A facile preparation of FePt-loaded few-layer MoS₂ nanosheets nanocomposites (F-MoS₂-FePt NCs) and their application for colorimetric detection of H₂O₂ in living cells

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Abstract
Background: Rapid and sensitive detection of H₂O₂ especially endogenous H₂O₂ is of great importance for series of industries including disease diagnosis and therapy. In this work, uniform FePt nanoparticles are successfully anchored onto Few-layer molybdenum disulfide nanosheets (F-MoS₂ NSs). The powder X-ray diffraction, transmission electron microscopy, UV–Vis spectra and atomic force microscopy were employed to confirm the structure of the obtained nanocomposites (F-MoS₂-FePt NCs). The prepared nanocomposites show efficient peroxidase-like catalytic activities verified by catalyzing the peroxidation substrate 4,4′-diamino-3,3′,5,5′-tetramethylbiphenyl (TMB) with the existence of H₂O₂.

Results: The optimal conditions of the constructed colorimetric sensing platform is proved as 35 °C and pH 4.2. Under optimal catalytic conditions, the detection limit for H₂O₂ detection reaches 2.24 μM and the linear ranger is 8 μM to 300 μM. Furthermore, the proposed colorimetric sensing platform was successfully utilized to detect the intracellular H₂O₂ of cancer cells (MCF-7).

Conclusions: These findings indicated that the F-MoS₂-FePt-TMB-H₂O₂ system provides a potential sensing platform for hydrogen peroxide monitoring in living cells.

Keywords: Few-layer MoS₂ nanosheets, FePt, Colorimetric, H₂O₂, Intracellular H₂O₂

Background
Hydrogen peroxide (H₂O₂) takes an essential position in many biochemical reactions, such as metabolism of proteins and carbohydrates. Furthermore, it can be used as a significant indicator of the occurrence of many serious disease especially cancer [1, 2]. Consequently, a sensitive, cost-effective, rapid and easy operation method for H₂O₂ determination would be demanded for bioassays and environmental applications [3]. Up to now, several techniques for H₂O₂ determination, such as chromatography [4], chemiluminescence, electrochemistry [5, 6] and colorimetric method [7], have been reported. Among these techniques, colorimetric route has several outstanding advantages, including visibility, low cost, easy automation, portability and operation convenience [8]. Although enormous progresses have been made, sensitive and rapid detection of H₂O₂ still remains highly need. Recently, due to its high selectivity, many nanomaterials were employed to construct colorimetric sensors to detect H₂O₂.

Conventional enzymes are especially effective when catalyze series of reactions under mild conditions. However, conventional enzymes have rigorous limitations in practical use because they usually show insufficient

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stability in cruel conditions, additionally, they are hard to purify and preserve [9]. Therefore, over the past few decades, an explosion of interests have been drawn to study enzyme-mimic materials aiming to get high efficiency without the mentioned shortcomings. To date, versatile nanomaterials, such as CoS NPs [10], Fe3O4 NPs [11, 12], Copper nanoclusters [7], metal—organic framework [13], WS2 nanosheets [14], graphene oxide [15, 16], and kinds of metals [5, 17, 18] are used to fabricate nano-enzymes and exhibit effective catalytic activities suggesting prospective potentials in numerous bio-field, accompanied by series of advantages, including cost-effective, simple process, readily available raw materials, easy purification of products, low cost and long guarantee period [19, 20].

Molybdenum disulfide (MoS2), with a graphene-like lamellar structure, is composed of S–Mo–S sandwich structure and held by weak van der Waals forces. The few-layer MoS2 nanosheets (F-MoS2 NSs) with excellent 2D structure possess a direct bandgap of 1.8 eV, which is much higher than the indirect bandgap in bulk MoS2 NSs (1.2 eV) [21]. Hence, great efforts have been devoted to prepare few-layer MoS2 NSs and they are applied in sensing, catalysis, supercapacitors and so on [22–27]. Furthermore, based on its super-large specific surface areas and abundant active edges, MoS2 NSs have been utilized as base material to integrate with series of nanomaterials to further improve their catalytic performance [28].

A variety of monometallic nanoparticles (MNPs), such as Ag [29, 30], Pd [31], Pt [27], Au [32, 33] and Co NPs [34] have been successfully decorated on 2D MoS2 NSs. The obtained MoS2-MNPs can enhance their intrinsic properties. However, it is extremely difficult to further enhance the catalytic efficiency. Therefore, bimetallic nanoparticles (BNPs) were developed to improve the catalytic abilities [35–41].

Platinum-based BNPs have been widely used as sensing materials in non-enzymatic H2O2 sensing platforms and they show excellent electronic and catalytic properties. Until now, few attempts have been made to study the peroxidase-like catalytic ability of FePt NPs. Therefore, both MoS2 and FePt NPs are expected to be employed together for the development of colorimetric sensor for H2O2 detection. In this work, few-layer MoS2 NSs (F-MoS2) loaded uniformly FePt NPs are prepared and the catalytic activity of the obtained NCs are systematically studied. Scheme 1 illustrates the technical route to prepare the NCs and the method to detect H2O2.

