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Electronic Supplementary Information

Highly active fluorogenic oxidase-mimicking NiO nanozymes

Dai Li,[a,b] Biwu Liu,[b] Po-Jung Jimmy Huang[b], Zijie Zhang[b], and Juewen Liu*[b]

a. National Institution of Drug Clinical Trial, Xiangya Hospital, Central South University, Changsha, 410008, China.

b. Department of Chemistry, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada.

Email: liujw@uwaterloo.ca

Materials and Methods

Chemicals. NiO (20 nm, US3356) and all the other metal oxides were purchased from US Research Nanomaterials (Houston, USA). 10-Acetyl-3,7-dihydroxyphenoxazine (Amplex Red, AR), 3,3',5,5'-tetramethylbenzidine (TMB) were form Sigma-Aldrich (St Louis, USA) and dissolved in DMSO to generate freshly prepared stock solutions. 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide (30 wt%) and resorufin were also purchased from Sigma-Aldrich and were dissolved in a fresh aqueous solution. 4',6-diamidino-2-phenylindole (DAPI), Alexa Fluor 488 phalloidin, fetal bovine serum (FBS), Dulbecco's modified eagle medium with nutrient mixture F-12 (DMEM/F-12), penicillin and streptomycin were from Thermo Fisher Scientific. Triton X-100 and 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) were from Sigma-Aldrich (St Louis, USA). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium acetate, acetic acid, 2-(*N*-morpholine) ethanesulfonic acid (MES), 3-(N-morpholino) propanesulfonic acid (MOPS), 2-Amino-2-(hydroxymethyl)-1,3-propanediol (Tris), sodium carbonate and sodium bicarbonate were obtained from Mandel Scientific (Guelph, ON, Canada). Nickel chloride and sodium hydroxide were from Sigma-Aldrich. Milli-Q water was used for all the experiments.

Oxidation assays. In a typical reaction, AR (1 μM) was dissolved in the HEPES buffer (50 mM, pH 7.5) and followed by adding NiO (final concentration of 1 mg mL^{-1}) or other metal oxides (MgO, TiO_2, Fe_3O_4, Fe_2O_3, CoO, CuO, ZnO, SnO_2, Y_2O_3, CeO_2). The fluorescence intensity was determined using a digital camera (with 470 nm excitation), and a SpectraMax M3 microplate reader (Molecular Devices LLC. CA), and the kinetics were recorded using a Varian Eclipse fluorescence spectrometer (Agilent Technologies, Santa Clara, CA) with excitation at 540 nm and emission at 580 nm at 25 °C. For the chromogenic substrates TMB and ABTS, the oxidization activities were determined using a digital camera and UV-vis spectrometry with a maximum adsorption at 652 nm and 420 nm respectively. For controlling pH, the buffers (50 mM) were acetate (pH 4.0 and 4.6), MES (pH 5.3), MOPS (pH 6.4), HEPES (pH 7.5), Tris (pH 8.5) and carbonate (pH 9.2).

TEM and DLS. The TEM sample was prepared by dropping an aqueous suspension of NiO on a copper grid and allowed to dry overnight before imaging on a Philips CM10 microscope. The size and ζ-potential of the metal oxides (200 μg mL^{-1}) were measured by DLS on a Nano ZS90 Zetasizer (Malvern) at 25 °C in the HEPES buffer (50 mM, pH 7.5).

XRD. XRD measurements were carried out using a PANalytical Empyrean X-ray diffractometer employing Co-Kα radiation (λ = 1.78901 Å). Samples were mounted on a silicon low background holder. XRD measurement shows that our NiO NPs were crystalline.

Cell culture and MTT assays. The HeLa cell line was obtained from the American Type Culture Collection (ATCC, MD, USA). The cells were cultured in DMEM/F12 medium, supplemented with 10% FBS and 100 U mL^{-1} penicillin, 100 mg mL^{-1} streptomycin at 37 °C using a humidified 5% CO_2 incubator. HeLa cells were seeded in 96-well plates at 50,000 cells per well in 100 mL of cell medium and incubated for 24 h. The culture medium was then replaced with 100 μL of freshly prepared culture medium containing 200 μg mL^{-1} of metal oxides (NiO,TiO_2, Fe_2O_3, CoO, ZnO, CeO_2). The cells were further incubated for 24 h, and then 10 μL of MTT stock solution (12 mg mL^{-1} in PBS) was added to each well to achieve a final concentration of 1 mg mL^{-1}, with the exception of the wells as blank to which 10 μL of PBS was added. After incubation for another 4h, 100 μL of the SDS-HCl solution (10% SDS in 0.01 M HCl) was added to the wells and incubated for another 2 h at 37°C. The absorbance was measured at 570 nm using a SpectraMax M3 microplate reader.

