Supporting Information

Free-Floating 2D Nanosheets with Superlattice Assembled from Fe_3O_4 Nanoparticles for Peroxidase-Mimicking Activity

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Chemicals

The following chemicals were used as obtained: iron (III) chloride ($FeCl_3 \cdot 6H_2O$, 98%, Sigma-Aldrich), oleic acid (OA, 90%, Sigma-Aldrich), 1-octadecene (ODE, 90%, Sigma-Aldrich), diethylene glycol (99%, DEG, Sigma-Aldrich), octyl ether (99%, TCI), cetyltrimethylammonium bromide (CTAB, 99%, Sigma-Aldrich), sodium oleate (97%, Sigma-Aldrich), hydroxylammonium chloride (99%,Sigma-Aldrich), sodium borohydride ($NaBH_4$, 98%, Sigma-Aldrich), cetyltrimethylammonium bromide (95%, Sigma-Aldrich), cetyltrimethylammonium bromide (99%, Sigma-Aldrich), cetyltrimethylammonium bromide (100.4 %, molecular biology grade, CalBioChem), horseradish peroxidase (HRP, 99%, Sigma-Aldrich, molecular weight (~44 kDa) includes the polypeptide chain (33,890 Da)), hydrogen peroxide (H_2O_2, 30%, Sigma-Aldrich), 3,3`,5,5`-tetramethylbenzidine (TMB, 240.35 g/mol, Sigma-Aldrich), polyvinylpyrrolidone (PVP, MW = 55,000, 99.9%, Sigma-Aldrich), hexane (99.9%, Sigma-Aldrich), dimethyl sulfoxide (DMSO, 99.9%, Sigma-Aldrich), N,N-dimethylformamide (DMF, 99.9%, Sigma-Aldrich), toluene (99.9%, Sigma-Aldrich), chloroform (99.9%, Sigma-Aldrich), methanol (absolute for analysis, 99%, Sigma-Aldrich), acetone and ethanol (absolute for analysis, ACS, 99.9%, Merck). All materials were used without further purification.

Synthesis of iron-oleate complex: In a typical synthesis of the iron-oleate complex, 10.8 g of iron chloride ($FeCl_3 \cdot 6H_2O$, 98%, Sigma-Aldrich) and 36.5 g of sodium oleate were dissolved in a mixed solvent composed of 40 mL ethanol, 40 mL deionized water and 80 mL hexane. The resulting solution was heated to 60 °C and kept at that temperature for four hours. The dark red-black iron oleate precursors were dissolved in 100 mL hexane, washed 3 times with warm DI water (~50 °C) and then separated in a separatory funnel. After washing, hexane was removed by evaporation, resulting in an iron-oleate complex in a waxy solid form.

Synthesis of iron oxide nanoparticles (NP**):**[1] The following is a typical synthetic procedure for monodisperse 7 nm iron oxide nanoparticles. Iron-oleate complex (4.8 g), synthesized as described above, and 0.76 g of oleic acid (90%, Sigma-Aldrich) were dissolved in 6 g octyl ether at room temperature. The mixture was heated to 110 °C and maintained for 60 min under Ar protection. The reaction mixture was then heated to 295 °C with a constant heating rate of 3 °C min^{-1} and then held 30 min. When the reaction temperature reached 295 °C, an obvious reaction occurred, and the initial

transparent solution became turbid and brownish black. The colloidal solution was washed 5 times using isopropyl alcohol/hexane (1:1 v/v) by sedimentation and redispersal using centrifugation (5000 rpm for 10 min). Finally, the Fe_3O_4 NPs were weighed and redispersed in chloroform at the desired NP concentration.

Synthesis of free-floating 2D nanosheets. In a typical synthesis of a clear NP-micelle solution (**Figure S1**), 60 μL OA were dissolved in a chloroform solution of 7-nm Fe_3O_4 NPs (4 mg) for 15 min using a vortex mixer. Then, an aqueous solution containing Cetyl-trimethylammonium bromide (CTAB) (100 μL, 20 mg/mL) was added to the mixture. Afterwards, the mixture was heated for 15 min. A clear, yellow NP-micelle solution was obtained.

Under vigorous stirring, a DEG solution (1 mL) containing 100 mg polyvinylpyrrolidone (PVP) was quickly injected into above Fe_3O_4 NP-micelle solution. The mixture was stirred at room temperature for 15 min and then heated to 83 ^oC and held for another 2 h. Finally, the resulting products were isolated by centrifugation and redispersed in ethanol.

