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# A simple route to $\text{CoFe}_2\text{O}_4$ nanoparticles with shape and size control and their tunable peroxidase-like activity†

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Recent studies have suggested that the physical and chemical properties of nanoparticles (NPs) strongly depend on local chemical composition, size, and shape. Here, we report a new precursor-mediated growth of monodisperse magnetic cobalt ferrite ( $\text{CoFe}_2\text{O}_4$ ) NPs with controlled size and shape.  $\text{CoFe}_2\text{O}_4$  NPs with near corner-grown cubic, near cubic and polyhedron shape can be successfully prepared by simply tuning the amount of iron and cobalt acetylacetonates in oleic acid. Interestingly, the product shape varies from near corner-grown cubic to starlike by only changing the reaction temperature from 320 °C to 330 °C. These  $\text{CoFe}_2\text{O}_4$  NPs exhibit size and shape-dependent peroxidase-like activity towards 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of  $\text{H}_2\text{O}_2$ , and thus exhibited different levels of peroxidase-like activities, in the order of spherical > near corner-grown cubic > starlike > near cubic > polyhedron; this order was closely related to their particle size and crystal morphology.  $\text{CoFe}_2\text{O}_4$  NPs exhibited high stability in HAc–NaAc buffer (pH = 4.0) and high activity over a broad pH (2.5–6.0). Furthermore, the Michaelis constants  $K_m$  value for the  $\text{CoFe}_2\text{O}_4$  NPs (0.006 mM) with TMB as the substrate was lower than HRP (0.062 mM) and  $\text{Fe}_3\text{O}_4$  NPs (0.010 mM). After further surface functionalization with folic acid (FA), the folate-conjugated  $\text{CoFe}_2\text{O}_4$  nanoparticles allow discrimination of HeLa cells (folate receptor overexpression) from NIH-3T3 cells (without folate receptor expression). Such investigation is of great significance for peroxidase nanomimetics with enhanced activity and utilization.

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## 1. Introduction

The ability to synthesize monodisperse magnetic nanoparticles (MNPs) with desired sizes and shapes is of great importance in exploring their applications in catalysis, sensors, microelectronics, and many other areas of nanotechnology.<sup>1</sup>  $\text{CoFe}_2\text{O}_4$  ferrite (CF), which is a well-known inverse spinel with  $\text{Co}^{2+}$  ions on B sites and  $\text{Fe}^{3+}$  ions distributed equally among A and B sites,<sup>2</sup> has attracted considerable attention for its potential applications such as in catalysis, as an anode material for lithium ion batteries, in biomedicine, and as enzymatic mimetics.<sup>3–6</sup> The properties can be additionally tuned by controlling the shape, size, and crystallinity of the nanocrystals. Various synthetic strategies have been devoted to preparing  $\text{CoFe}_2\text{O}_4$  nanoparticles with controlled size and shape. A range of methods, including coprecipitation, sol-gel methods,

thermal decomposition, combustion reaction, and hydrothermal synthesis.<sup>7–11</sup> Among chemical methods, thermal decomposition of mixed organic  $\text{Co}^{2+}$  and  $\text{Fe}^{3+}$  compounds, such as metal acetylacetonates, metal carbonyls, *etc.*, in high boiling point solvents has proven to be an ideal approach for preparing monodisperse  $\text{CoFe}_2\text{O}_4$  nanocrystals with high yield, narrow size distribution, and good crystallinity.<sup>12</sup> As we well known, the shape and size of the NPs using this method were controlled by adjusting the surfactant to precursor ratio, heating rate, stirring, and seed mediated growth during the reaction.<sup>13</sup> Despite this progress, these control methods require precise process management of environmental conditions or necessitate tedious multistep reaction that are not necessarily amenable to large scale NP synthesis. Thus, exploring facile and effective synthesis method is still a challenging task for the controlled synthesis of  $\text{CoFe}_2\text{O}_4$  NPs with desirable size, shape, and composition.

Exploitation of new functions of known nanomaterials is one of the most attractive aspects in nanoscience. Since magnetite nanoparticles were serendipitously discovered to have intrinsic peroxidase-like activity, they have been pursued as peroxidase nanomimetics to catalyze and detect some molecules.<sup>14</sup> In addition,  $\text{CoFe}_2\text{O}_4$  NPs have been found to possess intrinsic

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oxidase-like activity.<sup>15</sup> However, to date, very little has been explored on the effect of the shape and size on the catalytic properties of peroxidase nanomimetics of cobalt ferrite nanostructures and little is known about the effects of different CoFe<sub>2</sub>O<sub>4</sub> nanostructures on biocatalysis.

In this study, we demonstrate for the first time a general one-step synthesis of CoFe<sub>2</sub>O<sub>4</sub> NPs *via* the co-thermolysis of iron and cobalt acetylacetonates precursors in the solvent of oleic acid. The different shape (polyhedrons, near corner-grown cubic, near cubic and starlike) and different size (15, 25, 45 and 35 nm) could be readily tuned by only change the decomposition amount of Co and Fe precursors. Additional experiments have surprisingly revealed that enhancement the reaction temperature at 330 °C can induce growth starlike CoFe<sub>2</sub>O<sub>4</sub> NPs. Furthermore, we investigated the influence of various physical parameters like shape, size and surface area on the enzyme mimic properties of CoFe<sub>2</sub>O<sub>4</sub> NPs. Kinetic parameters ( $V_{\max}$  and  $K_m$ ) of all the nanoparticles were assessed, and a correlative investigation between the various physical features of CoFe<sub>2</sub>O<sub>4</sub> and its enzyme mimicking property is presented. On the basis of these findings, we have designed the folate-conjugated CoFe<sub>2</sub>O<sub>4</sub> nanoparticles as a nanoprobe to provide dual functionality by binding to folate-expressing cancer cells and facilitating detection by catalytic oxidation of sensitive colorimetric substrates (dyes).

