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ARTICLE

Hypochlorous Acid Activating MB-O to Release Methylene Blue for Photodegrading of Aβ Aggregates

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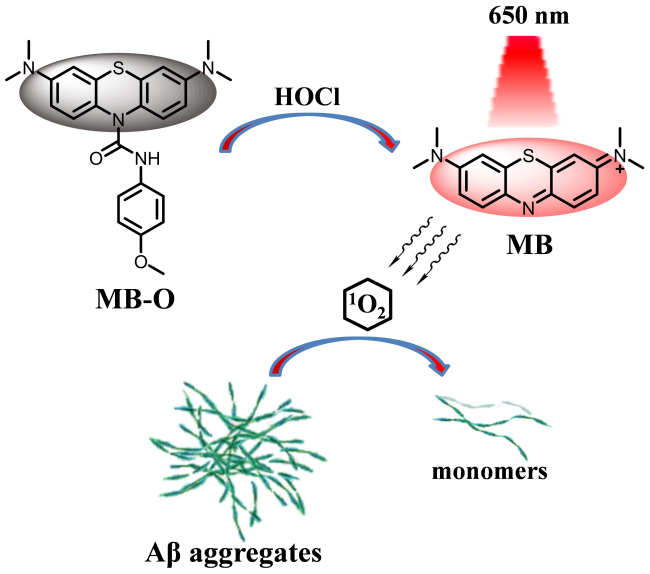
Amyloid-β (Aβ) aggregates are one of biomarkers of Alzheimer's disease (AD). It is well known that Aβ aggregates display neurotoxicty or cytotoxicity to neurons. Thus, degrading Aβ aggregates is crucial for exploring the treatment of AD. Moreover, the excessive production of HOCl in the AD brain is an important feature of the disease. Herein, a novel compound **MB-O** based on methylene blue (MB) skeleton was designed and synthesized. The probe **MB-O** can specifically react with HOCl, releasing the fluorophore MB with strong fluorescence intensity increase. More importantly, the released MB is capable of degrading Aβ aggregates under red light irradiation.

Introduction

Alzheimer's disease (AD), as one of the common neurodegenerative diseases, seriously affects the physical and mental health of the elderly.[1] Although the pathological mechanism of AD remains unclear yet, several hypotheses have been proposed to explore the pathogenesis of this disease.[2] One of them, amyloid cascade hypothesis is predominant in AD. According to amyloid cascade hypothesis, amyloid-β (Aβ) aggregates are closely related to the progress of AD owing to the neurotoxicity. Thus, it is urgently important to degrade Aβ aggregates. Besides, it is well established that the overproduction of reactive oxygen species, such as hypochlorous acid (HOCl), are linked well with the progression of AD.[3] Therefore, it is of great significance to develop a kind of hypochlorous acid-activated probe capable of degrading Aβ aggregates for AD treatment.

It is well known that methylene blue (MB) has been used as a drug for the treatment of various diseases.[4] Numerous researchers have demonstrated that photosensitizers excessively producing highly reactive oxygen species (ROS) could degrade Aβ aggregates, leading to the reduction of Aβ-induced toxicity.[5] Importantly, it has been reported that MB can generate $^1O_2$ as a powerful photosensitizer to depolymerize Aβ aggregates.[6] It has become an increasingly important strategy for the treatment of AD.[7] However, majorities of photosensitizers may be phototoxic to normal issues because they do not reach the AD lesion. Therefore, it is critical to activate the photosensitizer in situ using overexpressed HOCl in the AD microenvironment.

In this article, a novel compoud **MB-O** was constructed by the reduced form of MB (leucomethylene blue, **MB-Cl**) with aniline through an amide bond (Scheme 1). The probe **MB-O** was stable in physiological conditions and can specifically react with HOCl, accompanying with remarkable fluorescence intensity and obvious colour change (colourless to blue). In the presence of HOCl, the probe **MB-O** can release fluorophore MB fleetly to depolymerize Aβ aggregates under light irradiation. In addition, **MB-O** is biocompatible and capable of reacting with endogenous HOCl in living cells.



