## One-pot and one-step synthesis of bioactive Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites and their application in detection of urea

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## **Experimental section**

## **Reagents and Materials**

Zinc chloride (ZnCl<sub>2</sub>), Ferrous chloride (FeCl<sub>2</sub>•4H<sub>2</sub>O) and urea were purchased from Xilong Chemical Co., LTD. Urease was purchased from J&K Scientific LTD. Bromcresol purple was purchased from Institute of Tianjin Jinke Fine Chemical Industry. Aqua ammonia was commercially available and analytical grade. All the reagents used in this work were analytical purity without further purification. Deionized water was used in all procedure.

## **Characterization**

The samples were characterized via X-ray diffraction (XRD) on a D8 Focus X-ray diffractometer with a scanning rate of 0.02 °/s. The morphologies of the samples were examined via transmission electron microscopy (TEM, JEM-2100, JEOL) and scanning electron microscopy (SEM, Models 4300 and 4800, Hitachi). The magnetic hysteresis loops of the samples were recorded on a physics property measurement system (PPMS-9 Quantum Design Co.). X-ray photoelectron spectra (XPS) was used to study the surface chemical compositions and the valence states of the nanocomposites. Ultraviolet and visible spectrophotometer (UV-Vis) was used to analysis the catalytic results of urease/ZnFe<sub>2</sub>O<sub>4</sub>.



Fig. S1 (a) UV-Vis spectrophotometer spectra of the catalytic results catalyzed by pure ZnFe<sub>2</sub>O<sub>4</sub> and Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites and (b) UV-Vis spectrophotometer spectra of pure urease and Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites.

To demonstrate the proof of composite results of urease and  $ZnFe_2O_4$  nanoparticles, the catalytic activities of the nanoparticles are compared as Figure S1a, showing that the catalytic activity of Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites obtained by urease is obviously higher than the pure ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles. This result mainly owes to the existence of urease.

The UV-Vis spectrophotometer spectra of pure urease and Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites were also measured to further prove the combination of urease and ZnFe<sub>2</sub>O<sub>4</sub> nanoparticles and the consequence is shown in Figure S1b. From the spectrum, a sharp and narrow peak of urease can be found at about 195 nm, and for Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites, the peak can also be found at the same peak position. The consequence of Figure S1 can demonstrate that urease has been well combined with ZnFe<sub>2</sub>O<sub>4</sub> to obtaine Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites.



Fig. S2 (a) UV-Vis spectrophotometer spectra of the catalytic results catalyzed by Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites after bathed in different buffer solutions ranging from 7 to 11, (b) the tolerance properties to pH of Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites.

To evaluate the tolerance to pH, the below experiment was designed: equivalent amount of Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites were bathed in different buffer solutions ranging from 7 to 11 for an hour. Subsequently, wash the samples with deionized water to remove redundant buffer solutions. The detection procedure was repeated to compare the difference of catalytic ability. The UV-Vis spectra show that as the increase of pH value, the peak values show a slight change (Figure S2a). Figure S2b shows the tolerance properties to pH of Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites based on Figure S2a. With the increase of pH value, the catalytic ability of samples remains relatively constant. Namely that the Urease/ZnFe2O4 nanocomposites possess well tolerance properties to pH. Because our detection is based on the decomposition of urea and the system is alkalescent, the tolerance to pH is mainly designed buffer in alkaline solutions ranging from 7 to 11.



Fig. S3 (a) UV-Vis spectrophotometer spectra of the catalytic results catalyzed by Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites after air-dried for different time ranging from 0 to 48h, (b) the tolerance properties to air-dry of Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites.

To evaluate the tolerance to air-dry, the below experiment was designed: equivalent amount of Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites were air-dried for different time ranging from 0 to 48 h. Subsequently, the detection procedure was repeated to compare the difference of catalytic ability. The UV-Vis spectra show that as the extending of time, the peak values show a slight decrease (Figure S3a). Figure S3b shows the tolerance properties to air-dry of Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites based on Figure S3a. With the extending of time, the catalytic ability of samples decreases slightly. Yet after air-dried for 48 h, the catalytic ability can remain nearly 85%. Namely that the Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites possess well tolerance properties to air-dry.

Figure S2 and Figure S3 all confirm the Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites can possess rather stability in complex and harsh environment, indicating that the Urease/ZnFe<sub>2</sub>O<sub>4</sub> nanocomposites can be used in many complex environments to achieve the detection of urea.