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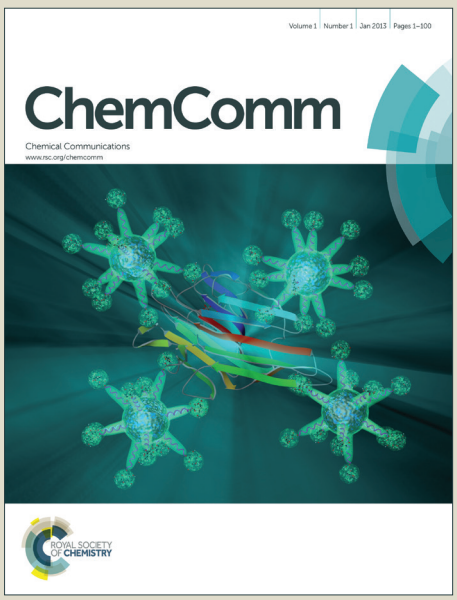

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ARTICLE TYPE

Synthesis of mixed valence state Ce-MOF as oxidase mimetics for colorimetric detection of biothiols

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We demonstrate that a facile and rapid *in situ* partial oxidation synthetic strategy to fabricate mixed valence state Ce-MOF (MVCM), which exhibits intrinsic oxidase-like activity. Furthermore, on the basis of the excellent catalytic activity of the MVCM, a colorimetric approach to high-throughput detects biothiols in serum samples was established.

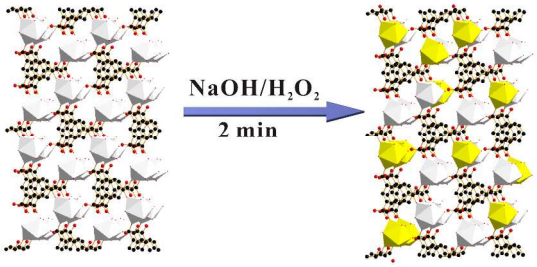
Colorimetric sensing has attracted much attention in the field of analytical chemistry due to the potential for direct visual readout. It has advantages of simplicity, rapidity, and cheapness as well as the fact that there is no requirement for any sophisticated instrumentation. As is well known that the color changes of detection event is the key challenge for colorimetric sensing.[1] Now, one of the more popular colorimetric sensing mainly focuses on enzyme catalytic chromogenic substrates such as 3,3',5,5'-tetramethylbenzidine (TMB), 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) and o-phenylenediamine (OPD). However, the nature enzyme inherent instability restricts its application. Therefore, development of artificial enzymes is necessary.

To date, artificial enzyme mimics have attracted special interest recently owing to the remarkable merits that they offer over natural enzymes, such as highly stable and low-cost.[2, 3] Since Gao *et al.*[4] reported that inert ferromagnetic nanoparticles possess the intrinsic horseradish peroxidase (HRP)-like activity, a great deal of excellent work about the enzyme mimics has been done, including carbon dots,[5] carbon nanotubes,[6] graphene oxide,[7] Pt-MoO_3 nanosheets,[8] gold nanoparticles,[9] Co_3O_4 nanoparticles,[10] and CeO_2 nanoparticles.[11] These enzyme mimics are certainly attractive, but most of them are unstable and prone to be aggregated and settled in aqueous solutions, which might affect their catalytic activity due to the decrease of specific surface area.[12] To address this issue, introduction of surface ligands are necessary, however, tedious surface modification have restricted the catalytic activity of these nanomaterials.

Very recently, metal-organic framework (MOF)—a new type of functional materials have attracted considerable attention in the field of enzyme mimics. In particular, Fe (III)-based MOF such as MIL-53, MIL-68, MIL-100 and MIL-88 have been reported to show intrinsic peroxidase-like catalytic activity.[13-15] Owing to their large surface areas, high stability and unsaturated metal sites, MOF artificial enzyme mimics exhibit excellent catalytic activity compared with the above mentioned nanomaterials. But that, unfortunately, only Fe-based MOF and HKUST-1[16] show intrinsic peroxidase-like catalytic activity. On the other hand, previous works[13-16] only focus on peroxidase-like catalytic activity. Thus, developing more types of MOF artificial enzymes still remains a major challenge.

