Supplementary materials

Label-free electrochemical immunosensor based on biocompatible nanoporous Fe_3O_4 and biotin-streptavidin system for sensitive detection of zearalenone

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Materials and methods

Reagents. $FeCl_3 \cdot 6H_2O$, ethylene glycol, sodium acetate, ethanediamine, ninhydrin, glutaraldehyde solution, N-hydroxysuccinimide ester (biotin-NHS), streptavidin, biotin, bovine serum albumin (BSA) (96-99%), $NaHCO_3$ and dimethyl formamide (DMF) were obtained from Sigma-Aldrich company. $K_3Fe(CN)_6$ and glutaraldehyde (GA) used in this study were purchased from Sinopharm Chemical Reagent Ltd Co. (Beijing, China). Zearalenone monoclonal antibody (anti-ZEN, Ab) and ZEN (99.07%) were purchased from Beijing Wanger Biotechnology Co., Ltd (Beijing, China). A stock solution mixture of ZEN (0.1 $mg \cdot mL^{-1}$) was prepared in methanol and stored at 4^oC in an amber glass volumetric flask. Working mixed standards were prepared by transferring 1 mL of stock solution mixture to separate vials and evaporating to dryness under a dry stream of nitrogen. Phosphate-buffered solutions (PBS, 0.13 $mol \cdot L^{-1}$) at various pH values were prepared by mixing the stock solutions of 0.1 $mol \cdot L^{-1}$ KH_2PO_4 and 0.1 $mol \cdot L^{-1}$ Na_2HPO_4 at different volume ratios to appropriate pH value. PBS was used as electrolyte for all electrochemical measurements. Ultrapure water was used throughout the experiment. All other chemicals were of analytical reagents grade and used without further purification. Human urine samples were supplied by volunteers and informed consent was obtained from all human subjects.

Apparatus. All electrochemical measurements were performed on a CHI760D electrochemical workstation (Shanghai CH Instruments Co., China). The Electrochemical impedance spectroscopy (EIS) was measured with a Model IM6e (Zahner Elektrik, Germany). Transmission electron microscope (TEM) images were obtained from a Hitachi H-800 microscope (Japan). UV/Vis measurements were carried out by using a Lambda 35 UV/Vis Spectrometer

(PerkinElmer, USA). Surface area measurements were performed on Micromeritics ASAP 2020 surface area and porosity analyzer (Quantachrome, United States). The samples were out-gassed overnight (12 h) under nitrogen prior to adsorption measurement. YM-10 columns were purchased from Millipore (USA). X-Ray Powder Diffraction (XRD) pattern was acquired by a D8 FOCUS X-ray diffraction spectrometer (Bruker, German) with a Cu Kα target at a scan rate of 0.03º 2θ s^{-1} from 10º to 80º. X-ray photoelectron spectroscopy (XPS) analysis was performed on ESCALAB 250 X-ray photoelectron spectrometer with an Al Kα radiation source (1486.6 eV). Fourier transform infrared spectroscopy (FTIR) spectra were carried out by using KBr pellet in the range of 4000-450 cm^{-1} on Spectrum One FTIR Spectrometer (PerkinElmer).

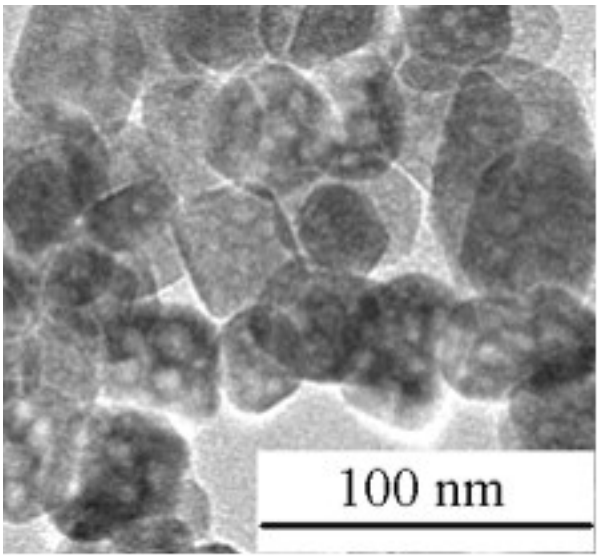

Figure S1. TEM image of nanoporous Fe_3O_4.