Small-angle X-ray scattering (SAXS) measurements. SAXS experiments were performed on the BL16B1 beam-line of the Shanghai Synchrotron Radiation Facility (SSRF). The photon source of the bending magnet of SSRF delivered X-rays of 5 keV to 20 keV. The beam-line optics consisted of a Si(111) flat double crystal monochromator (DCM). The detectors were Mar165 CCD for SAXS. For our SAXS measurements, energy of 10 keV (0.124 nm) and a sample-to-detector distance of 5 m were chosen. Scattering from the sample was transmitted in vacuum through a Kapton window to strike the detector. The q ranges were 0.03~3.6 nm^{-1} for SAXS measurement at 10 keV. Three mg of 2D nanosheets were put on a Compton belt for measurement, and the measurements were performed at room temperature using an angle dispersive synchrotron X-ray source. The *d* spacing between superlattice planes was calculated as: $d=\frac{2\pi}{q}$ and $d = \frac{4\pi \sin\theta}{\lambda}$.

Catalyzed oxidation. Unless otherwise stated, steady-state kinetic assays were carried out at 30 ^oC in a 2-mL tube with 0.67 μg Fe_3O_4 sheets (**Figure 1**) or Fe_3O_4 NPs in 500 μL reaction buffer (0.2 M NaAc, pH 4.5) in the presence of 530 μM H_2O_2 for Fe_3O_4 nanosheets and Fe_3O_4 NPs, using 800 μM 3,3',5,5'-tetramethylbenzidine (TMB) as the substrate. In a typical experiment, 30 μL H_2O_2 were added

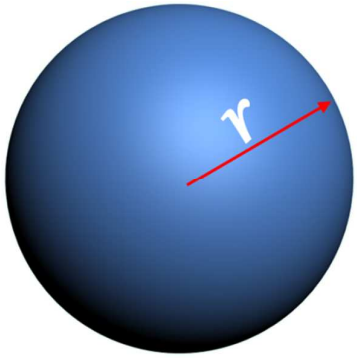
to 430 μL reaction buffer at different pH values at 30 ^{o}C and vortexed for 3 min. Then, 40 μL TMB (10 mM) were added to the mixture and vortexed for another 3 min. Finally, 0.67 μL Fe_3O_4 nanosheets (1 mg/mL) were quickly added to the mixture. Immediately after the substrates were added, color changes were observed. All reactions were monitored according to the maximum intensity of absorbance in time-scan mode at 652 nm using a Cary Bio-100 UV/Vis spectrometer (Varian).

To test the effect of different temperatures at pH 4.5, 430 μL of reaction buffer were kept at the desired temperature for 3 min. Then, 30 μL H_2O_2 were added to the reaction buffer, vortexed for 1 min, and held at that temperature for 3 min. Then, 40 μL TMB (10 mM) were added to the mixture and vortexed 1 min. The mixture was maintained at the desired temperature for another 3 min. Finally, 0.67 μL Fe_3O_4 nanosheets, or Fe_3O_4 NPs (1 mg/mL), was quickly added to the mixture, and the absorbance was monitored at 652 nm.

Materials Characterization

Morphology of the samples was characterized with a transmission electron microscope (TEM) system (JEOL Model JEM-2010F) operating at 200 kV. FTIR measurements were conducted on a Perkin Elmer Instruments Spectrum GX FTIR spectrometer at room temperature from 600 to 4000 cm^{-1}. A total of 32 scans were recorded at a resolution of 2 cm^{-1} for averaging each spectrum. UV-Vis measurements were performed with a Cary Bio-100 UV/Vis spectrometer (Varian). Atomic force microscopy (AFM) (Digital Instruments) was used to determine the thickness of the 2D nanosheets. NMR spectra were recorded on a Varian Unity spectrometer (400 MHz for ^{1}H NMR). All spectra were examined using MestReNova 8.1 (Mnova) software and displayed without the use of the signal suppression function.

Calculation of the number of Fe_3O_4 nanoparticles (NPs) ($N_{particles}$)



Scheme of one Fe_3O_4 NP

For one Fe_3O_4 NP, the radius is r. The density, mass and volume are ρ, m_1 and V_1. The mass of one Fe_3O_4 NP (m_1) was calculated as

$$m_1 = \rho \bullet V_1 \quad (1)$$

$$V_1 = \frac{4}{3}\pi r^3 \quad (2)$$

$$m_1 = \rho \bullet V_1 = \rho \bullet \frac{4}{3}\pi r^3 \quad (3)$$

The number of Fe_3O_4 NPs ($N_{particles}$) was calculated as

$$N_{particles} = \frac{m_{Total}}{m_1} = \frac{m_{Total}}{\rho \bullet V_1} = \frac{m_{Total}}{\rho \bullet \frac{4}{3}\pi r^3} \quad (4)$$

where ρ=5.17 g/cm^3, d=2r=7±0.5 nm, and m_{Total} is the total mass that was weighed in the experiment for m_{Total}=4 mg and $N_{particles}$=4.310 x 10^{15} in 4 mg total mass.