Scheme 1. The structure of **MB-O** and schematic diagram of degradation of Aβ aggregates

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Results and Discussion

The rapid release of MB

To evaluate the possibility of the probe **MB-O** releasing MB under the stimulation of HOCl, the spectral signals were recorded in PBS at room temperature. The probe **MB-O** displayed significant emission enhancement at 686 nm with the addition of HOCl (Figure 1A, B). The proposed mechanism by which **MB-O** reacts with HOCl was shown in Figure S1. The amide carbonyl group with an electron-deficient carbon atom is easily attacked by HOCl, which causes the amide cleaved and released fluorophore MB. The fluorescence intensity of probe **MB-O** dramatically enhanced (around 500-fold) after adding 10.00 μM HOCl, along with a solution colour change from colourless to remarkable blue (Figure 1A). These results suggested that the probe **MB-O** could release fluorophore MB rapidly under the stimulation of HOCl. Furthermore, the LOD of the probe **MB-O** was determined by fluorescence titration (HOCl concentrations ranging from 0.00 μM to 4.00 μM), and the LOD of **MB-O** was estimated to be 6.50 nM (Figure 1C, D). This indicated that the probe **MB-O** was sensitive to HOCl at a very low concentration. The above results demonstrated that the probe **MB-O** exhibited high sensitivity to HOCl and could release MB rapidly.

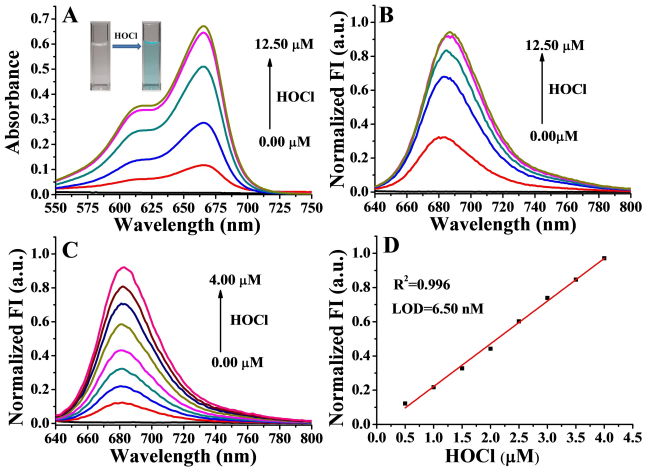

Figure 1. Changes in (A) absorption and (B) emission spectrum of **MB-O** (10.00 μM) towards the different concentrations of HOCl (0.00, 2.50, 5.00, 7.50, 10.00, 12.50 μM) in PBS buffer and the inset was a picture of the colour change of the solution before and after the reaction of **MB-O** with HOCl (12.50 μM); (C) The emission spectrum of **MB-O** (10.00 μM) under continuous titrated with HOCl (0.00–4.00 μM) in PBS buffer; (D) The linear regression curve was fitted according to fluorescence intensity at 686 nm in the range of HOCl from 0.00 to 4.00 μM. (PBS pH = 7.4, λ_{ex} = 620 nm, λ_{em} = 686 nm).

The stability of the probe

One of the crucial performances of the probe **MB-O** is the stability under physiological conditions. Therefore, we evaluated the stability the probe **MB-O** against some other substances (Figure 2). A variety of substances were incubated with the probe **MB-O** in PBS solution separately, including reactive oxygen/nitrogen species (ROS/RNS: H_2O_2, TBHP, ROO•, O_2^-, $ONOO^-$, •OH and TBO•), several anions (CH_3COO^-, CO_3^{2-}, SO_4^{2-}, Cl^-, F^-, I^-, NO_2^- and $S_2O_4^{2-}$), cations (Fe^{3+}, Ni^{2+}, Mg^{2+}, Fe^{2+}, Al^{3+}, Ca^{2+}, K^+, Cu^{2+} and NH_4^+), and various amino acids (Pro, Ser, Ala, Gln, Val, Thr, Ile, Gly, Tyr, Met, Trp, Phe, Cys, Glu, and Lys). It can be seen that these substances did not trigger **MB-O** to emit obvious fluorescence change. Even in extreme conditions, the probe **MB-O** showed excellent selectivity toward HOCl (Figure S2). These results clearly indicated that the probe **MB-O** was very stable under physiological conditions and can only be activated by HOCl.