**Results and discussion**

**Characterization of FePt nanoparticles, F-MoS2 NSs and F-MoS2-FePt NCs**

The way to prepare F-MoS2-FePt NCs is depicted in Scheme 1, while the experiments details are described in the experimental section. In this work, few-layer MoS2
NSs (F-MoS₂) are obtained by exfoliating bulk MoS₂ via lithium intercalation–exfoliation. To investigate the thickness of the as-prepared MoS₂ NSs, atomic force microscopy (AFM) is utilized to measure as-prepared MoS₂ NSs. As shown in Additional file 1: Figure S1, the altitude of as-prepared MoS₂ NSs is around 2 nm, implying as-prepared MoS₂ NSs have 2–3 layers [25, 42].

Then TEM is employed to characterize the obtained nanomaterials. As illustrated in Fig. 1A, FePt NPs show uniform spherical morphology and the diameter is 4 nm. The lattice fringes of as-prepared FePt NPs can be seen obviously in Fig. 1B, the adjacent fringe spacing is about 0.224 nm, corresponding to the (111) lattice planes of FePt [43, 44]. To equally disperse FePt NPs on the surface of F-MoS₂ NSs, the obtained FePt NPs are firstly transformed from the organic phase to aqueous phase via ligand exchange. The zeta potential of FePt-DMSA is −25 mV, indicating the successful modification of FePt NPs by dimercaptosuccinic acid (DMSA) (Additional file 1: Figure S2). After modified with DMSA, FePt NPs can be well-dispersed in water. Then, the thiolated FePt NPs could be easily anchored on the defect-rich edge sites of F-MoS₂ NSs. As shown in Fig. 1C, FePt NPs are successfully anchored onto the surface of F-MoS₂ NSs. Bulk MoS₂ NSs are thicker and more visible than the exfoliated F-MoS₂ NSs, as shown in Additional file 1: Figure S3. The lattice fringe spacing of the loaded nanoparticles is also 0.224 nm, similar to the monodispersed FePt NPs, as illustrated in Fig. 1D.

Powder X-ray diffraction is utilized to further confirm the crystalline structure of as-prepared F-MoS₂ NSs, FePt NPs and F-MoS₂-FePt NCs. The data of X-ray diffraction are displayed in Fig. 2a. The exfoliated F-MoS₂ NSs exhibit series of highlighted peaks in accordance with the reported ultrathin MoS₂ nanosheets [33]. The XRD spectra of FePt NPs show four peaks in accordance with the reported FePt NPs [45]. Nearly all peaks of FePt NPs and F-MoS₂ NSs are found in the XRD patterns of F-MoS₂-FePt NCs, shown in Fig. 2a. All data show that F-MoS₂-FePt NCs are successfully prepared.

**Fig. 1** TEM image of FePt NPs (A), HRTEM image of FePt NPs (B), TEM image of F-MoS₂-FePt (C) and HRTEM image of F-MoS₂-FePt (D)
Furthermore, UV–Vis spectra are employed to characterize the obtained nanomaterials. As presented in Fig. 2b, after modified with FePt NPs, the strong absorbance of the exfoliated F-MoS 2 NSs is covered by sufficient FePt NPs, which show no obvious absorbance band from 400 to 900 nm. These results demonstrate that FePt NPs is successfully anchored on the surface of F-MoS 2 NSs.

Peroxidase-like activity of the obtained FePt, F-MoS 2 NSs and F-MoS 2-FePt NCs

To investigate the peroxidase-like catalytic activities of F-MoS 2-FePt NCs, 4,4′-diamino-3,3′,5,5′-tetramethylbiphenyl (TMB) is selected as chromogenic substrate to induce the color reaction. As depicted in Fig. 3A, a prominent absorption peak of the oxidation products at 652 nm is observed, while the other three systems do not have any well-developed peaks ranging from 400 to 800 nm. As shown in the inset of Fig. 3A, in the presence of F-MoS 2-FePt NCs and H 2O 2, the TMB solution turns blue promptly. However, the TMB solution remains colorless in the absence of either H 2O 2 or F-MoS 2-FePt NCs. As illustrated in Fig. 3B, the absorbance of F-MoS 2-FePt/TMB/H 2O 2 at 652 nm climbs rapidly and maintains constantly within 100 s, indicating TMB could be oxidized rapidly, while the absorbance of the reference experiment remains unchanged. The results prove that the obtained F-MoS 2-FePt NCs possess efficient peroxidase-like catalytic activity.