Calculation of the number of OA molecules (N_{OA})

For OA molecules, N_A is Avogadro constant (N_A =6.022 x 10^{23} mol^{-1}), n is the number of mole, M_{OA} is the molecular weight, and m is total mass. The number of OA molecules (N_{OA}) was calculated as

$$N_{OA} = n \bullet N_A \quad (5)$$

$$n = \frac{m}{M_{OA}} \quad (6)$$

$$m = \rho \bullet V \quad (7)$$

The number of OA molecules (N_{OA}) was calculated as

$$N_{OA} = n \bullet N_A = \frac{m}{M_{OA}} \bullet N_A = \frac{\rho \bullet V}{M_{OA}} \bullet N_A \quad (8)$$

where ρ=0.887 g/cm^3, M_{OA}= 282.46 g/mol, and the oleic acid content of the purchased material was 90% (OA, 90%, Sigma-Aldrich).

Calculation of the number of OA molecules stabilized on the surface of one Fe_3O_4 NP (N_0)

$$N_0 = \frac{N_{OA}}{N_{particles}} \quad (9)$$

The N_{OA} and N_0 were calculated as follows:

V_{OA}	N_{OA}	N_0
0 μL	0	0
10 μL	1.702 x 10^{19}	3.949 x 10^3
30 μL	5.106 x 10^{19}	1.185 x 10^4
35 μL	5.957 x 10^{19}	1.382x 10^4
40 μL	6.808 x 10^{19}	1.580x 10^4
50 μL	8.510 x 10^{19}	1.974 x 10^4
60 μL	1.021 x 10^{20}	2.369 x 10^4

The table of calculation of N_{OA} and N_0

These data were used to prepare **Figure 2f**.

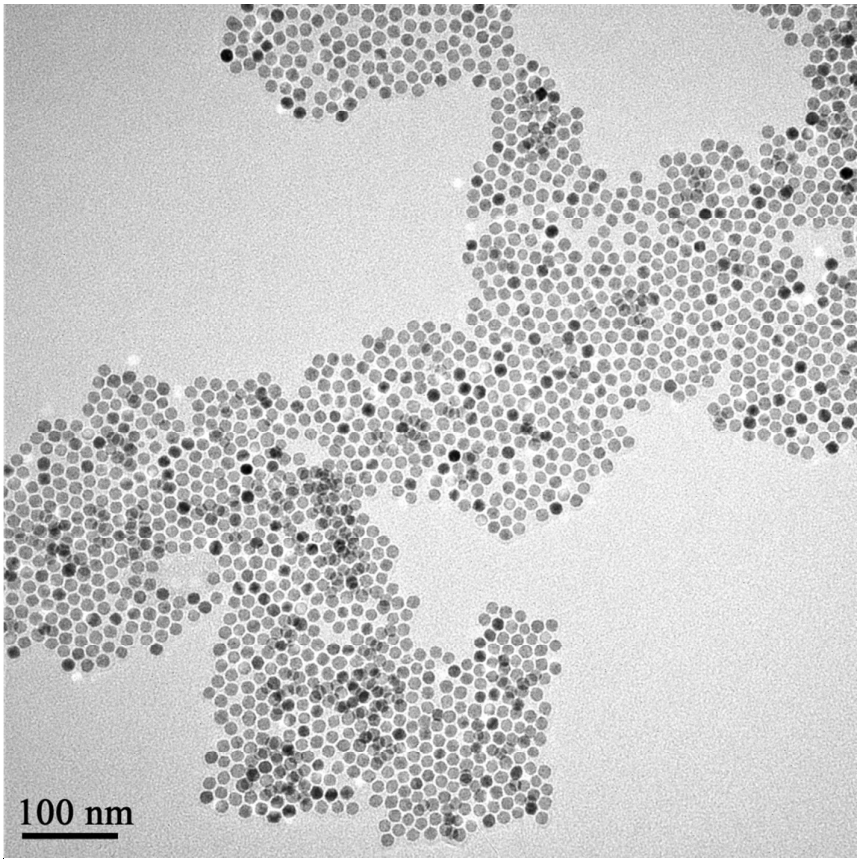


Figure S1 TEM images of Fe_3O_4 NP micelles.