The anti-interference ability of the probe

To investigate the interference of cellular reductants to **MB-O**, the anti-interference ability was determined using several reductants, including N-acetylcysteine (NAC), glutathione (GSH), glucose and aldehyde (Figure S3).[8] Because both NAC and GSH were capable of consuming HOCl to a certain extent, the increased concentration of them gradually diminished the intensity of **MB-O**. However, the higher fluorescence intensity of

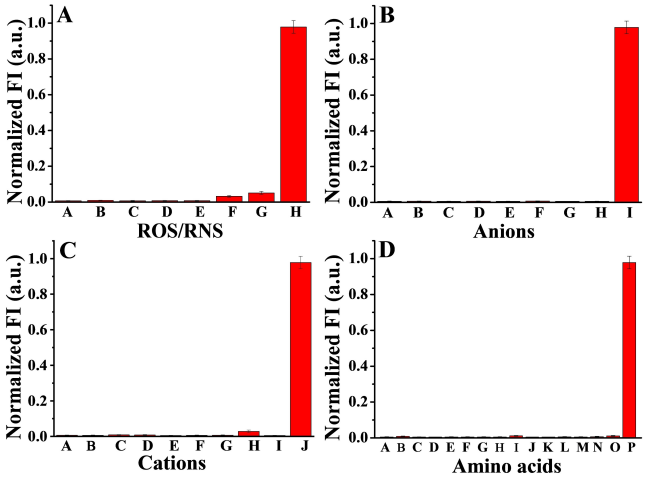

Figure 2. The fluorescence intensity of **MB-O** (10 μM in PBS, pH = 7.4) at 686 nm after the addition of other analytes. (A) Several ROS/RNS (from A to H: H_2O_2, TBHP, ROO•, O_2^-, $ONOO^-$, •OH, TBO• and HOCl) were tested with concentrations of 100 μM; (B) A wide range of anions (from A to I: CH_3COO^-, CO_3^{2-}, SO_4^{2-}, Cl^-, F^-, I^-, NO_2^-, $S_2O_4^{2-}$ and HOCl) were studied with concentrations of 100 μM; (C) A series of cations (from A to J: Fe^{3+}, Ni^{2+}, Mg^{2+}, Fe^{2+}, Al^{3+}, Ca^{2+}, K^+, Cu^{2+}, NH_4^+ and HOCl) were examined with concentrations of 100 μM; (D) Some amino acids (from A to P: Pro, Ser, Ala, Gln, Val, Thr, Ile, Gly, Tyr, Met, Trp, Phe, Cys, Glu, Lys and HOCl) were tested with concentrations of 100 μM. The excitation and emission wavelength ($\lambda_{ex}/\lambda_{em}$) are 620/686 nm.

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the probe **MB-O** was still obtained in the presence of HOCl compared to the probe itself (Figure S3). The fluorescence increases for NAC and GSH (40 μM) were 20.2- and 8.8-fold, respectively. In addition, the high concentrations of glucose and aldehyde did not pose interference to **MB-O**. To verify the anti-interference against pH, the PBS solutions of different pH (vary from 2 to 12) were prepared (Figure S4). There was little fluorescence when only **MB-O** was added at all PBS solutions with different pH. In the presence of 10 μM HOCl, the fluorescence intensity of **MB-O** exhibited dramatically enhancement at a wide range of pH from 2 to 10. Taken together, the probe **MB-O** exhibited excellent anti-interference ability in complex solution environment, indicating its great potential for biological applications.