## 2. Experimental

### 2.1. Chemicals and instrumentation

Fe(acac)<sub>3</sub> (99.9%), Co(acac)<sub>2</sub> (99%), oleic acid (OA, technical grade, 90%), 1,2-hexadecanediol (HDD, technical grade, 90%), phenyl ether, and polyethylene glycol (MW = 4000) were purchased from Sigma Aldrich. Folic acid, dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS), 3,3',5,5'-tetramethylbenzidine (TMB), *o*-phenylenediamine (OPD), 3,4-dihydroxybenzaldehyde and terephthalic acid were from Aladdin in China. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%), sodium acetate anhydrous (NaAc, A.R.) and acetic acid (HAc, A.R.) were obtained from Beijing Chemicals Inc (Beijing, China). All chemicals were used without further purification, except DMF, CHCl<sub>3</sub>, were used as anhydrous. Aqueous solutions were prepared with double-distilled water (dd-H<sub>2</sub>O) from a Millipore system (>18 MΩ cm). 1, ω-diaminopolyoxyethylene (MW = 4000), PEG-3,4-dihydroxybenzylamine (DIB-PEG-NH<sub>2</sub>) were synthesized according to the published method.<sup>16</sup> All the dialysis bags (MWCO 8000-14000) were obtained from Shanghai Med.

The TEM measurements were carried out with Philips EM 420 (120 kV) under ambient conditions deposition of the hexane or H<sub>2</sub>O dispersions of the particles on amorphous carbon coated copper grids. The hysteresis loop was obtained at 300 K with a LakeShore 7400 VSM system. The fluorescence spectra were recorded on a Shimadzu RF-5301 spectrofluorophotometer. UV-vis absorbance measurements experiments were carried out on a UV 1750 spectrophotometer (Shimadzu, Japan). The absorbance was acquired on a 721E visible spectrophotometer (Shanghai). The cytotoxicity assay

was detected by a microplate reader (Nanjing Huadong Electronics Group Co., Ltd. DG5033A – microplate reader).

### 2.2. Synthesis of CoFe<sub>2</sub>O<sub>4</sub> nanoparticles

For synthesis of 15 nm CoFe<sub>2</sub>O<sub>4</sub> nanoparticles, Fe(acac)<sub>3</sub> (8 mmol), Co(acac)<sub>2</sub> (4 mmol) and oleic acid (15 mL) were mixed and magnetically stirred under a flow of nitrogen. The mixture was heated to 120 °C under nitrogen atmosphere with vigorous stirring, and then kept at that temperature for 1 h. The obtained solution was then heated to 320 °C, and the solution color gradually became black, indicating that the magnetic nanoparticles were being formed. After refluxing for 4 h, the solution was cooled to room temperature, and a black precipitate was obtained upon addition of 50 mL of isopropyl alcohol and centrifuged. The product was washed with petroleum and ethanol (1/4, v/v) 3 times. Finally, the product was redispersed in hexane.

Under similar reaction conditions, the different size and shape CoFe<sub>2</sub>O<sub>4</sub> NPs could be produced by simply controlling the amount of Fe(acac)<sub>3</sub> and Co(acac)<sub>2</sub>. For example, the near corner-grown cubic NPs with 25 nm was synthesized using 2 mmol of Fe(acac)<sub>3</sub> and 1 mmol of Co(acac)<sub>2</sub>, and the near cubic NPs with 45 nm was prepared using 4 mmol of Fe(acac)<sub>3</sub> and 2 mmol of Co(acac)<sub>2</sub>. The amount of Fe(acac)<sub>3</sub> and Co(acac)<sub>2</sub> that synthesized star-shaped nanoparticles with 35 nm was same with the amount of that near corner-grown cubic NPs expect using 330 °C.

4 nm spherical CoFe<sub>2</sub>O<sub>4</sub> NPs are prepared according to literature.<sup>17</sup> Fe(acac)<sub>3</sub> (2 mmol), Co(acac)<sub>2</sub> (1 mmol), 1,2-hexadecanediol (10 mmol), oleic acid (6 mmol), and phenyl ether (20 mL) were mixed and magnetically stirred under a flow of nitrogen. The mixture was heated to 200 °C and kept at this temperature for 30 min, and then, under a blanket of nitrogen, the mixture was heated to reflux (265 °C) and kept at this temperature for another 30 min. The dark red-brown mixture was cooled to room temperature by moving the heat source. Under ambient conditions, ethanol (40 mL) was added to the mixture, and a dark red-brown material was precipitated and separated *via* centrifugation (6000 rpm, 3 min). The dark red-brown product was dissolved in hexane (Fig. S1†).

### 2.3. Synthesis of CoFe<sub>2</sub>O<sub>4</sub>-DIB-PEG-NH<sub>2</sub> (1a) and CoFe<sub>2</sub>O<sub>4</sub>-DIB-PEG-NH<sub>2</sub>-FA (1b)

DIB-PEG-NH<sub>2</sub> (100 mg) was dissolved in CHCl<sub>3</sub> (10 mL), and then the CoFe<sub>2</sub>O<sub>4</sub> nanoparticles (10 mg) was added. The mixture was stirred 24 h at room temperature. The modified CoFe<sub>2</sub>O<sub>4</sub> nanoparticles (water-soluble nanoparticles, abbreviated as 1a) were precipitated by adding petroleum ether, and collected by centrifugation at 4500 rpm (3 min). The product was washed with ethanol and petroleum (1/4, v/v) 3 times. Finally, the product was redispersed in double-distilled water.