Note: The hydrophobic van der Waals interactions between the hydrocarbon chain of the ligands (oleic acid) and the hydrocarbon chain of the surfactant (CTAB) caused these NP micelles to disperse in aqueous solution.[2]

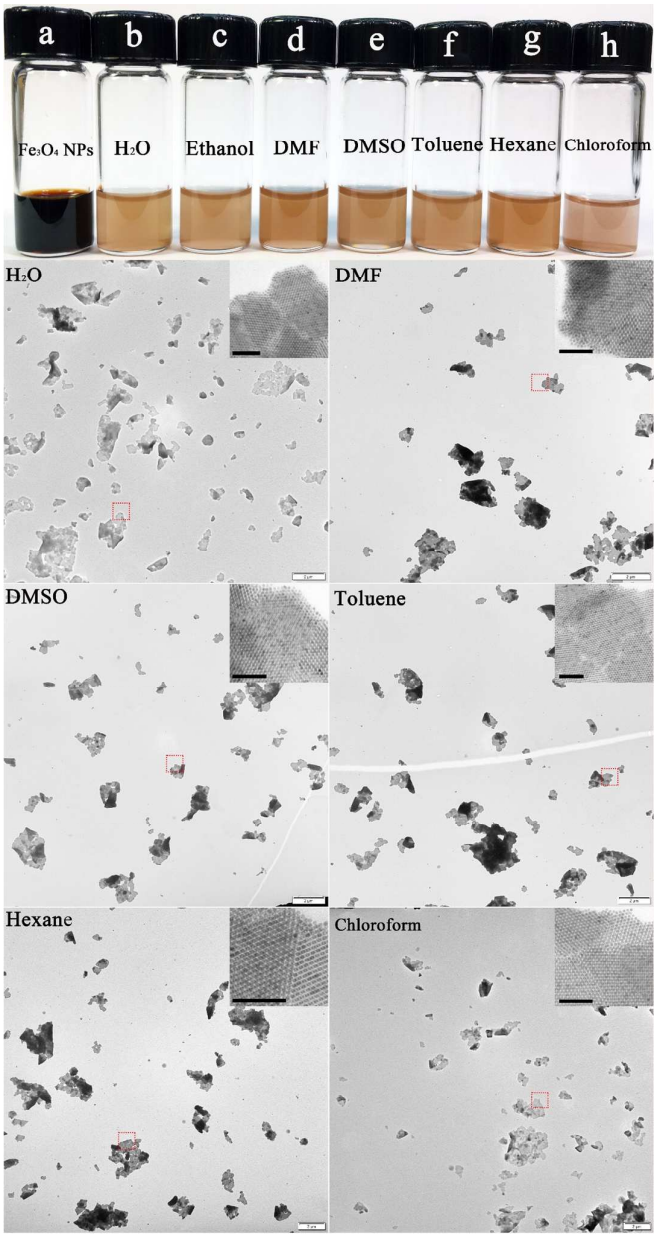


Figure S2 The stability of 2D nanosheets stored in different solvents for 90 days: a) Fe_3O_4 NPs building blocks; b) DI-water; c) ethanol; d) DMF; e) DMSO; f) toluene; g) hexane; h) chloroform. The insert scale bars are 100 nm

Note: To investigate the stability of 2D nanosheets, we dispersed 2D nanosheets at the same concentration in different solvents and kept them 90 days at room temperature. The usual storage solvent is ethanol, providing a yellow color. In **Figure S2b-h**, the color of all these solutions is yellow, which is totally different from that of their building blocks - Fe_3O_4 NPs (black, **Figure S2a).** Our TEM results for the samples shown in **Figure S2b-h** confirmed the samples to be nanosheets. From the stability experiments, these 2D nanosheets exhibited excellent stability in different solvents (DI-water, ethanol, DMF, DMSO, toluene, hexane, chloroform) for 90 days without disaggregation into their Fe_3O_4 NP building blocks (black, **Figure S2a**).

