INTRODUCTION

As Mother Nature’s marvelous creation, enzymes realize high catalytic efficiency in cells. This function is promising for ever-expanding applications in catalysis (1, 2), biosensors (3–6), drug delivery (7–9), molecular machines (10–14), etc., but the implementation is challenged by the reduced lifetime and efficiency of enzymes under general working conditions, which are far different from the cellular environment. One way to tackle these challenges is directed evolution of enzymes via altering the protein molecular structure (15). Another way is to design a biomimicking microenvironment to protect the enzymes so that their functionality can be retained in an unfavorable environment. As one important example of the latter strategy, metal-organic frameworks (MOFs), (16–18) composed of metal ions and organic linkers joined together, have porous structure and balanced rigidity and flexibility that resemble the cellular environment. Pioneering works have shown the promise of encapsulating enzyme in MOFs to allow better functionality under general conditions (19–23). Among them, the de novo approach such as coprecipitation and biomimetic mineralization is simply mixing the enzymes, metal ions, and organic ligands under ambient condition in bulk solution (24–33) to produce enzyme-encapsulated MOF composites. This approach is an efficient, straightforward, and scalable way to prepare MOF biocomposites that can protect enzyme and other fragile biomolecules such as nucleic acids (19–23, 25–33). The activity of enzyme-MOF composites synthesized via such coprecipitation method, however, is still far from satisfactory. For example, enzymes [horseradish peroxidase (HRP), lipase, catalase, etc.] embedded in zeolitic imidazolate frameworks such as ZIF-8 and ZIF-90 only displayed less than ~10% activity of their free counterparts (25, 30, 31). To improve the enzyme activity, it is urgent to investigate how enzymes, metal ions, and organic ligands interact with each other to form enzyme-MOF composites in the coprecipitation process. Unfortunately, this system is difficult to study in bulk solution, owing to the incapability of controlling the mixing process with sufficient spatial and temporal precision.

Microfluidics holds promise to address this challenge. Manipulating fluids at small scales (micrometers), microfluidics not only realizes effects predictable according to scaling law, such as enlarged surface-to-volume ratio and shortened diffusion distance (34), but also realizes new hydrodynamic characters such as laminar flow profile caused by low Reynolds number, in contrast to the turbulent flow nature in macroworld (35). These features allow precise manipulation of liquids with high resolution both spatially and temporally to obtain mixing conditions impossible in bulk synthesis (36, 37). Micro- and nanostructures have been synthesized in microfluidic systems (38, 39), including MOFs (40–42). Nevertheless, to our best knowledge, there has not been any report on the synthesis of enzyme-MOF composites on microfluidic chips, which is far more complicated than the formation of MOFs only.

In this work, we synthesized enzyme-MOF composites via a special mixing manner in microfluidic flow, which, to our surprise, generated products with markedly improved enzyme activity (Fig. 1). We used a three-way mixing scheme inside a microfluidic laminar flow system, which realized precisely controlled diffusive mixing condition and allowed one component to join the system certain seconds after (fig. S1). In this work, we synthesized enzyme-MOF composites via a special mixing manner in microfluidic flow, which, to our surprise, generated products with markedly improved enzyme activity (Fig. 1). We used a three-way mixing scheme inside a microfluidic laminar flow system, which realized precisely controlled diffusive mixing condition and allowed one component to join the system certain seconds after (fig. S1). We found that the continuously changed concentrations of MOF precursors in the gradient mixing on-chip resulted in structural defects in products. This defect-generating phenomenon enables multimodal pore size distribution in MOFs and therefore allows improved access of substrates to encapsulated enzymes while maintaining the protection to the enzymes. Thus, the as-produced enzyme-MOF composites showed much higher (~one order of magnitude) biological activity than those from conventional bulk solution synthesis. This work suggests that while microfluidic flow synthesis is currently underexplored, it is a promising strategy in producing highly active enzyme-MOF composites.

RESULTS AND DISCUSSION

Synthesis of enzyme-MOF composites in the microchannel

A double-Y–shaped microfluidic channel was used to synthesize enzyme-MOF composites. The second Y junction was designed for adding one type of reactant slightly (a few seconds, depending on flow rate) after the first two reactants met. We designed three
schemes of mixing (Fig. 2A). In scheme 1, zinc ions (Zn\(^{2+}\)) and 2-methylimidazole (2-MeIM) were mixed first, while in schemes 2 and 3, protein was introduced in the first mixing step. During each trial, the product was collected cumulatively from the outlet, followed by centrifuging and washing steps. For comparison, mixtures of reactants with the same feeding ratio as in microfluidic synthesis were added into a beaker and mixed through stirring (Fig. 1).

