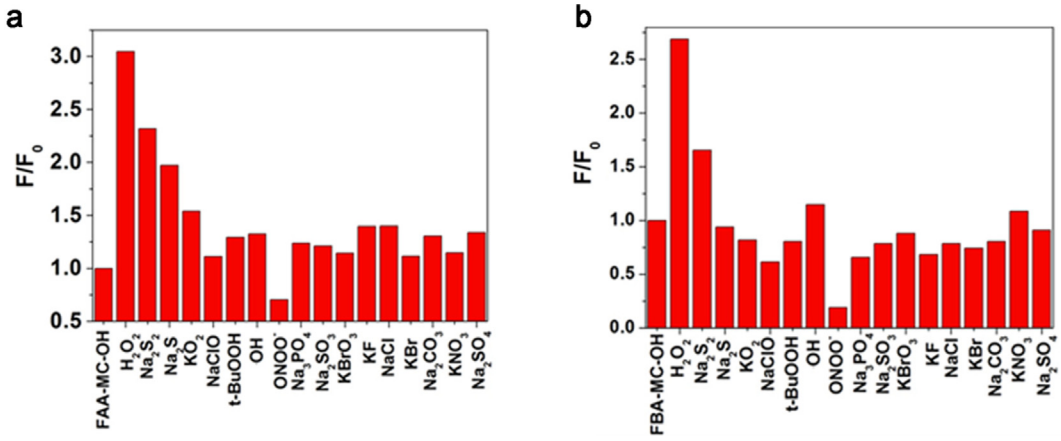

Fig. 6. Fluorescence response of **FAA-MC-OH** (10 μ M, a) and **FBA-MC-OH** (10 μM, b) in the presence of H_2O_2 and other relevant agents (100 μM) in PBS buffer (10 mM, pH = 7.40) in the 15 min. (λ_{ex} = 325 nm).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2021.120131.

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