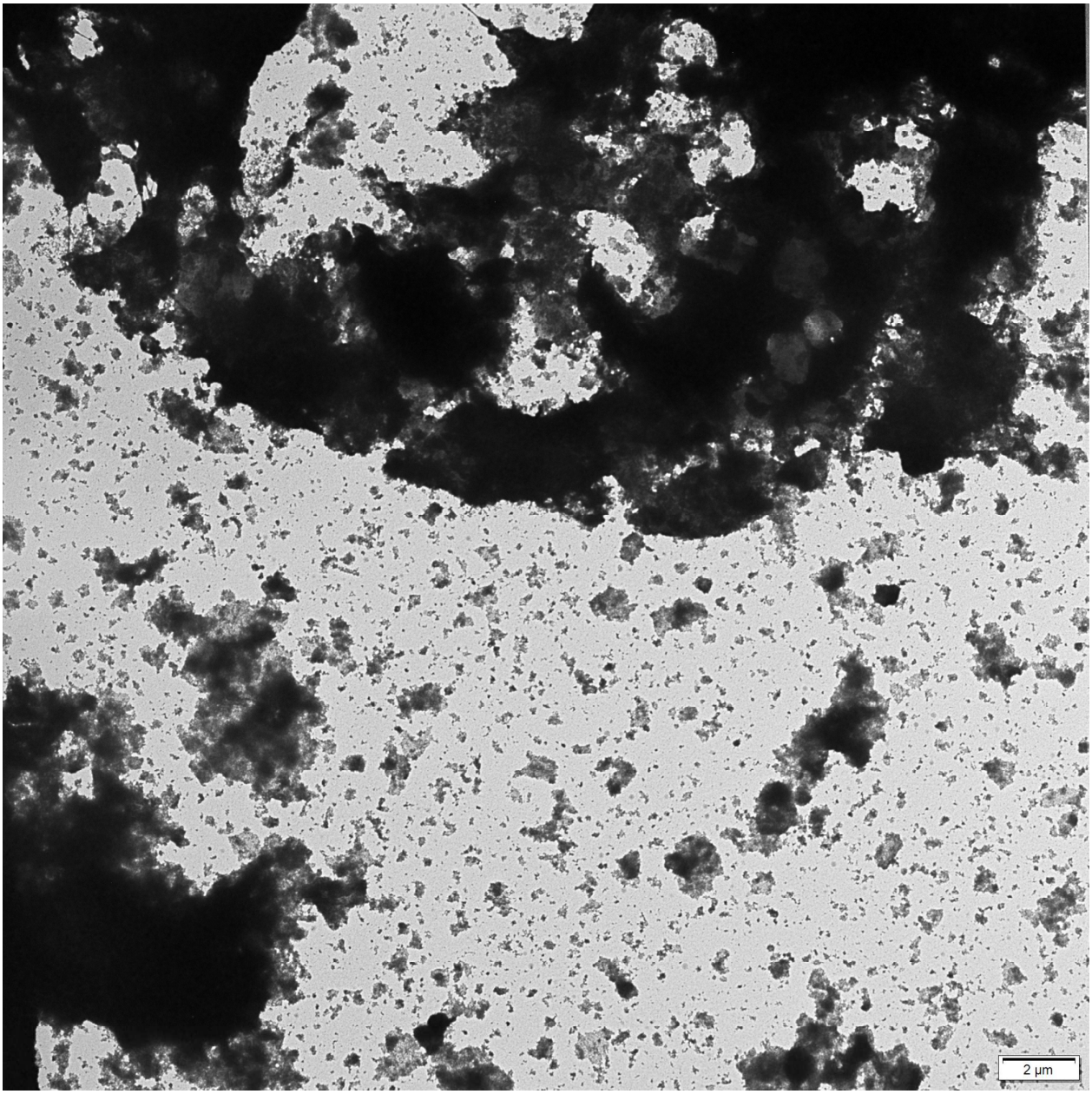


Figure S3 Samples prepared from Fe_3O_4 NPs assembled without CTAB, 32 µL OA.