UV spectrum is utilized to estimate the catalytic activities of F-MoS 2-FePt NCs, bulk MoS 2 NSs, F-MoS 2 NSs and FePt NPs. As illustrated in Fig. 3C, the absorbance of F-MoS 2-FePt NCs reaches the highest value among all the materials. Moreover, the absorbance of F-MoS 2 NSs is much higher than bulk MoS 2 NSs, which is attributed to the higher specific surface area and more exposed active sites. Furthermore, the time-dependent mode of the UV–Vis spectra at 652 nm for these materials is also investigated. As depicted in Fig. 3D, the UV spectra of F-MoS 2-FePt NCs at 652 nm reach the balance within 100 s and the highest value is obtained, which indicates the strong synergistic effect between F-MoS 2 NSs and FePt NPs [46].

Similar to other enzyme-mimic systems, temperature and pH play vital roles in the catalytic activities of F-MoS 2-FePt NCs. As depicted in Fig. 4a, the absorption at 652 nm keeps relatively high between 20 and 50 °C and reaches the maximum value at about 40 °C. Similarly, the influence of the pH of the TMB solution is also investigated as the pH varies from 2.2 to 8. As depicted in Fig. 4b, higher UV–Vis absorption is acquired when the TMB solution is kept weakly acidic, which indicates that the weakly acidic environment would be beneficial to the oxidation of TMB. However, when the solution is kept neutral or basic, the UV–Vis absorption is relatively low, which is mainly attributed to the reason that under basic solution, more OH − groups are absorbed on F-MoS 2-FePt NCs, occupying active sites of F-MoS 2-FePt NCs for the further reaction with H 2O 2 [47]. In summary, 35 °C and weakly acidic condition (pH = 4.2) are chosen as the optimum conditions.

Kinetic investigation of F-MoS 2-FePt NCs as peroxidase mimics

Under optimal conditions, TMB and H 2O 2 are chosen as the substrates to study the steady-state kinetic of the prepared F-MoS 2-FePt NCs. As illustrated in Fig. 5a, b, when TMB or H 2O 2 is catalyzed in certain concentration range, normative Michaelis-Menten curves are acquired.
Michaels-Menten constant \( (K_m) \), which represents the affinity between substrates and catalyst, and the initial reaction velocity \( (V_{max}) \) are reckoned from the L-B plot, the results are displayed in Fig. 5c, d. Based on the calculation, for the obtained F-MoS\(_2\)-FePt NCs, the \( K_m \) value is 0.2225 mM and the relevant \( V_{max} \) is \( 2.9458 \times 10^{-8} \) M s\(^{-1}\).
Correspondingly, the $K_m$ and $V_{\text{max}}$ values with TMB are 0.4283 mM and $1.7857 \times 10^{-8}$ M s$^{-1}$. Compared with other reported artificial enzymes and Horseradish peroxidase, the $K_m$ and $V_{\text{max}}$ are much smaller, which represents higher affinity between F-MoS$_2$-FePt NCs and the substrates (H$_2$O$_2$ and TMB) [40, 48–50].

**Catalytic mechanism**

Based on the prominent advantages such as cost-effective and high stability, p-Phthalic acid (TA) is applied to detect hydroxyl radicals (OH·) produced by the decomposition of H$_2$O$_2$. Fluorescence spectrometer is carried out to monitor the production generated from the combination of TA and hydroxide radical. As displayed in Fig. 6, as the amount of F-MoS$_2$-FePt NCs varies from 10 to 50 $\mu$g mL$^{-1}$, the fluorescence intensity decreases monotonically, which is caused by the reduction of hydroxide radical inhibited by high concentration of F-MoS$_2$-FePt NCs. According to the literatures, electron transfer mechanism is applied to explain these catalytic activities, as shows in Fig. 7 [51–53]. F-MoS$_2$-FePt NCs facilitate electrons shift between H$_2$O$_2$ and TMB. TMB can be easily absorbed on F-MoS$_2$-FePt NCs, this is because the higher affinity ($K_m$=0.4283) and donated lone-pair electrons from the amino groups cause the increase of the electron density and mobility on the NCs, as a result, the electron transfer to H$_2$O$_2$ is facilitated and the oxidation of TMB is speed up [54].

**Detection of H$_2$O$_2$ and sensing of the intracellular H$_2$O$_2$**

The determination of H$_2$O$_2$ is carried out under the optimal conditions using UV–Vis absorption spectra ranging from 250 nm to 800 nm. As illustrated in Fig. 8a, with the increasing of H$_2$O$_2$ the UV absorption at 652 nm of the colorimetric system increases gradually. More importantly, the absorbance is in proportion to H$_2$O$_2$ concentration, providing a linear detection range from 8 to 300 $\mu$M (Fig. 8b). The corresponding image of the linear detection range of H$_2$O$_2$ is shown in the inset of Fig. 8a, which indicates that as the concentration decreases from
300 to 8 μM, the color of these solution turns from dark blue to baby blue, the detection limit is reckoned to be 2.24 μM. When compared with other nanomaterial-based colorimetric sensing platforms the linear detection range of the constructed sensing platform (F-MoS2-FePt-TMB-H2O2) is more wide and a lower limit of detection is obtained, as listed in Additional file 1: Table S1 [48, 50, 55, 56].