To the best of our knowledge, there have not hitherto been any reports on pure MOF possess oxidase-like catalytic activity. In this communication, we developed a facile and rapid *in situ* partial oxidation synthetic strategy to fabricate mixed valence state Ce-MOF (MVCM), and demonstrated that the prepared MVCM has intrinsic oxidase-like catalytic ability for the first time. As a proof of concept, we investigated the oxidase-like property of MVCM by catalyzing the oxidation of TMB. Furthermore, based on the oxidase mimics catalytic ability, MVCM was used successfully for high-throughput colorimetric detection of biothiols in serum samples.

The MVCM was synthesized by *in situ* partial oxidation Ce-MOF in the presence of $NaOH/H_2O_2$ mixed solution (Scheme 1) (see detailed synthesis in the Supporting Information).



Scheme 1 Rapid synthesis of MVCM. Ce^{3+} white, Ce^{4+} yellow(shown as polyhedra), O red, C black.

Photograph (Fig.S1) showed that the white Ce-MOF translated into yellow MVMC after treatment with $NaOH/H_2O_2$, which is mainly due to a change in the oxidation state from Ce^{3+} to Ce^{4+}.[17] The high-resolution XPS spectra of Ce 3d (Fig.1) indicates the presence of a mixed valence state(Ce^{3+}/Ce^{4+}) in MVCM, and the ratio of Ce^{3+}/Ce^{4+} was found to be 2.9/1. The peaks at 881.6, 898.1, 900.2 and 916.1eV are associated to Ce^{4+}, while the peaks at 884.6 and 903.2 eV are associated to Ce^{3+}.[18] The crystalline structures of the as-prepared Ce-MOF and MVCM were analyzed by a powder XRD technique (Fig. 2). Results show that the as-obtained Ce-MOF was crystalline, which was coincident with previously reported in literature.[19] After partial oxidation,

MVCM still remains the crystalline structures of Ce-MOF. It has been proposed that the crystallinity and structure are two of the most important parameters toward the activity and stability of the enzyme mimics.[20] Therefore, good crystalline structures of MVCM is advantageous to catalytic activity. SEM investigations (Fig. S2 c and d) revealed that the as-prepared Ce-MOF is well defined aligned nanobars and has sharp edges. As can be seen from the micrographs of MVCM (Fig. S2 a and b) that the original morphology of the parent Ce-MOF is mimicked.

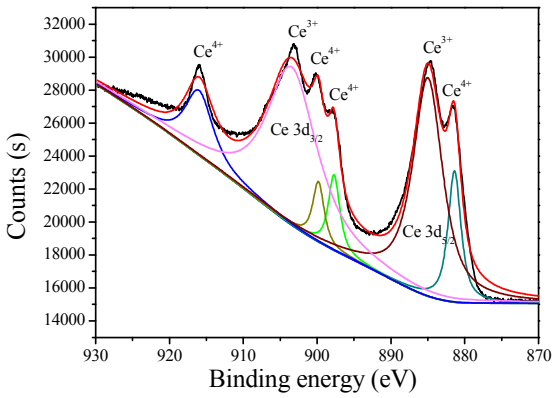


Fig. 1 XPS spectrum of as-prepared MVCM: high-resolution Ce 3d binding energy spectrum, showing the presence of both $Ce^{3+}$and Ce^{4+} valence states.