The low Reynolds number of the flows inside the microchannel makes the flows laminar. Thus, the mixing between reactants is stream-stream diffusion dependent, a precisely predictable fashion with a determinable concentration distribution alongside the microchannel, which is markedly different from the chaotic and uncontrollable mixing process in macroworld. This makes it possible to control the mixing in a reliable distance-to-time transformation. Through the calculations of velocity distribution (fig. S2) and concentration distribution (fig. S3) in the microchannel, it was found that when Zn\(^{2+}\) and 2-MeIM were injected from the first Y junction, they had enough time to mix before reaching protein molecules at the second Y junction (scheme 1 in fig. S4). Similarly, the mixing of Zn\(^{2+}\) with protein and that of 2-MeIM with protein were also completed before adding the third component (schemes 2 and 3 in fig. S4). In this microchannel, we synthesized cytochrome c (Cyt c)–MOF composites in three representative mixing manners (fig. S6). For comparison, we synthesized Cyt c–MOF composites in bulk solution as well.

Figure 2 (A to C) show bright-field images of microchannels captured during the reactions. Deposition (indicated by arrows) occurred at the bottom of the channel, indicating an interface in the laminar flow. A second black line (indicated by dashed ovals) appeared at the expected interface between the two liquid streams of protein and 2-MeIM (see Fig. 2, A and C), but no deposition left in the channel after reaction. This interesting observation validated a possible mechanism of the formation of protein–MOF composites proposed in a previous study, suggesting that protein molecules adsorb 2-MeIM molecules first and then induce the biomimetic mineralization of MOF skeletons around protein molecules (26).

Structural features of enzyme-MOF composites induced by gradient mixing

To study the fundamental reason of the markedly increased activity of enzyme-MOF composites from gradient mixing, we synthesized GOx-MOF composites prepared by scheme 1 of microfluidic flow synthesis. Further analysis showed that the XRD peaks of microfluidic synthesized enzymes-MOFs shifted to the low angle (fig. S9), indicating the increased lattice constant, which is probably due to the defects in composites.

Gradient mixing for the synthesis of enzyme-MOF composites

To investigate the difference between our microfluidic flow synthesis and the bulk solution synthesis, it is crucial to understand the difference in mixing conditions first. Numerical calculations on the ratio of molar concentration between 2-MeIM and Zn\(^{2+}\) (see sections S3 and S13 for details) suggest that despite the feeding molar ratio of 40, owing to the laminar flow mixing, the ratio between 2-MeIM and Zn\(^{2+}\) at the interface where protein molecules contacted MOF precursors quickly climbed from 50 to 100 at the very beginning stage (within 0.5 mm after the third inlet), followed by a continuous decrease to approximately 80 in mixing process of scheme 1 (Fig. 3, A and B). Similar but mild changes of ratio of reactants were also observed for schemes 2 and 3 (Fig. 3, A and B). This continuously changed molar ratio between 2-MeIM and Zn\(^{2+}\) in microfluidic synthesis was much different from the case in bulk solution synthesis. As shown in Fig. 3A, we labeled in orange color the range of reactant molar ratio (around 25 to 40) that are common in bulk solution synthesis (43) and found that the ratio of reactants in the three schemes were beyond the common range, especially in the case of scheme 1.

To further investigate whether the special mixing manner in microfluidic synthesis would change the performance of the products, we prepared glucose oxidase (GOx)–MOF composites using scheme 1 of microfluidic flow synthesis (SEM images in fig. S10). The GOx-MOF composites prepared in scheme 1 had a weight percentage of GOx loading at 8.45% (fig. S11) and showed ~98% activity of native GOx (Fig. 3C). This, to the best of our knowledge, is the highest record of enzymatic activity of enzyme-MOF composites prepared by the coprecipitation process (24–33).