Folic acid (0.044 g, 0.1 mmol) was dissolved in 3 mL of dry dimethyl sulphoxide (DMSO) into which 0.040 g (0.15 mmol) of dicyclohexylcarbodiimide (DCC) and 0.014 g (0.12 mmol) of hydroxysuccinimide (NHS) were added. The reaction mixture was stirred for 12 hours at room temperature and then added

with DIB-PEG-NH<sub>2</sub> (200 mg). After another 12 hours of reaction, the product was precipitated by adding diethyl ether (20 mL) and dried *in vacuo*. <sup>1</sup>HNMR of DIB-PEG-NH<sub>2</sub>-FA (DMSO, 400 MHz):  $\delta$  = 8.64 (1H, s, C8), 7.65 (2H, d,  $J$  = 8.4 Hz, C13 and C15), 7.22 (1H, m, C26), 6.99 (1H, d,  $J$  = 1.6 Hz, C24), 6.75 (1H, m, C25), 6.63 (2H, d,  $J$  = 8.4 Hz, C12 and C16), 4.48 (2H, d,  $J$  = 6.0 Hz, C9), 4.32 (1H, m, C18), 3.64 (2H, dd,  $J$  = 6.0, 4.4 Hz, C21), 3.51 (~345H, bs, PEG3800), 3.19 (2H, td,  $J$  = 11.0, 5.4 Hz, C22), 2.54 (1H, s, C23), 2.25 (2H, m, C20), 1.93 ppm (2H, m, C19). The CoFe<sub>2</sub>O<sub>4</sub>-DIB-PEG-NH<sub>2</sub>-FA (**1b**) was synthesized by DIB-PEG-NH<sub>2</sub>-FA and the CoFe<sub>2</sub>O<sub>4</sub> nanoparticles with the similar reaction conditions mentioned above.

#### 2.4. Leaching of iron ions from CoFe<sub>2</sub>O<sub>4</sub> and Fe<sub>3</sub>O<sub>4</sub> NPs

To investigate the leaching of iron ions from the water-soluble CoFe<sub>2</sub>O<sub>4</sub> and Fe<sub>3</sub>O<sub>4</sub> NPs in the presence of HAc-NaAc buffer (pH = 4.0), they were put into dialysis bags and incubated them in the HAc-NaAc buffer. After incubating for 60 min, the leaching solution from the dialysis bags was added into H<sub>2</sub>O<sub>2</sub>-TMB system and their catalytic activities were tested. The same concentration iron of NPs as control was also measured.

#### 2.5. Mechanism of peroxidase-like activity of **1a**

10  $\mu$ L of 0.4 mM terephthalic acid solution dissolved in DMF was added into 2 mL the HAc-NaAc buffer containing 500  $\mu$ L of 10 mM H<sub>2</sub>O<sub>2</sub> and different concentration of **1a** (0  $\mu$ g and 30  $\mu$ g).<sup>18</sup> Then the mixture solution was incubated at 37 °C for 10 h. After that, the reaction solution was detected by the fluorescence spectra under the 315 nm excitation. Under the same condition, the reaction solution without H<sub>2</sub>O<sub>2</sub> was also measured.

#### 2.6. The catalytic oxidation of TMB by **1a** and steady-state kinetic assays

To investigate the peroxidase-like activity of the **1a**, the catalytic oxidation of TMB in the presence of H<sub>2</sub>O<sub>2</sub> was tested. 500  $\mu$ L of 100 mM H<sub>2</sub>O<sub>2</sub> and 20  $\mu$ L of 15 mM TMB (prepared freshly) were added to 2 mL of HAc-NaAc buffer (pH = 4.0). Then, a certain amount of **1a** suspension was added into the above mixture. The oxidation reaction progress was monitored at 37 °C with 2 min intervals by recording the absorption spectra in a scanning kinetics mode. Steady-state kinetic assays were carried out at 37 °C in 0.2 M HAc-NaAc buffer (pH = 4.0) in the presence of **1a**. For TMB as a substrate, the H<sub>2</sub>O<sub>2</sub> concentration was fixed at 100 mM. For H<sub>2</sub>O<sub>2</sub> as a substrate, the TMB concentration was fixed at 15 mM. All the reactions were monitored at 653 nm using a 721E visible spectrophotometer. The apparent kinetic parameters were calculated based on the function  $v = V_{\max} \times [S]/(K_m + [S])$ , where  $v$  is the reaction velocity,  $V_{\max}$  is the maximal reaction velocity,  $[S]$  is the concentration of substrate and  $K_m$  is the Michaelis constant.

#### 2.7. Cytotoxicity and bioassay assay

The cytotoxicity of the **1b** *in vitro* was evaluated by performing methyl thiazolyl tetrazolium (MTT) assay of the HeLa cells

incubated with the different nanoparticles. Cells were seeded into a 96-well cell culture plate with a density of  $5 \times 10^4$  cells per well in DMEM with 10% FBS at 37 °C under 5% CO<sub>2</sub> for 24 h. Then, different concentrations (0, 10, 20, 40, 60, 100  $\mu$ g mL<sup>-1</sup> in DMEM) of **1b** were added into the plate respectively. The cells were incubated for 24 h at 37 °C under 5% CO<sub>2</sub>. Thereafter, MTT (20 mL, 5 mg mL<sup>-1</sup>) was added to each well and the plate was incubated for 4 h at 37 °C. After the addition of DMSO (100  $\mu$ L per well), the cell plate was vibrated at 37 °C for 10 min. The optical density was measured at 492 nm using a microplate reader (Nanjing Huadong Electronics Group Co., Ltd. DG5033A – microplate reader).

In the bioassay assay of **1b**, the HeLa and NIH-3T3 cells were seeded into a 96-well cell culture plate with gradient density (0, 10<sup>2</sup>, 10<sup>3</sup>,  $5 \times 10^3$ , 10<sup>4</sup>,  $5 \times 10^4$ , 10<sup>5</sup> cells per well) in DMEM with 10% FBS. After incubated 2 h at 37 °C under 5% CO<sub>2</sub>, the cells were washed twice with PBS, 120  $\mu$ L HAc-NaAc buffer solution, 50  $\mu$ L of 10 mM H<sub>2</sub>O<sub>2</sub> and 30  $\mu$ L of 1.5 mM TMB were added in sequence. The cell plate was vibrated at 37 °C for 20 min. The optical density was measured at 653 nm using a microplate reader (Nanjing Huadong Electronics Group Co., Ltd. DG5033A – microplate reader). Different concentrations (0, 5, 10, 20, 30, 40  $\mu$ g mL<sup>-1</sup> in DMEM) of **1b** that incubated with certain amounts of cells ( $5 \times 10^4$  cells per well) were also detected.