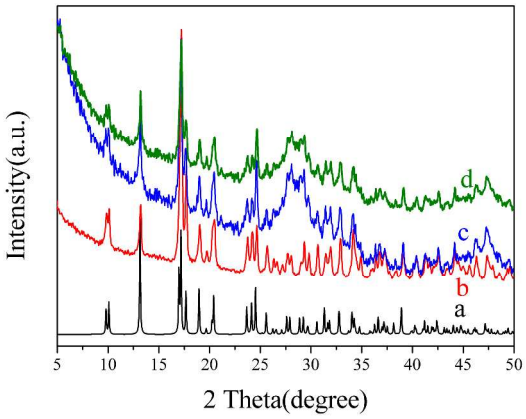


Fig. 2 XRD patterns of (a)simulated Ce-MOF, (b)as-synthesized Ce-MOF, (c)MVCM and (d) after catalytic reaction MVCM.

The FT-IR spectra (Fig. S3) of Ce-MOF and MVCM showed that they have similar functional groups features. The characteristic peaks at 1612-1557, 1435-1373 cm^{-1}, and 531cm^{-1} belong to the stretching vibrations $\nu_{assy(-COO-)}$ and $\nu_{sym(-COO-)}$ of the carboxylate ions, and the Ce-O stretching vibration, respectively.[21] The above result further indicated that the structure of Ce-MOF was not destroyed during the synthesis of MVCM. TGA curve (Fig. S4) exhibits two step major weight loss (adsorption water and organic ligand), and reveal that the MVCM is stable up to 500℃ (means that MVCM could work at high temperature, this feature is very useful in some catalyses (such as automotive exhaust catalytic)). Considering the results supported by XRD, SEM, XPS etc. we can conclude that the MVCM was successfully synthesized.

To investigate the intrinsic oxidase-like catalytic activity of MVCM, TMB as a typical chromogenic substrate was chosen (because TMB is a benign and noncarcinogenic color reagent).[22] As shown in Fig. 3, MVCM can quickly catalyze the oxidation of TMB without the need for additional oxidizing agents (e.g., H_2O_2), and a blue color appears in the solution. For comparison, the catalytic activity of Ce-MOF at the same conditions was also tested, but the solution was colorless, indicating that only Ce^{3+} Ce-MOF lack of catalytic oxidation capacity. Instead, MVCM shows the excellent intrinsic oxidase-like catalytic activity. The mechanism of the oxidase-like activity of MVCM toward TMB maybe that the MVCM possess Ce^{3+}/Ce^{4+} system which " spontaneously" recycle and can flip-flop between the two in a redox reaction. [23] Therefore, when TMB buffer was added into MVCM, Ce^{4+} quickly oxidize TMB to form blue oxTMB and itself chage into Ce^{3+}, and then Ce^{3+} "spontaneously" recycle to Ce^{4+} (Fig. S5). To investigate whether the Ce-MOF treatment with only NaOH or H_2O_2 also possess oxidase-like catalytic activity, contrast test was performed at the same conditions. As shown in Fig. S6, the oxidase-like catalytic activity of Ce-MOF treatment with only NaOH or H_2O_2 was poor, this maybe due to the lower +4/+3 ratios efficient in their oxidase mimetic activity.