To validate whether the high activity was because of the relatively high molar ratios between 2-MeIM and Zn\(^{2+}\), we synthesized GOx-MOF composites in bulk solution at ratios between 2-MeIM and Zn\(^{2+}\) ranging from 50 to 100. All the products showed less than 15% of the activity of native GOx. This result suggested that the continuous change of ratio (for example, ranging from 50 to 100 with an average gradient of 2.0 × 10\(^{-3}\)/m along the channel in scheme 1), rather than the high ratio (for example, ratio of 80 to 100), enabled the high catalytic activity.

Fig. 1. Schematics of microfluidic laminar flow synthesis and bulk solution synthesis of enzyme-MOF composites. (A) Synthesis of enzyme-MOF composites in a microfluidic laminar flow. Zinc nitrate, 2-MeIM, and enzymes were introduced into the three inlets; enzyme-MOF composites were formed in the microchannel via diffusion-based gradient mixing. (B) Synthesis of enzyme-MOF composites in bulk solution. The reactants were added into the beaker and mixed through stirring.

The TGA, bicinchoninic acid colorimetric protein assay, and inductively coupled plasma mass spectrometry results suggest that the weight percentage of loading for Cyt c in MOFs is around 4%. It is worth mentioning that both the morphology under electron microscope (SEM) and TEM (transmission electron microscope); Fig. 2, D to F, and fig. S8) and the powder x-ray diffraction (XRD) patterns of Cyt c–MOF composites (Fig. 2H) indicate a reduced crystallinity of the microfluidic-prepared enzyme-MOF composites, compared to the composites and pure MOFs obtained in bulk solution synthesis. Further analysis showed that the XRD peaks of microfluidic synthesized enzyme-MOFs shifted to the low angle (fig. S9), indicating the increased lattice constant, which is probably due to the defects in composites.
were characterized with extended x-ray absorption fine structure (EXAFS) (Fig. 4A). The short-range local structures around Zn atoms illustrated by R-space information were obtained through Fourier transformation. For the peaks at 1.9 Å, which correspond to the coordination of Zn and N atoms, a good agreement was observed between the samples prepared by microfluidic flow synthesis and those from bulk solution synthesis (Fig. 4A), suggesting that the Zn-N coordination also mainly presented in the microfluidic flow–synthesized MOFs. Furthermore, the fitted R-space EXAFS data showed that the normalized apparent local coordination number of the Zn atoms in microfluidic flow–synthesized MOFs turned out to be 4.286, higher than that from bulk solution synthesis, which was normalized as 4 (fig. S15 and table S2). The higher apparent coordination number of Zn atoms suggested that in the GOx-MOF composites prepared by microfluidic synthesis, a smaller number of Zn atoms presented in the frameworks and interacted with N atoms. This loss of Zn atoms hence caused coordination defects.

The defects caused by the loss of Zn atoms were also confirmed by the results of inductively coupled plasma optical emission spectrometry (ICP-OES) and elemental analysis. The ratios of nitrogen to zinc in control samples (GOx-MOF composites from bulk solution synthesis at the ratios between 2-MeIM and Zn$^{2+}$ from 40 to 100) did not change obviously (from 3.98 to 4.26) (table S3), compared to that of the product prepared with the commonly used ratio of 40 (4.41). In contrast, the GOx-MOF composite prepared by microfluidic flow synthesis has a statistically higher ratio of nitrogen to zinc (4.41) according to Student’s $t$ test, also proving the loss of Zn atoms in the frameworks. These results are in accordance with low-angle shift of XRD peaks, which was caused by the loss of zinc atoms, resulting in a larger lattice constant.

Such defects led to change in porosity of the products. In the results of $N_2$-sorption isotherm, the pore size distribution calculated by density functional theory (DFT) displayed only micropores (~1 nm) presented in control samples as mentioned earlier (table S4), which is similar to the standard MOFs prepared at the ratio of 40. The GOx-MOF composites prepared by microfluidic flow synthesis, however, have a wide pore distribution from 1 to 6 nm (Fig. 4B). It is highly possible that the mesopores are caused by the coordination defects. The above results suggest that the continuously changed ratio of 2-MeIM to Zn$^{2+}$ in the laminar flow of microfluidic synthesis caused the formation of defects in the composites. These defects created mesopores ($44, 45$) in the composites, which, in turn, facilitated the substrate transportation and improved the apparent enzyme activity. This is demonstrated by the data showing that the substrates are smaller than the mesopores (fig. S22) and, more importantly, by numerical calculation (fig. S23) showing that the substrate diffusion rate largely increased with the introduction of mesopores. This spontaneous process can serve as a general method to introduce mesopores in MOF particles, which is more convenient compared to existing methods and better protects the biomolecules during the
synthesis. Existing methods for generating mesopores in MOFs usually use templating strategies (46, 47), which are multistep processes that require calcination or chemical reaction to remove the template, and thus are complicated or may damage the activity of enzymes. Physically induced defects in MOF structures were also reported in a work involving temperature variations, where the gas mixture separation performance was improved on an MOF membrane (48). However, the method in the current work is more favorable to enzyme immobilization because of its mild condition.