## 3. Results and discussions

### 3.1. Nanoparticle characterization and morphology

The shape, size, and structure of the as-synthesized CoFe<sub>2</sub>O<sub>4</sub> nanostructures have been investigated using transmission electron microscopy (TEM). Fig. 1 and S1† show representative images of four different nanostructures under different amounts of Fe(acac)<sub>3</sub> and Co(acac)<sub>2</sub>. By merely changing the decomposition amount of Co and Fe precursors, the nanocrystals can be tuned in the form of near corner-grown cubic (Fig. 1A and B), near cubic (Fig. 1D and E), and nanopolyhedrons (Fig. 1G and H). Interestingly, by only changing the reaction temperature from 320 °C to 330 °C, the product shape varies from near corner-grown cubic (Fig. 1D and E) to starlike (Fig. 1J and K). All of the products have narrow size distributions (Fig. 1C, F, I and L), and the average diameters of the NPs are  $24.5 \pm 5.3$ ,  $45.2 \pm 15.1$ ,  $13.8 \pm 4.6$ , and  $32.1 \pm 4.2$  nm, respectively.

Generally, the particle size mainly depends on the nuclei speed. The rapid nuclei speed obtains the small size particle, and the large particle is formed when the nuclei speed is slow.<sup>19</sup> When the precursor concentration is high, the nuclei speed is fast. So the size decreases with the increasing precursor concentration. But the NPs size then decreased to 25 nm at the low precursor concentration. That is largely because the nuclear concentration is too low, limiting the further growth of nanoparticles. The shape of a nanocrystal was mainly determined by the ratio of the growth rates of different crystallographic planes.<sup>20</sup> Several studies have reported that controlling the nucleation and growth dynamics by changing the heating rate, temperature, and the precursor concentration can result in the formation of differently shaped nanocrystals. In our



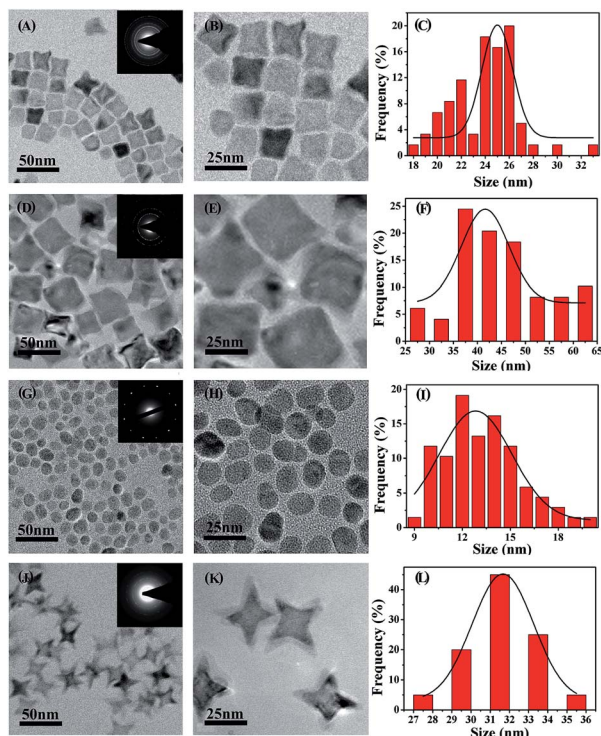
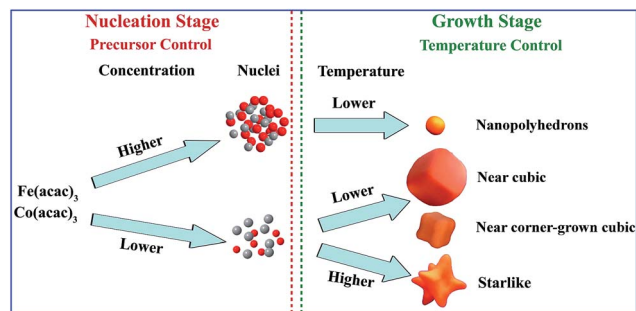


Fig. 1 TEM images of the as synthesized  $\text{CoFe}_2\text{O}_4$  NPs with different shape and size. (A and B) near corner-grown cubic; (D and E) near cubic; (G and H) nanopolyhedrons; (J and K) starlike. The size distribution histograms of as-prepared  $\text{CoFe}_2\text{O}_4$  NPs with the different average sizes of (C)  $24.5 \pm 5.3$  nm, (F)  $45.2 \pm 15.1$  nm, (I)  $13.8 \pm 4.6$  nm, and (L)  $\sim 32.1 \pm 4.2$  nm.

experiments, only oleic acid was used as the solvent and surfactant and the total volume is the same. In the low concentration of precursors, only cubic  $\text{CoFe}_2\text{O}_4$  NPs are obtained. However the shapes of the obtained  $\text{CoFe}_2\text{O}_4$  NPs are nanopolyhedrons in the higher concentration. These are likely because the seeds growth in the different crystal planes is sensitive to the precursor concentration. When the precursors concentration was high, the growth rates on various planes are fast that the differential growth is not significant, leading to a nanopolyhedrons shape. The gradual decrease in precursors concentration triggered the difference in the growth rate of various planes, resulting in a cubic shape. The formation of the starlike shape at  $330^\circ\text{C}$  may be the continuous growth along the eight corners of the intermediate cubic shape.<sup>21</sup> The dependence of the shape and size on the precursor concentration and reaction temperature is shown in Scheme 1.