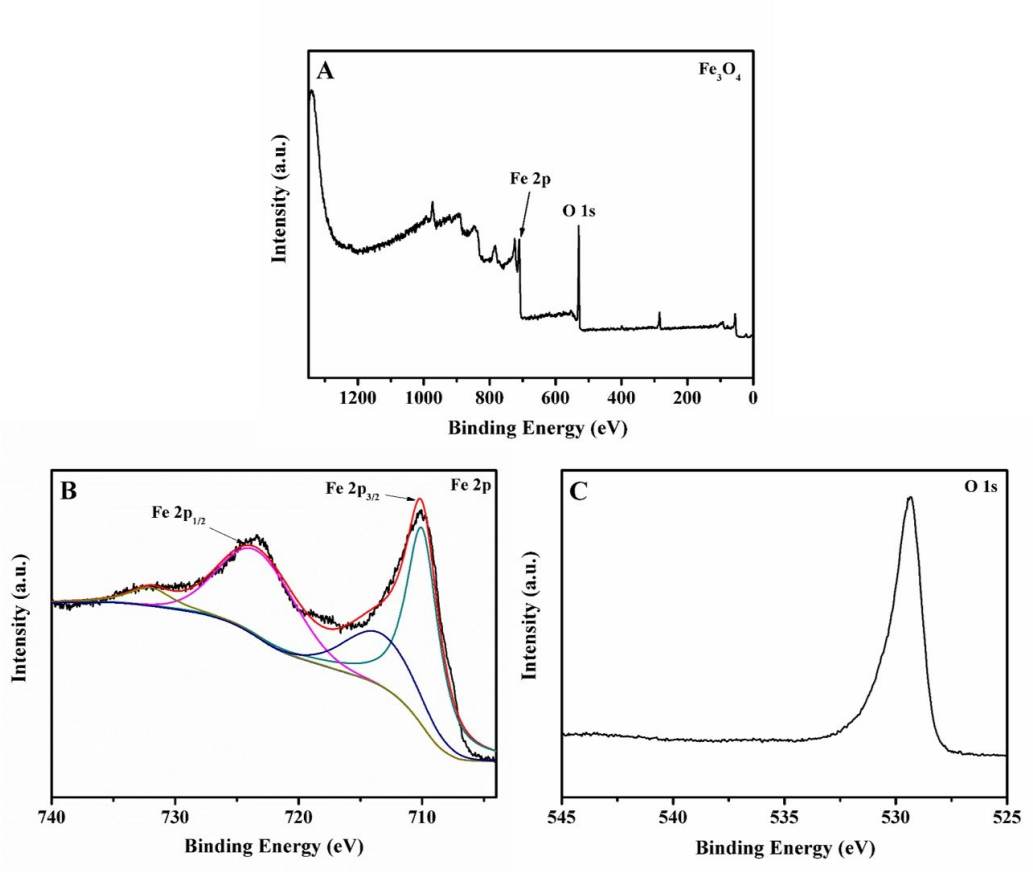

Figure S2. XPS spectrum of the nanoporous Fe_3O_4 (A) in the Fe 2p (B) and O 1s (C) regions.

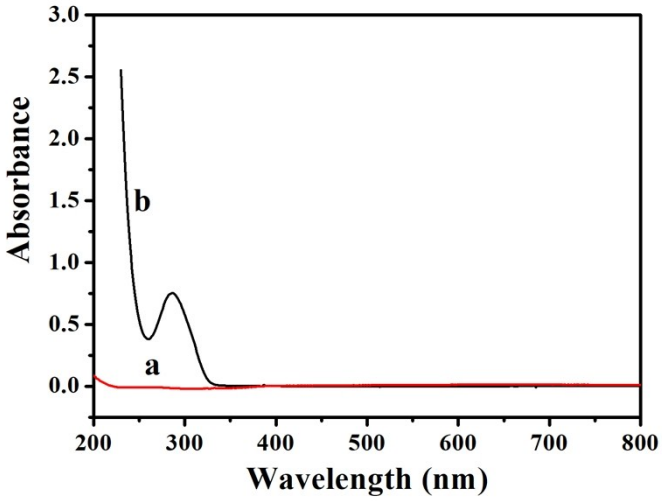

Figure S3. UV-vis spectroscopy of the nanoporous Fe_3O_4 (a) and streptavidin-Fe_3O_4 (b).

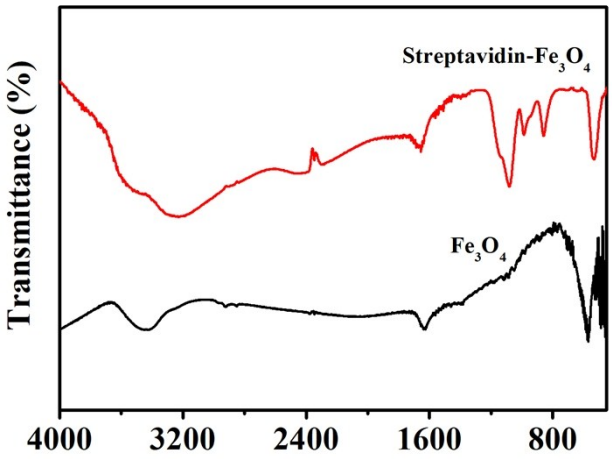

Figure S4. FTIR spectra of the nanoporous Fe_3O_4 and streptavidin-Fe_3O_4.

Table S1 Results for the determination of ZEN in urine by the prepared immunosensor.

Urine sample ($ng \cdot mL^{-1}$)	The addition content ($ng \cdot mL^{-1}$)	The detection content ($ng \cdot mL^{-1}$)	RSD (%, n=5)	Recovery (%)
0.17	1.00	1.20, 1.15, 1.23, 1.11, 1.13	4.3	99.4
	5.00	5.14, 4.83, 5.09, 5.10, 5.32	3.4	98.5
	10.0	10.5, 10.7, 10.0, 9.84, 10.4	3.5	101