To test the generality of this method, we prepared enzyme-MOF composites using another enzyme, HRP (SEM images in fig. S10). The HRP-MOF composites prepared in scheme 1 had a weight percentage of HRP loading at 4.71% (fig. S11) and had ~63% activity, compared to native HRP, which was much higher than that from bulk solution synthesis (Fig. 4D). Moreover, at an optimum flow rate (1 μl min⁻¹) (fig. S17), the HRP-MOF and GOx-MOF composites prepared with scheme 1 displayed higher activity than those from schemes 2 and 3 (Fig. 4, C and D). In addition to more pronounced gradient mixing in scheme 1 (Fig. 3A), an additional plausible reason is that in scheme 1, enzyme molecules were encapsulated during the growth of MOF nanocrystals and thus close to the surface of particles, allowing better access to substrate molecules. This hypothesis has been validated by replacing enzyme with ~5-nm-diameter gold nanoparticles in the synthesis, which resulted in a higher density of gold dots presented close to the surface of MOF particles (fig. S18). Meanwhile, the stability of enzyme-MOF composites prepared on-chip against high temperatures and protease digestion was also superior compared to their natural counterparts (Fig. 4, E and F), similar to those prepared by bulk solution synthesis. Enhanced reusability and storage stability were also observed. After five times of reuse, the enzyme-MOF–prepared on-chip still retained 73% activity, similar to those prepared in bulk solution (fig. S20); 15 days storage at room temperature did not affect the enzymatic activity of the microfluidic synthesized composites, similar to those prepared in bulk solution (fig. S19). In contrast, the control sample of enzyme adsorbed on bare MOF particles displayed poor stabilities against protease digestion (Fig. 4E) and reuse (fig. S20). All the above results demonstrated that the enzyme molecules were embedded in MOF particles.

Therefore, in conclusion, the increased activity of microfluidic synthesized composites is mainly due to the defects in MOFs, which facilitated substrate diffusion. At the same time, while all the composites prepared by microfluidic synthesis showed remarkably increased activity compared to bulk solution synthesis, the composites synthesized with different mixing schemes by microfluidics also displayed some differences in activity, probably caused by the different locations of enzyme in MOFs.

Meanwhile, the defect property could be controlled by adjusting the flow rate of the reactants into the microchannel. When the flow rate of scheme 1 was increased to 5 and 20 times of the original flow rate, the pore width calculated by DFT increased from 3 to 6 nm to

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**Fig. 3. Investigation on the gradient mixing for the synthesis of enzyme-MOF composites in the microchannel.**

(A) Molar ratio between 2-MeIM and Zn²⁺ at the positions where the enzyme has a maximum probability of diffusion into the growing MOF crystals alongside the microchannel in three mixing schemes. The area in orange color indicates the common range of concentration ratios in bulk solution synthesis of enzyme-MOF composites. (B) Simulation results (shown as heat map) of the molar ratio between 2-MeIM and Zn²⁺ alongside the microchannel where all three reactants meet up. The dashed lines alongside the microchannel reflect the position where the enzyme has a maximum probability of diffusion into the growing MOF crystals. (C) Relative activities of native glucose oxidase (GOx) and GOx-MOFs synthesized in microchannel with mixing scheme 1 and GOx-MOFs synthesized in bulk solution with ratios of 2-MeIM to zinc nitrate ranging from 50 to 100, respectively.
4 to 10 nm (five times flow rate) and 7 to 12 nm (20 times flow rate) (the pore size distribution is shown in fig. S21), and the pore volume also showed some growth (0.173 m³ g⁻¹ for the original flow rate, 0.322 m³ g⁻¹ for five times flow rate, and 0.281 m³ g⁻¹ for 20 times flow rate). Under all these tested conditions, the flow inside the microchannel is still laminar according to the calculation of Reynolds number (2.16 for five times flow rate and 8.64 for 20 times flow rate). Nevertheless, compared with the original condition of scheme 1, the average gradient in the accelerated flow has increased significantly (2.0 × 10⁴/m for the gradient of the original flow rate of scheme 1; 1.0 × 10⁵/m for five times flow rate; 5.4 × 10⁶/m for 20 times flow rate) (fig. S5), which could have contributed to the increased pore width and pore volume. This result suggests that the degree of defects could be controlled to a suitable point when using the microfluidic laminar flow synthesis method.