The crystallinity and structure of the  $\text{CoFe}_2\text{O}_4$  NPs were also confirmed by powder X-ray diffraction (XRD). As shown in Fig. 2A, the peak position and relative intensity of all diffraction peaks for the four products match well with standard powder diffraction data. The half-peak width of  $\text{CoFe}_2\text{O}_4$  NPs decreases with the increase of NPs sizes. The average sizes of the NPs, estimated with Scherrer's formula, are consistent with the sizes observed in the TEM images. The room-temperature hysteresis loop of the ferrites was measured using a vibrating sample



Scheme 1 Schematic illustration of the shape and size control of  $\text{CoFe}_2\text{O}_4$  through adjusting the amount of precursors and heating temperature.

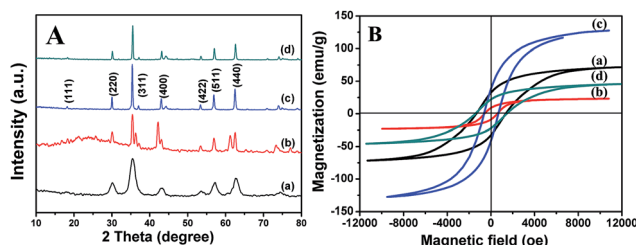


Fig. 2 (A) XRD patterns of  $\text{CoFe}_2\text{O}_4$  NPs with the different average sizes (a)  $13.8 \pm 4.6$  nm, (b)  $24.5 \pm 5.3$  nm, (c)  $32.1 \pm 4.2$  nm and (d)  $45.2 \pm 15.1$  nm. (B) Magnetic hysteresis loops of (a)  $13.8 \pm 4.6$  nm, (b)  $24.5 \pm 5.3$  nm, (c)  $32.1 \pm 4.2$  nm and (d)  $45.2 \pm 15.1$  nm  $\text{CoFe}_2\text{O}_4$  NPs measured at 298 K.

magnetometer (VSM). The magnetization curves, as shown in Fig. 2B, display relatively high saturation magnetization. The saturation magnetizations ( $M_s$ ) of nanopolyhedrons ( $13.8 \pm 4.6$  nm), near corner-grown cubic ( $24.5 \pm 5.3$  nm), starlike ( $32.1 \pm 4.2$  nm) and near cubic ( $45.2 \pm 15.1$  nm) were 71.7, 23.1, 127.6 and 45.7  $\text{emu g}^{-1}$ , respectively, which indicated starlike NPs exhibited higher saturation magnetizations than near cubic and near corner-grown cubic NPs, and the nanopolyhedrons NPs exhibited medium saturation magnetizations. Generally, the moment is dependent on the NP size, and the truncated octahedral nanostructures possess lower  $M_s$  values than spherical nanostructures. The 15 nm NPs has larger particle size than that of 25 nm and 45 nm NPs, which is generally believed to be due to the presence of a magnetic dead or antiferromagnetic layer on the surface of these two NPs.<sup>22</sup> About this phenomenon, we will focus on the systematic investigation in future studies. The hysteresis curves of four samples suggest their ferromagnetic behavior.

The  $\text{CoFe}_2\text{O}_4$  nanoparticles were also characterized by X-ray photoelectron spectroscopy (XPS). In the  $\text{Co}2p$  spectrum (Fig. 3E), the main peak at 798.2 eV with the satellite peak at 803.8 eV and that at 781.0 eV with the satellite peak at 786.9 eV belong to  $\text{Co}2p_{1/2}$  and  $\text{Co}2p_{3/2}$ , respectively. The two main peaks and satellite peaks near them confirmed the presence of  $\text{Co}^{2+}$ .<sup>23</sup> The  $\text{Fe}2p$  spectrum in Fig. 2D exhibited one peak at  $\sim 716$  eV, which is identified as the surface peak of  $\alpha\text{-Fe}_2\text{O}_3$ .<sup>24</sup>

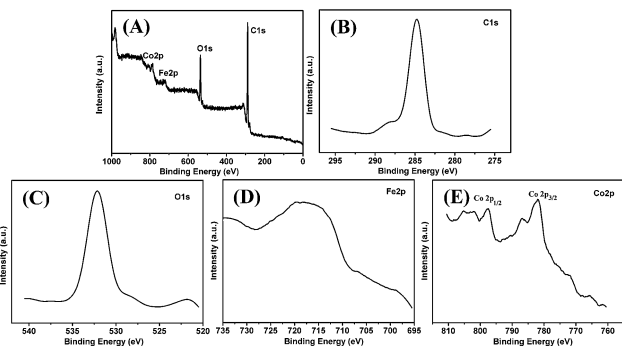


Fig. 3 (A) XPS spectra of  $\text{CoFe}_2\text{O}_4$  NPs; (B) the spectrum in the  $\text{C}1\text{s}$  region; (C) the spectrum in the  $\text{O}1\text{s}$  region; (D) the spectrum in the  $\text{Fe}2\text{p}$  region; (E) the spectrum in the  $\text{Co}2\text{p}$  region.

### 3.2. The tunable peroxidase-like activity of the $\text{CoFe}_2\text{O}_4$ NPs

All of the monodispersed nanoparticles described above exhibited a hydrophobic surface. To modify the surface into a hydrophilic surface, the ferrite nanoparticles were treated with PEG-3,4-dihydroxy benzyl amine (DIB-PEG- $\text{NH}_2$ ). After modification with DIB-PEG- $\text{NH}_2$ , all of the ferrite nanoparticles described above could be dispersed in water easily and the suspensions were stable (Scheme S1†).