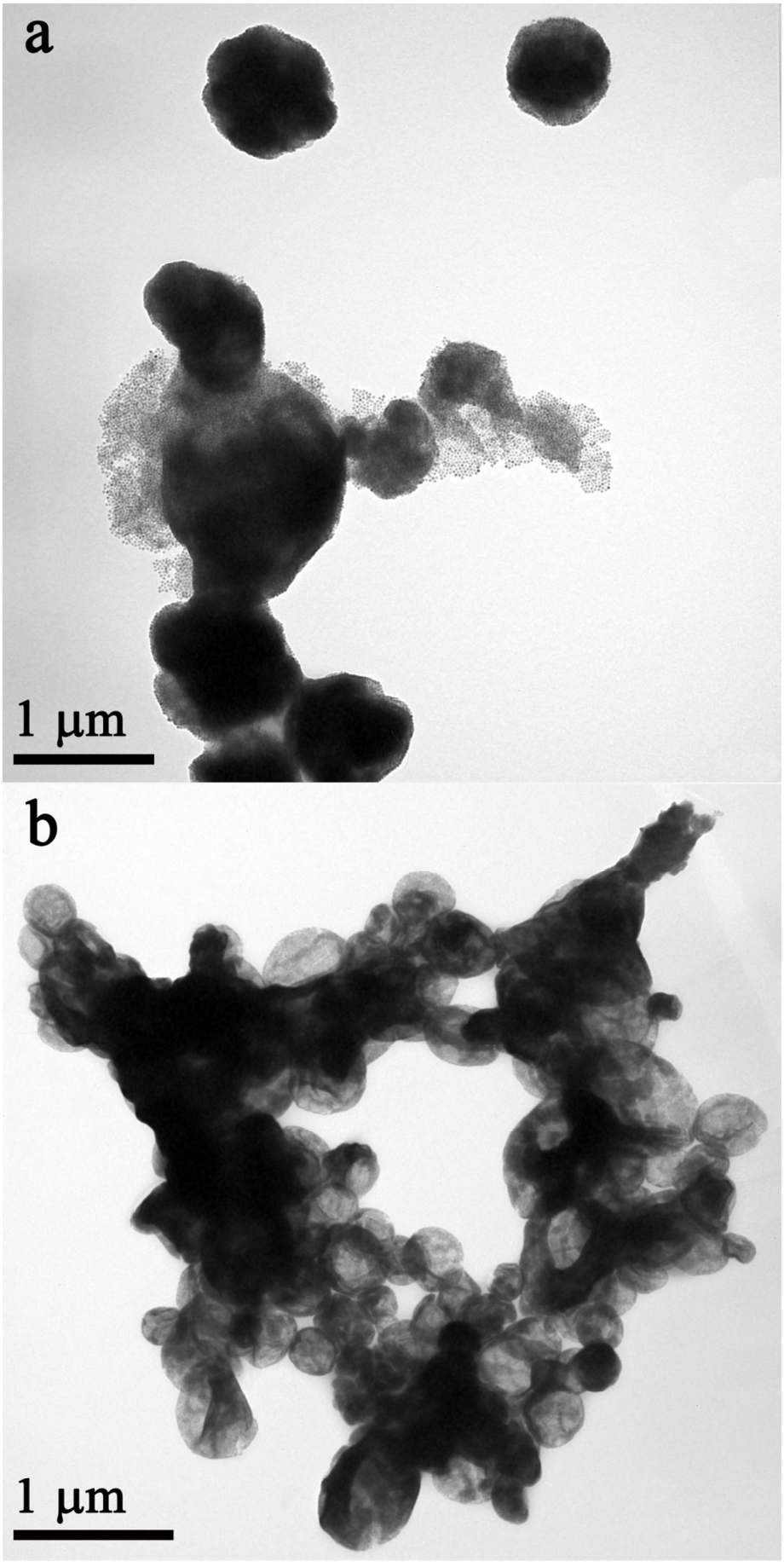


Figure S4 Samples prepared from Fe_3O_4 NPs assembled by adding a) 10 μL and b) 40 μL OA to stabilize Fe_3O_4 NPs.