To verify the feasibility of the detection of H2O2 in living cells, the obtained sensing platform is utilized to detect the intracellular H2O2. To improve the stability in the culture medium, the obtained NCs is modified with SH-PEG-FA. Then the established colorimetric sensing platform is further utilized to detect the intracellular H2O2 in MCF-7. After 4 h' co-incubation with F-MoS2-FePt-PEG-FA, 0.2 mM TMB and 100 μM H2O2 are added.
into the plate and incubated for another 40 min. After co-
incubation the cells are subjected to the electron micro-
scope and the images are shown in Fig. 8. Compared
with the cells only treated with NCs and TMB (Fig. 9A),
the MCF-7 cells treated with NCs, TMB and H$_2$O$_2$ turn
to clear blue (Fig. 9B). Without FA receptor on the cell
membrane of normal cell (L02), nearly none NCs can
be endocytosed (Additional file 1: Figure S5B). When
treated with TMB only, MCF-7 remains colorless, as
depicted in Additional file 1: Figure S5 A. The sensitive
and selective of intracellular detection indicates that the
F-MoS$_2$-FePt-PEG-FA have the potential for monitoring
of H$_2$O$_2$ in living cells.

**Conclusion**

In this work, a sensitive and rapid colorimetric sensing
platform for H$_2$O$_2$ detection utilizing F-MoS$_2$-FePt NCs
as artificial enzyme is constructed. The uniformly pre-
pared FePt NPs are anchored on the surface of exfoli-
ated few-layer MoS$_2$ NSs by a facile operation. Series of
experiments are carried out to verify the peroxidase-like
catalytic activity of the obtained NCs. Under optimal
conditions, the linear range of H$_2$O$_2$ detection is between
8 and 300 μM and the detection limit is 2.24 μM. Com-
pared with other reported methods, F-MoS$_2$-FePt NCs-
based colorimetric sensing platform for H$_2$O$_2$ detection
is a sensitive, simple and cost-effective method. To
improve the stability and transmembrane performance
of F-MoS$_2$-FePt NCs, the surface of the prepared NCs is
modified by SH-PEG-FA for intracellular H$_2$O$_2$ detection,
which indicates that the sensor could be applied in living

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**Fig. 8** a The absorption spectra of H$_2$O$_2$ with various concentration and the corresponding images are shown in the inset picture. These experiments conditions are shown as follows: 1.4 mL CPBS (pH 4.2), 200 μL TMB (1 mM), 200 μL F-MoS$_2$-FePt HNPs (20 μg mL$^{-1}$) and different amount of H$_2$O$_2$. b Dose–response curve for H$_2$O$_2$ determination

**Fig. 9** Images of MCF-7 incubated with F-MoS$_2$-FePt-PEG-FA NCs and TMB (A) F-MoS$_2$-FePt-PEG-FA and TMB (B)
cells testing and has potential in disease diagnosis and therapy.

**Additional file**

Additional file 1: Figure S1. The atomic force microscopy (AFM) images of the as-prepared Few-layers MoS2 nanosheets. Figure S2. The potential distribution of FePt-DMSA NPs. Figure S3. a TEM image of bulk MoS2 sheets; b HRTEM of MoS2-FePt. Figure S4. Image of FePt NPs before and after transferred from lipophilic to hydrophilic by DMSA via ligand exchange reaction. For the two phases, the upper layer is n-hexane, the lower is the water. Figure S5. a Images of MCF-7 cells incubated with TMB (1 mM) and b L02 cells incubated with F-MoS2-FePt-PEG-FA and TMB (1 mM). Table S1. Comparison of the linear range and the detection limit of H2O2 by means of different sensors.

**Authors’ contributions**

ZH performed experiments; ZD, XH, BY and QL drew the TOC, scheme and figures; YY wrote the paper with support from XZ. All authors contributed to the general discussion. All authors read and approved the final manuscript.

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**Acknowledgements**

Financial support from the National Natural Science Foundation of China (Grant Nos.: 21675073, S1872150), Primary Research and Development Plan of Shandong Province (2017GGX20115) and Shandong Province Natural Science Foundation (Nos.: ZR2017B0870, ZR2018MB034) are gratefully acknowledged.

**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

All data generated or analyzed during this study are included in the article and Additional file.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

Not applicable.

**Studies involving human participants, human data or human tissue**

Not applicable.

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Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 6 September 2018 Accepted: 11 February 2019 Published online: 13 March 2019

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