Photodegradation of Aβ42 aggregates

Previous reports showed that the behavioural defects in transgenic mouse models could be effectively improved via removing pre-existing amyloid deposit.[9] To evaluate the possibility of the probe **MB-O** for photo-depolymerizing Aβ42 aggregates, the singlet oxygen ($^1O_2$) productivity was firstly evaluated by two singlet oxygen capture agents: DPA (9, 10-diphenylanthracene) and DPBF (1, 3-Diphenylisobenzofuran). As showed in Figure 3A and B, under red light (650 nm, 0.31 W/cm^2, 20 min) irradiation and the existence of HOCl, the absorbance intensities of both probes were significantly diminished due to the excellent $^1O_2$ productivity of the probe **MB-O**.

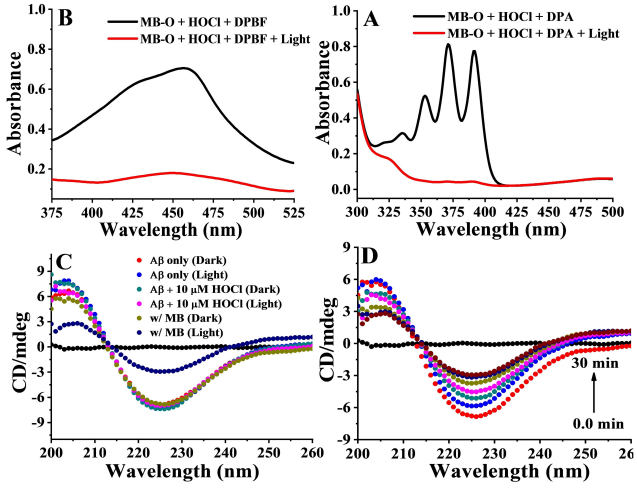

Figure 3. The $^1O_2$ productivity and light-induced photodegradation of Aβ42 aggregates by **MB-O**. The absorption spectrum analyses of (A) DPA and (B) DPBF in the presence of **MB-O** (containing HOCl) with or without red light irradiation. (C) The CD spectrum of Aβ42 aggregates (40 μM) under various conditions. (D) Time-dependent CD spectrum changes of Aβ42 aggregates in the presence of 10 μM **MB-O** (containing 10 μM HOCl) under light conditions (650 nm, 0.31 W/cm^2). "Light" in (A), (B), and (C) refers to red light (650 nm, 0.31 W/cm^2) for 20 min; "w" in (C) and (D) is the abbreviation of " Aβ + 10 μM HOCl".

Then the photo-depolymerize Aβ42 aggregates assays were launched using thioflavin T (ThT) as an indicator under dark or light conditions. ThT possesses high affinity toward protein aggregates and is widely used in AD pathological protein staining.[10] According to the experimental results, the fluorescence intensity of ThT was significantly diminished (~ 73 %) in the presence of **MB-O** (containing 10 μM HOCl) under continuous irradiation by red light (650 nm, 0.31 W/cm^2) for 20 min. On the contrary, the fluorescence rare decreased in the absence of red light irradiation (Figure S5). By contrast, only Aβ42 aggregates or HOCl did not trigger ThT fluorescence changes under red light irradiation. To further verify the effect of disaggregation, circular dichroism (CD) experiment was carried out to examine the secondary structure changes of Aβ42 aggregates (Figure 3C, D). From the CD spectrum, the positive and negative band peaks of Aβ42 aggregates were presented at 195 nm and 226 nm, respectively. The intensity of the two peaks in CD spectrum was gradually decreased in the presence of **MB-O** (containing 10 μM HOCl) with the continuous red light (650 nm, 0.31 W/cm^2) irradiation. This further confirmed thar the photosensitizer MB was successfully released from the probe **MB-O** with the addition of HOCl, and degraded Aβ42 aggregates under red light irradiation.