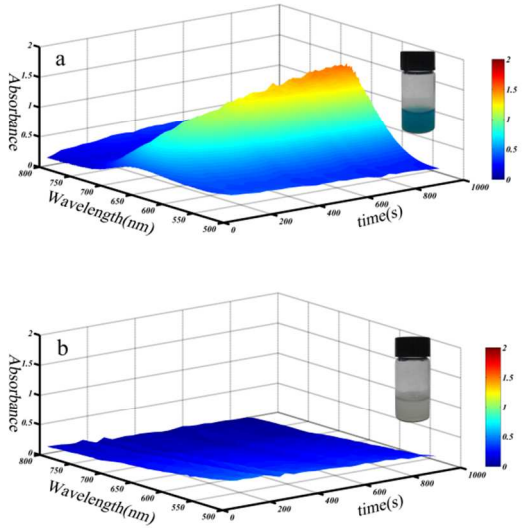


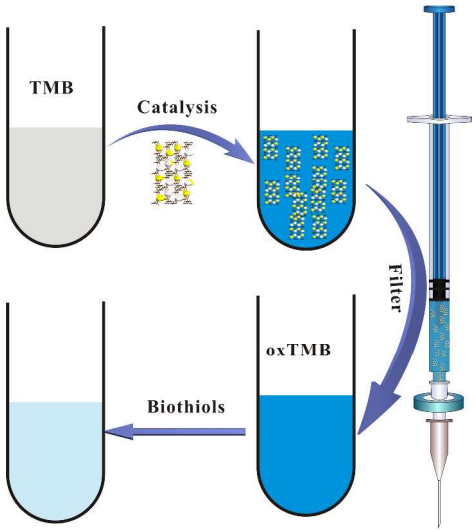
Fig. 3 Catalytic activity of MVCM (a) and Ce-MOF (b).(inset: the corresponding photographs)

In order to obtain the best performance of the MVCM, the synthetic conditions were evaluated (Fig. S7). Under the different synthesis conditions, the catalytic activities of the obtained MVCM at different Ce^{3+}/Ce^{4+} ratios were presented in Fig. S8. From Fig. S9, we can find that the catalytic activities of MVCM were related to the solution pH. It performs optimally oxidation activity at acidic pH values, and as the pH value of the buffered solution increases, the catalytic activity decreases. To further study the catalytic properties, the steady-state kinetics of MVCM for TMB was determined and a typical Michaelis-Menten curve was obtained (Fig. S10). Maximum initial velocity (Vmax) and Michaelis–Menten constant (K_m) were calculated using the Lineweaver–Burk equation. Based on Lineweaver–Burk plots, the K_m and V_{max} of MVCM were calculated to be 0.37 μM and 5.5 μM s^{-1}, respectively. Compared to the previous report[10] of CeO_2

with 3.8 μM and 0.7 μM s^{-1}, MVCM had higher affinity and activity for TMB, this maybe due to the large surface areas of MVCM and the π-π stacking interaction between MVCM and TMB. It means that the MVCM first binds and reacts with the TMB, then releases the product oxTMB. For an excellent artificial enzyme mimics the reusability is essential and the reusability test of the MVCM was investigated by conducting the experiment independently for 5 times, results show that MVCM is stable and recyclable (Fig. S11).

On the basis of the excellent catalytic activity of MVCM, a colorimetric approach to detect biothiols in serum samples was established. As illustrated in scheme 2, first, the MVCM was added to TMB in NaAc buffer to generate blue color oxTMB. The MVCM is then removed from the solution by filter to avoid disproportionation reaction. The obtained blue color oxTMB solution was stored in the dark at 4°C until use (the experimental result shows that blue color oxTMB was stable for at least 10 days, which is a requirement in high-throughput detection of samples). Then, biothiols or serum was added, blue color oxTMB transforms back into colorless TMB.

Base on quantified by reducing oxTMB, calibration curves and performance characteristics of the method for detecting GSH, Hcy and Cys were investigated. As shown in Fig. S12 and Table S1, good linear relationships and low detection limits were obtained for GSH, Hcy and Cys. In order to study the selectivity of the biothiols detection, the possible coexisting substances control experiments were taken under the same conditions of biothiols testing and the results were displayed in Fig. S13. As expected, these possible coexisting substances in human blood serum gave negligible responses compared to that of biothiols. The standard addition method was used to detect the level of biothiols in human blood serum (table S2), the recoveries for Cys measurement at three spiked samples ranged from 96.4 to 103.0%, RSD (n=3) values were less than 2.3%. All the characteristic parameters of the method validated that this colorimetric method can be used to detect biothiols in real samples.



Scheme 2 Schematic of the biothiols detection.

In summary, we have demonstrated the facile and rapid synthesis of MVCM, and explored its intrinsic oxidase-like catalytic activities. On the basis of the excellent catalytic activity, a simple and sensitive colorimetric method for the high-throughput determination of biothiols was developed. This work demonstrates that *in situ* partial oxidation synthetic strategy for fabricate MVCM artificial enzyme is an effective way. Therefore, we expected that MVCM holds great promise in sensors, antioxidant compounds screening and catalysts.

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Notes and references

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