Peroxidase-like behaviors of different water-soluble NPs (**1a**) were investigated spectrophotometrically at room temperature using 3,3',5,5'-tetramethylbenzidine (TMB) and *o*-phenylenediamine (OPD) as the peroxidase substrate in the presence of  $\text{H}_2\text{O}_2$ . First, TMB and  $\text{H}_2\text{O}_2$  were used to test the catalytic activity of the different **1a**. As shown in Fig. 4, all the four different shaped **1a** chosen were found to catalyze the reaction with the peroxidase substrate TMB in the presence of  $\text{H}_2\text{O}_2$ , producing a blue coloured product and exhibiting similar spectral features with their characteristic absorbance at 652 nm, except for the variations in absorbance intensity. This absorption arises from the oxidation product of TMB, similar to the phenomena observed for the commonly used horse radish peroxidase enzyme.<sup>25</sup> The intensity might have relevance to the catalytic activities of different **1a**. In addition, similar catalytic performance was observed when OPD was used as the substrate in place of TMB. As shown in Fig. 4A, with any of **1a**, the solutions gave an orange color with maximum absorbance at 450 nm, which originated from the oxidation product of OPD;<sup>25</sup> but there was still a difference in absorbance intensity for different **1a**. In contrast, it was found that **1a** or  $\text{H}_2\text{O}_2$  alone failed to give significant color change to either TMB or OPD solution. These results indicate that all of **1a** has peroxidase-like activity toward typical peroxidase substrates in the presence of  $\text{H}_2\text{O}_2$ , but different **1a** might exhibit different catalytic activities. From the ICP, We also found that the leaching iron ions from the  $\text{CoFe}_2\text{O}_4$  NPs are only 1.85%, whereas the leaching iron ions from the  $\text{Fe}_3\text{O}_4$  NPs are 2.78%, indicating the  $\text{CoFe}_2\text{O}_4$  NPs have high stability. We compared the activity of the leaching solution with that of NPs under the same conditions. As shown in Fig. S5,† the leaching solution had no activity, demonstrates

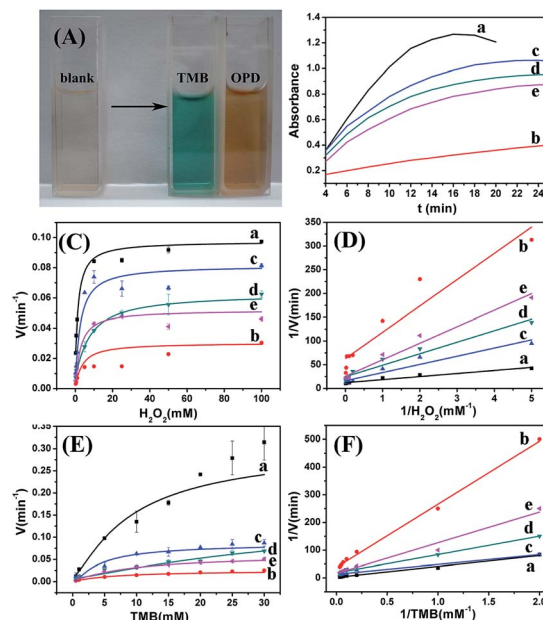


Fig. 4 (A) Photographs show production of colored product upon addition of  $\text{CoFe}_2\text{O}_4$  NPs to TMB and OPD at pH 4.0. (B) The time-dependent UV absorbance curve of the HAC–NaAc solution (pH = 4.0) containing 10.0 mM  $\text{H}_2\text{O}_2$  and 1.5 mM TMB in the presence of an equal amount of  $\text{CoFe}_2\text{O}_4$  NPs for 25 min. (C) The reaction rate of different NPs in 2 mL HAC–NaAc (pH = 4.0) in the presence of 1.5 mM TMB and  $\text{H}_2\text{O}_2$  with different concentrations at room temperature. (D) Double-reciprocal plots of activities of different NPs at a fixed concentration of TMB (1.5 mM) and varying concentrations of  $\text{H}_2\text{O}_2$ . (E) The reaction rate of different NPs in 2 mL HAC–NaAc (pH = 4.0) in the presence of 10 mM  $\text{H}_2\text{O}_2$  and TMB with different concentrations at room temperature. (F) Double-reciprocal plots of activities of different NPs at a fixed concentration of  $\text{H}_2\text{O}_2$  (10 mM) and varying concentrations of TMB. (a)  $4.1 \pm 0.3$  nm, (b)  $13.8 \pm 4.6$  nm, (c)  $24.5 \pm 5.3$  nm, (d)  $32.1 \pm 4.2$  nm and (e)  $45.2 \pm 15.1$  nm  $\text{CoFe}_2\text{O}_4$  NPs.

that the observed reaction cannot be attributed to leaching of iron ions into solution, but occurs on the surface of the NPs.

To investigate the catalytic activities of different **1a**, the substrates TMB and  $\text{H}_2\text{O}_2$  were selected for use in a model system in the following experiments. To acquire an optimal experimental condition, the effects of pH and temperature on the catalytic activities of the **1a** were investigated. We measured the peroxidase-like activity of 4 nm, 15 nm, 25 nm, 35 nm and 45 nm **1a** while varying the pH from 2.5 to 6.0, the temperature from 20 °C to 50 °C, and the  $\text{H}_2\text{O}_2$  concentration from 0.01 to 100 mM. From the relative catalytic activity (Fig. S2†), the optimized pH value and temperature are 4.0 and 37 °C. Therefore, we adopted pH 4.0 and 37 °C as standard conditions for subsequent analysis of **1a** activity. Likewise, the optimal  $\text{H}_2\text{O}_2$  concentration was 10 mM (Fig. S3†). Most notably,  $\text{CoFe}_2\text{O}_4$  NPs exhibited high activity over a broad pH range (2.5–6.0). As shown in Fig. S2,† the peroxidase-like activity of  $4.1 \pm 0.3$  nm  $\text{CoFe}_2\text{O}_4$  NPs could reach about 23.4% at pH 6.0 compared to that at pH 4.0.