In summary, we synthesized enzyme-MOF composites in a microfluidic laminar flow diffusive gradient mixing environment, of which the reaction condition is difficult to be realized in conventional bulk solution synthesis. Under a continuously changed ratio of reactants along the microchannel, the resulted products showed coordination defects and therefore mesopores. This multimodal distribution of pore sizes ensured the immobilization of enzymes and, in the meantime, reduced resistance to mass transfer, which is believed to be responsible for nearly an order-of-magnitude higher enzymatic activity than that of the enzyme-MOF composites prepared from conventional bulk solution synthesis. This defect-assisted synthesis suggested a new strategy to enhance the activity of enzyme-MOF composites. Compared to existing strategies for generating mesopores that usually use templates and are thus complicated or may damage enzyme activity during template removal processes, our method generated the mesopores spontaneously in a single step without using any template. The unique gradient mixing nature of microfluidic laminar flow synthesis strategy allows controllable, continuous, and fast synthesis of enzyme-MOF composites with improved activity, which we expect to become a new general approach for inducing defects into the enzyme-MOF composites and other biocomposites.

**MATERIALS AND METHODS**

**Materials**

2-MeIM, GOx, glucose, trypsin, 2,2’-azinobis(2-ethylbenzothiazoline-6-sulfonate) (ABTS), and gold nanoparticles [optical density (OD) 1, Au NPs (nanoparticles) suspended in 0.01% tannic acid with 0.04% trisodium citrate] were purchased from Sigma-Aldrich. Zinc nitrate hexahydrate and hydrogen peroxide (H₂O₂) were purchased from Alfa Aesar. Cyt c from equine heart and HRP were obtained from Hoffmann-La Roche. Other chemicals are all of analytical grade and were used as received without further purification.

**Microfluidic flow synthesis of enzyme-MOF composites**

Zinc nitrate (9.3 mg ml⁻¹), 2-MeIM (102.5 mg ml⁻¹), and enzyme (1 mg ml⁻¹) water solutions were introduced into the inlets of the microfluidic chip by a syringe pump, with a flow rate of 0.5 μl min⁻¹ (74900 Cole-Parmer, USA) at room temperature. Three trials corresponding to three mixing schemes were studied on a double-Y-shaped microfluidic chip. In scheme 1, enzyme molecules were added
into the main channel from the side channel after organic ligands and metal ions were mixed. For schemes 2 and 3, enzyme molecules were first mixed with metal ions and organic ligands, respectively, followed by adding organic ligands and metal ions from the side channel. The product was collected from the outlet continuously by centrifuging at 6000 rpm for 10 min (Neofuge 13/R, Heal Force, Life Science Instruments, Shanghai, China) and washed three times with deionized (DI) water. Last, to obtain powder of enzyme-MOF composites, the as-prepared product was lyophilized overnight.

**Bulk solution synthesis of enzyme-MOF composites**

First, 0.372 g of Zn(NO₃)₂·6H₂O was dissolved in 4 ml of DI water in a glass beaker under stirring. Then, 4.1 g of 2-MeIM was dissolved in another 40 ml of DI water. Both solutions were incubated in an ultrasonic bath for 5 min to obtain a homogeneous mixture. The zinc nitrate solution was mixed with the 2-MeIM solution under stirring, followed by the immediate addition of 4 ml of water solution containing Cyt c/HRP/GOx (4.5 mg ml⁻¹) and stirring the mixture at 500 rpm for 30 min at room temperature. Next, the mixture was centrifuged at 6000 rpm for 10 min at 4°C to recover the product, and the product was washed three times with DI water. Last, the product was lyophilized overnight. Note that since the growth of ZIF-8 crystals is very fast, the enzyme solution needs to be poured into the mixture immediately once the metal ions and organic ligands were mixed.