The release of MB in living cells

Ultimately, we assessed the practical application of **MB-O** for the possibility of releasing MB in living cells. PC12 cells and **MB-O** were incubated treated with or without lipopolysaccharides (LPS)/phorobol myristate acetate (PMA), and 4-Aminobenzoic acid hydrazide (ABAH). LPS and PMA can induce endogenous HOCl in PC12 cells, while ABAH is used for decreasing the intracellular HOCl level by inhibiting myeloperoxidase (MPO) activity.[11] As showed in Figure 4, A1–A3, no intracellular fluorescence could be observed while only 10 μM **MB-O** was incubated with PC12 cells. However, remarkable fluorescence appeared in the red channel (Figure 4, B1–B3) when PC12 cells incubated with **MB-O**, 1 μg/mL LPS and 500 ng/mL PMA. As showed in Figure 4, C1–C3, very weak red fluorescence could be observed following the co-incubation with 250 μM ABAH by suppressing MPO activity and decreasing the level of endogenous HOCl. These results indicated that **MB-O** can be induced by endogenous HOCl for releasing MB in PC12 cells.

Conclusions

In summary, a novel compound **MB-O** was designed and synthesized based on methylene blue skeleton. **MB-O** could be activated by HOCl to release MB capable of producing $^1O_2$ to degrade Aβ aggregates under red light irradiation. Moreover, **MB-O** exhibited excellent stability among various substances in vitro and was only sensitive towards HOCl. This work will provide new insights into the treatment for AD.

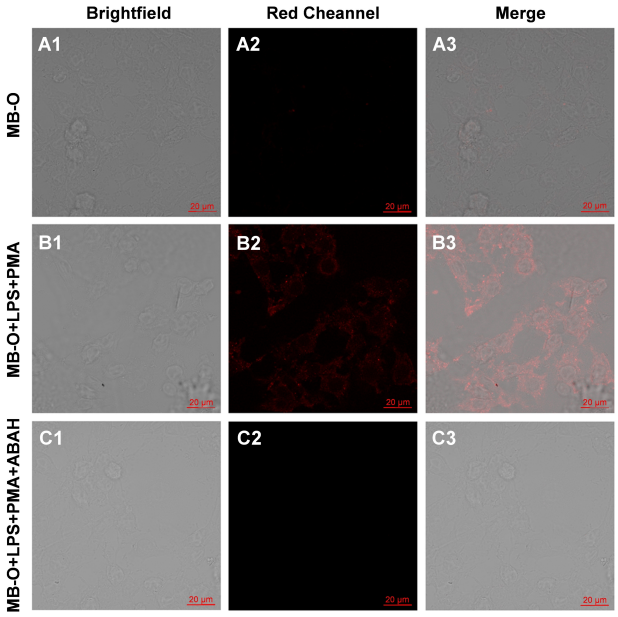

Figure 4. Fluorescence images of **MB-O** for endogenous HOCl. (A1–A3) Fluorescence images of PC12 cells alone. The PC12 cells were cultured with **MB-O** (10 μM) for 1 h and (B1–B3) further treated with LPS (1 μg/mL)/PMA (500 ng/mL) or (C1–C3) LPS (1 μg/mL)/PMA (500 ng/mL)/ABAH (250 μM) for another 1 h. Images from left to right: bright field, fluorescence field and merged images, fluorescence channel: 700 ± 50 nm, λ_{ex} = 633 nm. Scale bar: 20 μm.

Experimental Section

General materials and experimental methods: All commercially available compounds were obtained from pharmaceutical suppliers without further purification. The probe **MB-O** was dissolved in HPLC grade of ethanol to obtain a 1.0 mM stock solution. Hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), tert-butyl hydroperoxide (TBHP), 2, 2′-azobis(2-amidinopropane)dihydrochloride (ROO•), peroxynitrite ($ONOO^-$), and superoxide solution (O^{2-}) were prepared following the reported literature.[12] Hydroxyl radicals (•OH) was generated by Fenton reaction. $FeSO_4$ (5 equiv.) was added in the presence of H_2O_2 (1 equiv.). TBO• was prepared by adding $FeSO_4$ (5 equiv.) in the presence of TBHP (1 equiv.). Buffer solution (PBS, pH = 7.4) was prepared using deionized water.