Also, the time-dependent catalytic activities of the different **1a** were investigated under the optimized catalytic conditions. The results are given in Fig. 4B. The different **1a** showed

different levels of activity over the reaction time, in the order of spherical ( $4.1 \pm 0.3$  nm) > near corner-grown cubic ( $24.5 \pm 5.3$  nm) > starlike ( $32.1 \pm 4.2$  nm) > near cubic ( $45.2 \pm 15.1$  nm) > nanopolyhedrons ( $13.8 \pm 4.6$  nm). We also found that the catalytic activity decreases with increasing particle size under the similar morphology. Generally, the catalytic performance could be specifically regulated either by the crystal size or morphology with distinct crystallographic planes. The reason might be that different crystal sizes or planes exhibit different numbers of dangling bonds and different atom arrangement manners, which intrinsically determine the reactivity and selectivity of catalysts.<sup>26</sup>

In order to investigate the kinetic mechanism of the peroxidase activity of the **1a** and compare the peroxidase-like activities of the different kinds of NPs reported in this paper, apparent steady-state kinetic parameters for the peroxidase-like color reaction were determined by changing the concentration of the substrate (TMB and  $\text{H}_2\text{O}_2$ ) while keeping the other concentration constant. We observed that the oxidation reaction catalyzed by the different kinds of NPs follows a Michaelis-Menten behavior towards both components, TMB and  $\text{H}_2\text{O}_2$  (Fig. 4C and E). The Michaelis constants ( $K_m$ ) were determined to explore the correlation between the structures and the activities. The  $K_m$  values of the different kinds of NPs towards different substrates were obtained and are shown in Fig. 4D and F. The  $K_m$  values of spherical, nanopolyhedrons, near corner-grown cubic, starlike and near cubic with TMB as the substrate are 0.007, 0.055, 0.017, 0.024 and 0.035 mM, respectively, and with  $\text{H}_2\text{O}_2$  as the substrate the corresponding  $K_m$  values are 0.036, 0.228, 0.039, 0.066 and 0.111 mM, respectively (Fig. 4A). The results indicated that the spherical NPs with the size of 4 nm had the highest affinity for TMB and  $\text{H}_2\text{O}_2$ , and nanopolyhedrons NPs exhibited the lowest affinity for TMB and  $\text{H}_2\text{O}_2$ . However, the starlike NPs, near corner-grown cubic NPs, and near cubic NPs showed medium affinity. Interestingly, the apparent  $K_m$  value for the  $\text{CoFe}_2\text{O}_4$  NPs (4 nm) with  $\text{H}_2\text{O}_2$  or TMB as the substrate was lower than HRP (0.062 mM)<sup>27</sup> and  $\text{Fe}_3\text{O}_4$  NPs (0.010 mM, Table S3<sup>†</sup>), suggesting that the  $\text{CoFe}_2\text{O}_4$  NPs had higher affinity for  $\text{H}_2\text{O}_2$  or TMB than HRP and  $\text{Fe}_3\text{O}_4$  NPs which might be a reason for the enhanced peroxidase-like activity.

### 3.3. Mechanism of peroxidase-like activity of $\text{CoFe}_2\text{O}_4$ NPs

In general, in the magnetic MNPs catalysts for the oxidation of TMB, MNPs firstly bind and react with the  $\text{H}_2\text{O}_2$  molecules to generate the  $\cdot\text{OH}$ . Second, TMB was oxidized by  $\cdot\text{OH}$  to form a blue color product.<sup>28</sup> To evidence mechanism of  $\text{CoFe}_2\text{O}_4$  NPs catalysts for the oxidation of TMB, 50 mM  $\text{H}_2\text{O}_2$ , 0.4 mM terephthalic acid in DMF, and different concentrations (0, 5, 10, 15, 20, 25 and 30  $\mu\text{g}$ ) of the  $\text{CoFe}_2\text{O}_4$  NPs incubating in 0.2 mM HAc–NaAc buffer (pH = 4.0) at 40 °C for 10 h.

It was clearly shown that gradual increase of the fluorescence intensity was observed as the concentration of the  $\text{CoFe}_2\text{O}_4$  NPs increased, suggesting that the amount of generated  $\cdot\text{OH}$  was increased by the increase in  $\text{CoFe}_2\text{O}_4$  NPs. However, there was no fluorescence intensity in the absence of  $\text{H}_2\text{O}_2$  (Fig. 5B). The

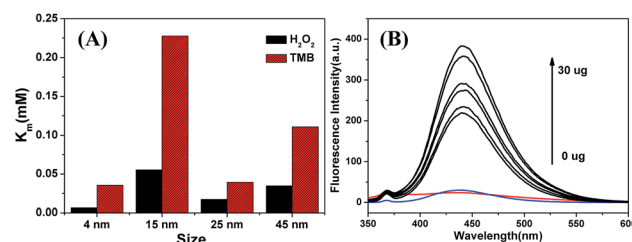


Fig. 5 (A) The Michaelis constants  $K_m$  values of the different kinds of  $\text{CoFe}_2\text{O}_4$  nanoparticles towards  $\text{H}_2\text{O}_2$  (the black bars) and TMB (the red bars). (B) Fluorescence spectral changes observed during the added of  $\text{CoFe}_2\text{O}_4$  (0  $\mu\text{g}$  to 30  $\mu\text{g}$ ) in terephthalic acid solution (excitation at 315 nm), the red line was without the  $\text{H}_2\text{O}_2$ , and the blue line was with 0  $\mu\text{g}$  NPs.