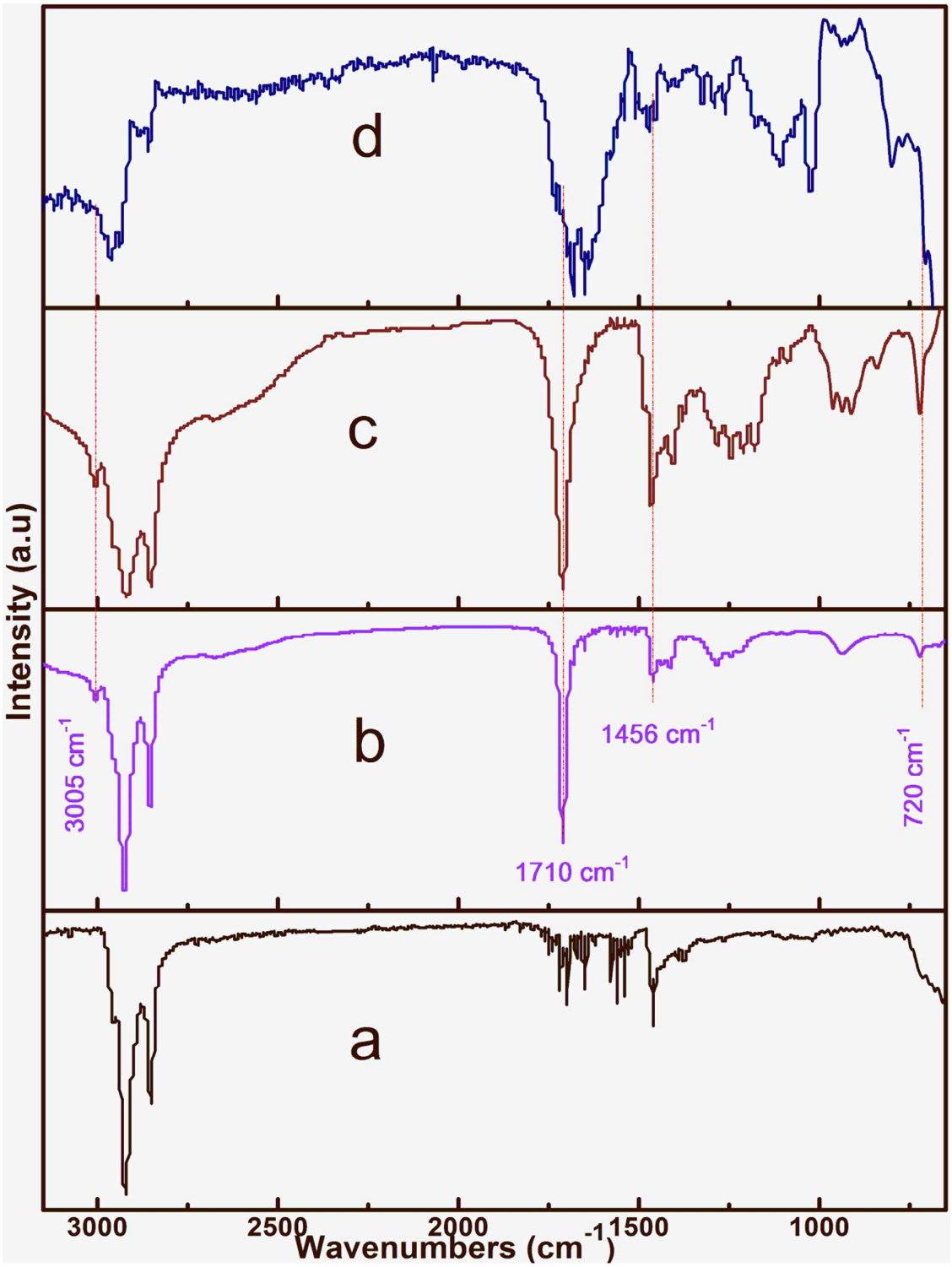


Figure S5 FTIR spectra of a) Fe_3O_4 NPs, b) OA-stabilized Fe_3O_4 NPs, c) Fe_3O_4 NP micelles and d) organic ligands on the 2D nanosheets (60 µL OA).

Note: Figure S5b-d shows the band at 1710 cm^{-1}, resulting from the stretching vibration of C=O in oleic acid, and the band at 1456 cm^{-1}, corresponding to the asymmetric (-COO-) stretch.[3,4,5] In addition, the 3005 cm^{-1} and 720 cm^{-1} peaks originating from the stretching vibration of the -CH=CH- group were observed.[6,7,8] These bands indicate that the oleic acid species are bonded on the surface of Fe_3O_4 NPs, as shown in **Figure S5b**.[9] Based on the FTIR spectra, oleic acid is present on the surface of the Fe_3O_4 nanosheets, as shown in **Figure S5d**.