Instruments: The absorption spectra were measured at room temperature by a UH5300 UV-Vis spectrophotometer, ranging from 550 to 750 nm. All fluorescence measurements were accomplished at room temperature with an F-4600 fluorescence spectrophotometer, ranging from 640 to 800 nm (λ_{ex} = 620 nm). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were measured on Bruker AV-400 or AV-600 NMR spectrometer, using $CDCl_3$ as solvents. HRMS analysis was performed on a TOF MS instrument.

Synthesis details of MB-O:

(1) To a solution of methylene blue (1.00 g, 3.13 mmol, 1.00 eq.) in 10 mL water, dichloromethane (DCM, 5.00 mL) and Na_2CO_3 (1.33 g, 12.52 mmol, 4.00 eq.) were added. The mixture solution was stirred at 40 °C under nitrogen atmosphere. Sodium dithionite (10.89 g, 12.52 mmol, 4.00 eq.) dissolved in 10 mL water was injected to the solution directly using a syringe device. After addition the reaction solution was stirred for 1 h at 40 °C under nitrogen atmosphere until the mixture became yellow. The reaction solution was cooled with an ice-water bath, and then bis(trichloromethyl)carbonate (0.56 g, 1.88 mmol, 0.60 eq.) dissolved in 5 mL of DCM was added dropwise. Above reaction solution was carried out under a nitrogen atmosphere at room temperature for 2 h. Then reaction solution was poured into ice-water, and the resulting mixture was extracted with DCM. The combined extracts were dried using anhydrous sodium sulfate and evaporated on a rotary evaporator then purified by column chromatography (**MB-Cl**, white solid, yield 42.8%; ethyl acetate: petroleum ether = 1:10). ^{1}H NMR (400 MHz, $CDCl_3$), δ = 7.38 (d, J = 8.1 Hz, 2H), 6.69 (d, J = 2.8 Hz, 2H), 6.61 (dd, J = 8.9, 2.8 Hz, 2H), 2.95 (s, 12H).

(2) **MB-Cl** (100 mg, 0.287 mmol, 1.00 eq.), Na_2CO_3 (91.0 mg, 0.861 mmol, 3.00 eq.), para-Anisidine (106 mg, 0.861 mmol, 3.00 eq.) dissolved in 3 mL of DCM was stirred for 8 h at 40 °C under nitrogen atmosphere. The reaction mixture was evaporated on a rotary evaporator then purified by column chromatography (**MB-O**, white solid, yield 42.8%; ethyl acetate: petroleum ether = 1:3). ^{1}H NMR (400 MHz, $CDCl_3$), δ = 7.42 (d, J = 8.8 Hz, 2H), 7.28 (d, J = 9.0 Hz, 2H), 6.84 (s, 1H), 6.78 (d, J = 9.0 Hz, 2H), 6.70 (d, J = 2.7 Hz, 2H), 6.63 (dd, J = 8.9, 2.8 Hz, 2H), 3.73 (s, 3H), 2.91 (s, 12H). ^{13}C NMR (100 MHz, CDCl3) δ = 155.7, 153.5, 149.1, 134.4, 131.8, 128.1, 127.2, 121.36, 114.0, 111.3, 111.1, 55.5, 40.7. HR-MS (ESI, m/z): calculated for C24H27 N4O2S $[M+H]^+$, 435.1845, found 435.1810.

Determination of limit of detection (LOD): The limit of detection (LOD) was determined according to the commonly used fluorescence titration method. The emission spectrum of probe **MB-O** in PBS buffer (10.00 μM) was collected for eleven times to confirm the standard deviation (σ). The linear regression curve was then fitted according to the data in the range of HOCl from 0.00 to 4.00 μM and obtained the slope (k) of the curve. Finally, the LOD was calculated using the following equation: LOD = 3σ/k

Preparation of Aβ42 aggregates: A solid form of Aβ42 was purchased from protein suppliers. The preparation of Aβ42 aggregates was carried out by dissolving the peptide (0.50 mg/mL) in PBS. The peptide solution was stirred at room temperature for 48 h until the solution becomes clear. The Aβ42 aggregates were kept in the refrigerator freezer.