**Determination of enzyme loading amounts in MOFs**

The loading amount of enzyme in composite was determined by TGA. In TGA experiment, 3 to 5 mg of samples was heated from room temperature to 600°C at a rate of 20°C min⁻¹ under air atmosphere. Compared with pure ZIF-8 MOFs, there was a weight loss occurring during the second stage attributing to the decomposition of enzyme. The loading amount of enzyme can be calculated using the weight loss before 400°C.

The concentration of unincorporated enzyme in the supernatant after synthesis of enzyme-MOF composites was measured by the size exclusion chromatography (SEC). For the SEC experiment, 20 μl of sample solution was injected and eluted using phosphate-buffered saline (PBS) (0.1 M) containing 0.1 M Na₂SO₄, 0.1% NaN₃ at an elution speed of 1 ml min⁻¹. The detection of protein was carried out by an ultraviolet–visible (UV-Vis) absorbance at 280 nm on a Shimadzu HPLC system, which was equipped with a TSK-GEL G2000SWXL column. The loading amount of enzyme in the composites was calculated by comparing the amount of unencapsulated enzyme with the total amount of free enzyme added into the reaction system in synthesis.

**UV analysis**

UV analysis was performed on a UV-Vis spectrophotometer (Cary 100, Agilent Technologies, USA). The relative activity of enzyme-MOF composites was calculated by comparing the slope with that of free native enzyme. All the enzymatic assays were carried out at the same protein concentration, with the activity of free enzyme in solution as 100%.

**Stability of enzyme-MOF composites at high temperature and in the presence of protease digestion**

For thermal stability test of free enzyme and enzyme-MOF composites, HRP (50 μg ml⁻¹) and HRP-MOF composite (2 mg ml⁻¹) water solutions were incubated at 60° and 70°C, respectively, for different time periods. Then, the increase of absorbance at 415 nm for 2 min was recorded at different time points immediately after adding 50 μl of HRP or HRP-MOF composites to the mixture solutions of 50 μl of ABTS (0.5 mM), 50 μl of H₂O₂ (0.3%, v/v), and 350 μl of PBS [50 mM (pH 7.2)]. The relative activity of HRP or HRP-MOF composites was calculated by comparing the relative activity with that of initial relative activity.

For trypsin digestion test, native HRP or HRP-MOF composites (with the same protein concentration at 50 μg ml⁻¹) was mixed with equal volume of trypsin solution (50 mg ml⁻¹) and incubated at 37°C in 50 mM PBS (pH 7.2) for 1, 2, 4, 8, and 24 hours. The enzyme activity was calculated from the slope of absorbance at 415 nm versus time curve after adding 50 μl of HRP or HRP-MOF composites to the mixture solutions of 50 μl of ABTS (0.5 mM), 50 μl of H₂O₂ (0.3%, v/v), and 350 μl of PBS [50 mM (pH 7.2)]. The relative activity of HRP or HRP-MOF composites was calculated by comparing the relative activity with that of initial relative activity. UV analysis was performed on a UV-Vis spectrophotometer (Cary 100, Agilent, USA) at room temperature.

**SEM analysis of pure MOFs and enzyme-MOF composites**

For the SEM analysis of pure MOFs and enzyme-MOF composites, samples were prepared by first suspending the composites in methanol and then dropped 1 to 5 μl of the sample solution onto a small piece of clean silica wafer (around 10 mm x 10 mm). After all methanol was evaporated, the silica wafer was then put to a carbon paste and sputter-coated it with a thin layer of conductive gold for 50 s to improve the electrical conductivity. The LEO 1530 Field Emission SEM was used for collecting images with an accelerating voltage of 15 kV.

**XAFS spectra of bulk solution and microfluidic flow synthesized ZIF-8 crystal**

The X-ray absorption data at the Zn K-edge of the samples were recorded at room temperature in transmission mode using ion chambers at beamline BL14W1 of the Shanghai Synchrotron Radiation Facility, China. The station was operated with a Si (211) double crystal monochromator. During the measurement, the synchrotron was operated at an energy of 3.5 GeV and a current between 150 and 210 mA. The photon energy was calibrated with the first inflection point of Zn K-edge in Zn metal foil.
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