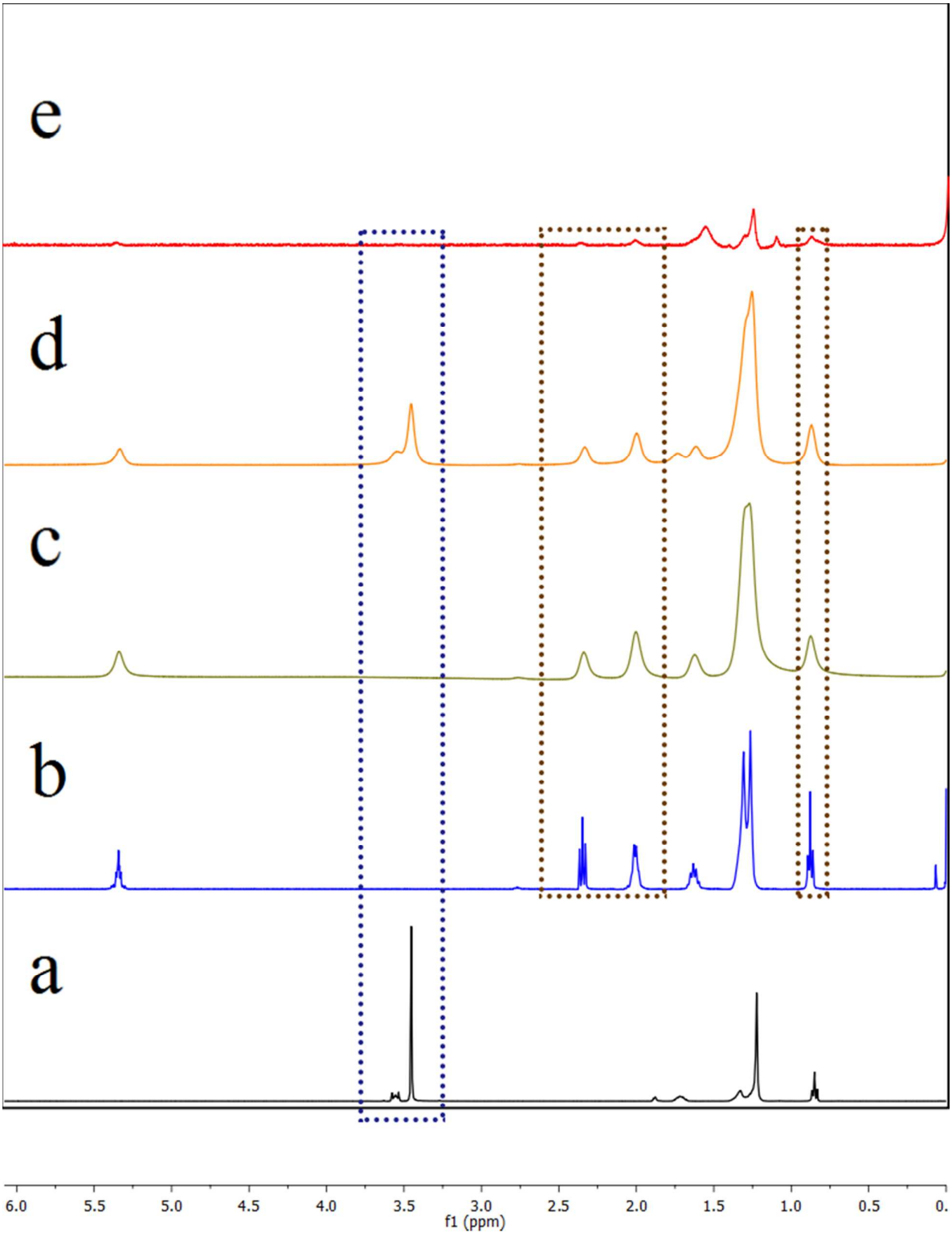


Figure S6 ^{1}H NMR spectra of a) CTAB, b) OA, c) OA on the Fe_3O_4 NPs, d) CTAB and OA on the Fe_3O_4 NP micelles, and e) the organic ligands (OA) on the 2D nanosheets (^{1}H NMR of Fe_3O_4 nanosheets was acquired after dissolving the sample in HCl).

Note: ^{1}H NMR spectrum in **Figure S6d** shows that the organic ligands on the surface of Fe_3O_4 NP micelles were both oleic acid and CTAB molecules. In contrast, the organic ligands on the surface of Fe_3O_4 nanosheets were only oleic acid, and CTAB was not measurable in **Figure S6e**. These ^{1}H NMR spectra results confirmed the loss of CTAB and presence of OA during the assembly process.

Table S1 Comparison of the kinetic parameters of Fe_3O_4 nanosheets and Fe_3O_4 NPs. [E] is the enzyme (or NP) concentration, K_m is the Michaelis constant, V_{max} is the maximal reaction velocity and K_{cat} is the catalytic constant, where $K_{cat} = V_{max}/[E]$.

	[E] (10^{-11} M)	Substrate	K_m (mM)	V_{max} (10^{-8} M s^{-1})	K_{cat} (10^2 s^{-1})
Fe_3O_4 nanosheets	2.40	TMB	0.201	15.02	62.80
Fe_3O_4 nanosheets	2.40	H_2O_2	0.186	15.80	65.82
Fe_3O_4 NPs	2.40	TMB	0.151	11.56	48.16
Fe_3O_4 NPs	2.40	H_2O_2	0.202	11.82	49.25

Note: K_m is an indicator of enzyme affinity to substrate, with a high K_m value representing a weak affinity and vice versa. **Table S1** shows that the apparent K_m value of Fe_3O_4 nanosheets with H_2O_2 as the substrate was significantly lower than that of Fe_3O_4 NPs. Thus, Fe_3O_4 nanosheets showed better affinity to H_2O_2 compared to Fe_3O_4 NPs. The maximum initial velocities (V_{max}) of Fe_3O_4 nanosheets are around 50% higher than the V_{max} values for Fe_3O_4 NPs.

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