Cell culture and confocal fluorescence imaging: The PC12 cells (Rat pheochromocytoma cells) were cultured in RPMI-1064 (Roswell Park Memorial Institute) supplemented with 10% fetal bovine serum (SFBS), 1% penicillin and 1% streptomycin sulphate. Then PC12 cells were seeded in a 96-well micro-assay culture plate at a density of 104 cells per well and then growth for 24 h in air (5% CO_2) at 37 °C. After that, the PC12 cells were pre-treated with 10 μM **MB-O** in RPMI-1064 solution for 60 min at 37 °C in air (5% CO_2) incubator. After washing with RPMI-1064 to remove the remaining **MB-O**, and the PC12 cells were further incubated with LPS (1 μg/mL)/PMA (500 ng/mL) or LPS (1 μg/mL)/PMA (500 ng/mL)/ABAH (250 μM) for 60 min. Cell culture medium was removed and cells were washed three times with RPMI-1064. The fluorescence images were recorded using an Olympus FV1000 confocal scanning system.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: fluorescence · methylene blue · hypochlorous acid · amyloid beta-peptides · Alzheimer's disease

[1] a) M. Goedert, M. G. Spillantini, *Science* **2006**, *314*, 777–781; b) M. Prince, R. Bryce, E. Albanese, A. Wimo, W. Ribeiro, C. P. Ferri, *Alzheimer's Dementia* **2013**, *9*, 63–75 e62.

[2] a) J. Hardy, G. Higgins, *Science* **1992**, *256*, 184–185; b) J. T. Coyle, P. Puttfarcken, *Science* **1993**, *262*, 689–695; c) X. Du, X. Wang, M. Geng, *Transl. Neurodegener.* **2018**, *7*, 2–9.

[3] a) S. Samanta, T. Govindaraju, *ACS Chem. Neurosci.* **2019**, *10*, 4847–4853; b) N. Lu, Y. Ding, R. Tian, Y.-Y. Peng, *Eur. J. Pharmacol.* **2016**, *780*, 142–147; c) J. Yang, Y. Yao, Y. Shen, Y. Xu, G. Lv, C. Li, *Z. Anorg. Allg. Chem.* **2020**, *646*, 431–436.

[4] a) E. Akoury, M. Pickhardt, M. Gajda, J. Biernat, E. Mandelkow, M. Zweckstetter, *Angew. Chem. Int. Ed.* **2013**, *52*, 3511–3515; *Angew. Chem.* **2013**, *125*, 3596–3600; b) C. Stack, S. Jainuddin, C. Elipenahli, M. Gerges, N. Starkova, A. A. Starkov, M. Jové, M. Portero-Otin, N. Launay, A. Pujol, N. A. Kaidery, B. Thomas, D. Tampellini, M. F. Beal, M. Dumont, *Hum. Mol. Genet.* **2014**, *23*, 3716–3732; c) M. Yuksel, K. Biberoglu, S. Onder, K. G. Akbulut, O. Tacal, *Biochimie* **2018**, *146*, 105–112; d) X. Duan, R. Lv, Z. Kong, *Z. Anorg. Allg. Chem.* **2020**, *646*, 1408–1411; e) Y. Yang, C. Zhu, R. Li, X. Gu, S. She, *Z. Anorg. Allg. Chem.* **2021**, *647*, 1560–1567; f) G.-L. Li, W.-D. Yin, Q.-L. Liu, X.-R. Gong, Y.-J. Zhao, G.-Z. Liu, *Z. Anorg. Allg. Chem.* **2021**, *n/a*; g) Q. Mao, X. Liu, Y. Li, Y. Jiang, J. Sun, *Z. Anorg. Allg. Chem.* **2021**, *647*, 731–741.