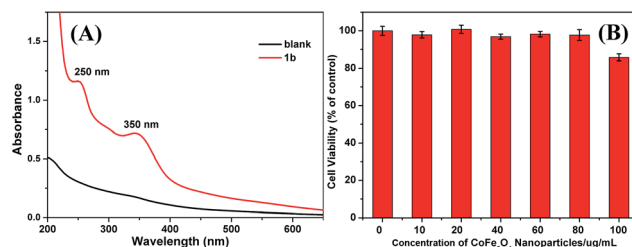


Fig. 6 (A) UV-vis detection of **1b**. The absorbance peaks at 250 nm and 350 nm belong to folic acid (FA). (B) *In vitro* cell viability of HeLa cells incubated with **1b** with different concentrations (0, 10, 20, 40, 60, 80, 100  $\mu\text{g mL}^{-1}$ ).

same phenomena was also happened in  $\text{ZnFe}_2\text{O}_4$  NPs–terephthalic acid– $\text{H}_2\text{O}_2$  system.<sup>27</sup> These results indicated that  $\text{CoFe}_2\text{O}_4$  NPs could decompose  $\text{H}_2\text{O}_2$  to generate the  $\cdot\text{OH}$  radical, and the catalytic mechanism for the oxidation of TMB is same as the chen' report.<sup>27</sup>

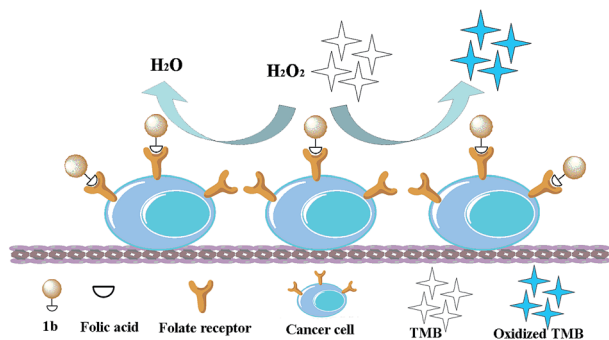
### 3.4. Cytotoxicity assay of **1b**

As one of the most commonly employed small molecules, folic acid (FA) could target the folate receptor present on the surface of tumor cells. Therefore, we used the FA to modify the 25 nm near corner-grown cubic  $\text{CoFe}_2\text{O}_4$  NPs. The resulting **1b** was detected by UV-vis spectra to confirm that if the FA molecules were conjugated to  $\text{CoFe}_2\text{O}_4$  nanoparticles. As shown in Fig. 6A, the characteristic absorption peak at around 250 nm and 350 nm appeared, respectively, which is from FA, indicating the presence of FA in **1b**. Prior to the determination of the expression of the folate receptor in cancer cells by **1b**, the cytotoxicity of the NPs was evaluated *via* an MTT assay of the viability of the HeLa cells. After the cells were incubated with different concentrations of **1b** for 24 h, no obvious decrease in cell viability was observed (Fig. 6B), which indicated that **1b** was low cytotoxicity in the given concentration range, and the **1b** was essential for further biological applications.

### 3.5. Targeting and detecting cancer cells

To investigate if **1b** could be used for specific detection of cancer cells (Scheme 2), HeLa cells are used, which was reported





Scheme 2 Colorimetric determination of target cancer cells based on **1b**.

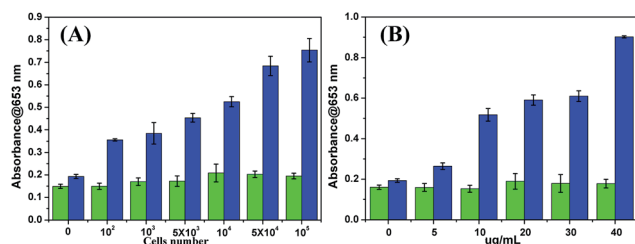


Fig. 7 Colorimetric determination of the expression of the folate receptor in cancer cells using **1b**-mediated ELISA detection of folate receptor-expressing cells. (A) The absorption values at 653 nm after 20 min depend on the number of cells. (B) The absorption values at 653 nm after 20 min with different concentrations of **1b**. TMB was used as the substrate. HeLa cells: blue bars, NIH-3T3 cells: green bars.

to overexpress folate receptor on the cell surface, and the NIH-3T3 cells has no folate receptor expression level for comparison.<sup>29</sup>

In our initial experiment, the 25 nm sized FA-coated CoFe<sub>2</sub>O<sub>4</sub> NPs (**1b**) was chosen as a model study. Then both the HeLa and NIH-3T3 cells were incubated with **1b** for 2 h and subjected to monitor at 653 nm using a microtiter plate reader. As shown in Fig. 7, the absorbance of **1b** incubated with HeLa cells at 653 nm was much higher than which incubated with NIH-3T3 cells. Result shows that with the increase of HeLa cells number or the concentrations of **1b**, the absorbance gradually improved. The absorbance, however, had not been matched by increasing the number of NIH3T3 cells or the concentrations of **1b**. This clearly indicates that the proposed **1b** is suited for the simple discrimination of FA-positive cancerous cells from normal cells.

## 4. Conclusion

In summary, we have developed a simple precursors mediated growth method to prepare monodisperse CoFe<sub>2</sub>O<sub>4</sub> NPs with controlled sizes and shape. Through controlled the amount of Fe(acac)<sub>3</sub> and Co(acac)<sub>2</sub>, near cubic, starlike, near corner-grown cubic and nanopolyhedrons CoFe<sub>2</sub>O<sub>4</sub> NPs with sizes of 45.2 ± 15.1, 32.1 ± 4.2, 24.5 ± 5.3 and 13.8 ± 4.6 nm, respectively, can be successfully fabricated, suggesting the amounts of iron and cobalt precursors play important roles in the manipulation of

the morphology and dimensions of CoFe<sub>2</sub>O<sub>4</sub> NPs. These CoFe<sub>2</sub>O<sub>4</sub> NPs had different size and exposed crystal planes, and thus exhibited different levels of peroxidase-like activities, in the order of spherical (4.1 ± 0.3 nm) > near corner-grown cubic (24.5 ± 5.3 nm) > starlike (32.1 ± 4.2 nm) > near cubic (45.2 ± 15.1 nm) > nanopolyhedrons (13.8 ± 4.6 nm). This indicates that selective fabrication of peroxidase nanomimetics with different size and shape is very important to harness their catalytic activities. Based on the peroxidase-like activity, the folic acid-conjugated CoFe<sub>2</sub>O<sub>4</sub> NPs can be used for discrimination of HeLa cells from normal breast cells. Our approach is not only useful in tuning the size, shape and catalytic performance of cobalt ferrite nanoparticles, but also offers a protocol to specifically recognize detect the cancer cells.

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