[5] a) B. I. Lee, Y. S. Suh, Y. J. Chung, K. Yu, C. B. Park, *Sci. Rep.* **2017**, *7*, 7523–7533; b) Z. Du, N. Gao, X. Wang, J. Ren, X. Qu, *Small* **2018**, *14*, 1801852; c) B. Ding, S. Shao, C. Yu, B. Teng, M. Wang, Z. Cheng, K.-L. Wong, P. a Ma, J. Lin, *Adv. Mater.* **2018**, *30*, 1802479; d) J. Xu, W. Han, P. Yang, T. Jia, S. Dong, H. Bi, A. Gulzar, D. Yang, S. Gai, F. He, J. Lin, C. Li, *Adv. Funct. Mater.* **2018**, *28*, 1803804.

[6] a) J. P. Tardivo, A. Del Giglio, C. S. de Oliveira, D. S. Gabrielli, H. C. Junqueira, D. B. Tada, D. Severino, R. de Fátima Turchiello, M. S. Baptista, *Photodiagn. Photodyn. Ther.* **2005**, *2*, 175–191; b) M. Wainwright, A. McLean, *Dyes Pigm.* **2017**, *136*, 590–600.

[7] a) A. Taniguchi, D. Sasaki, A. Shiohara, T. Iwatsubo, T. Tomita, Y. Sohma, M. Kanai, *Angew. Chem. Int. Ed.* **2014**, *53*, 1382–1385; *Angew. Chem.* **2014**, *126*, 1406–1409; b) M. Chang, M. Wang, Y. Chen, M. Shu, Y. Zhao, B. Ding, Z. Hou, J. Lin, *Nanoscale* **2019**, *11*, 10129–10136.

[8] a) J. J. Hu, N.-K. Wong, S. Ye, X. Chen, M.-Y. Lu, A. Q. Zhao, Y. Guo, A. C.-H. Ma, A. Y.-H. Leung, J. Shen, D. Yang, *J. Am. Chem. Soc.* **2015**, *137*, 6837–6843; b) X. Bai, Y. Huang, M. Lu, D. Yang, *Angew. Chem. Int. Ed.* **2017**, *56*, 12873–12877; *Angew. Chem.* **2017**, *129*, 13053–13057; c) L. Wang, H. Xu, J. Gao, J. Yao, Q. Zhang, *Coord. Chem. Rev.* **2019**, *398*, 213016; d) W. Ren, J. Gao, C. Lei, Y. Xie, Y. Cai, Q. Ni, J. Yao, *Chem. Eng. J.* **2018**, *349*, 766–774; e) Y. Wu, Y. Li, J. Gao, Q. Zhang, *SusMat* **2021**, *1*, 66–87.

[9] V. L. Villemagne, K. E. Pike, G. Chételat, K. A. Ellis, R. S. Mulligan, P. Bourgeat, U. Ackermann, G. Jones, C. Szoeke, O. Salvado, R. Martins, G. O'Keefe, C. A. Mathis, W. E. Klunk, D. Ames, C. L. Masters, C. C. Rowe, *Ann. Neurol.* **2011**, *69*, 181–192.

[10] a) H. Naiki, K. Higuchi, M. Hosokawa, T. Takeda, *Anal. Biochem.* **1989**, *177*, 244–249; b) H. Levine Iii, *Protein Sci.* **1993**, *2*, 404–410.

[11] a) A. J. Kettle, C. A. Gedye, C. C. Winterbourn, *Biochem. J.* **1997**, *321*, 503–508; b) A. J. Kettle, C. A. Gedye, M. B. Hampton, C. C. Winterbourn, *Biochem. J.* **1995**, *308*, 559–563.

[12] J. Yang, W. Zheng, Y. Shen, Y. Xu, G. Lv, C. Li, *J. Lumin.* **2020**, *226*, 117460.

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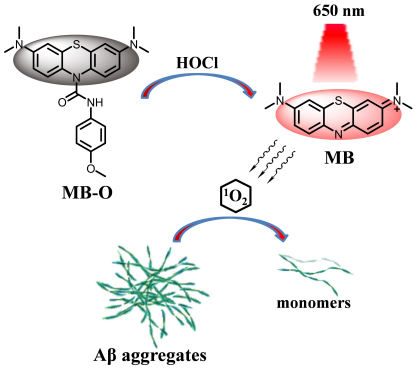

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