Confocal microscopy. HeLa cells were seeded onto 14 mm coverslips in 24-well plates with 50 000 cells per well and allowed to grow to 60% confluency. For cellular uptake tests, the cells were first incubated with 200 μg mL^{-1} of NiO and 10 μM AR or resorufin for 1 h at 37 °C. The cells were then washed twice with PBS buffer to remove non-internalized AR, NiO or resorufin. Then the cells were washed twice with 500 mL PBS buffer and fixed with fresh 4% paraformaldehyde for 10 min at room temperature. The cells were counterstained with DAPI for the cell nucleus, Alexa Fluor 488 phalloidin for the cell actin following the manufacturer's instructions. The coverslips were mounted on glass microscope slides with a drop of antifade mounting medium (Sigma-Aldrich Co., USA) to reduce fluorescence photobleaching. The prepared microscope slides were visualized under a laser scanning confocal fluorescence microscope (LSM510Meta, Carl Zeiss Inc., Thornwood, NY).

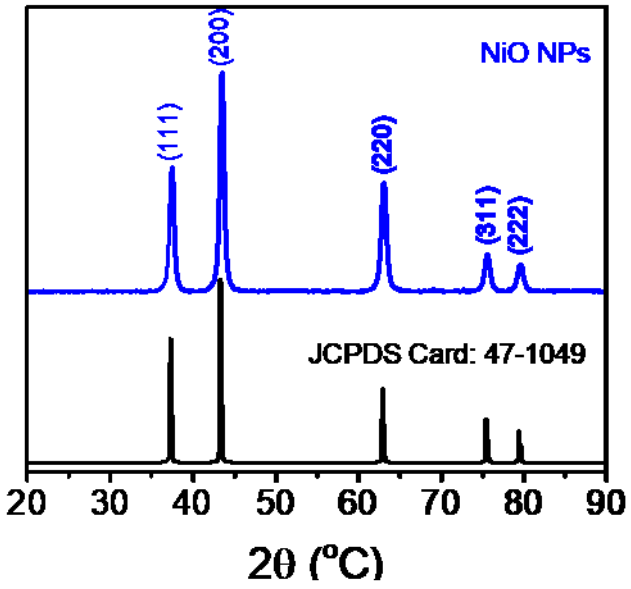

Figure S1. Powder X-ray Diffraction experiments were carried out to characterize the NiO NPs using a PANalytical Empyrean X-ray diffractometer employing Cu-Kα radiation (λ = 1.78901 Å. The pattern matches well with that of NiO confirming the composition of our materials.

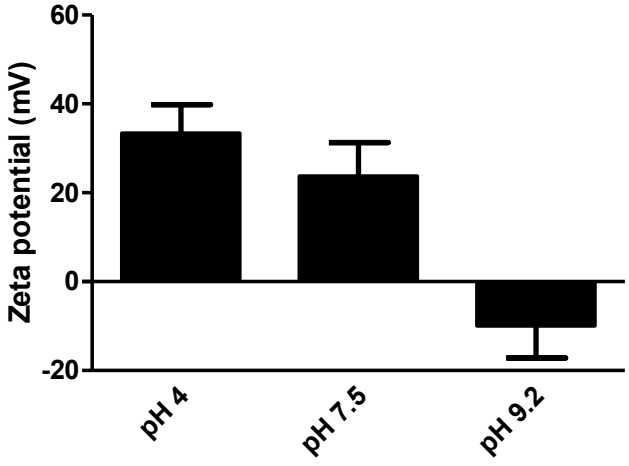

Figure S2. Zeta-potential of the NiO nanoparticles dispersed in buffers of different pH's. The pH was controlled by 50 mM acetate buffer (pH 4); HEPES buffer (pH 7.5); and carbonate buffer (pH 9.2).

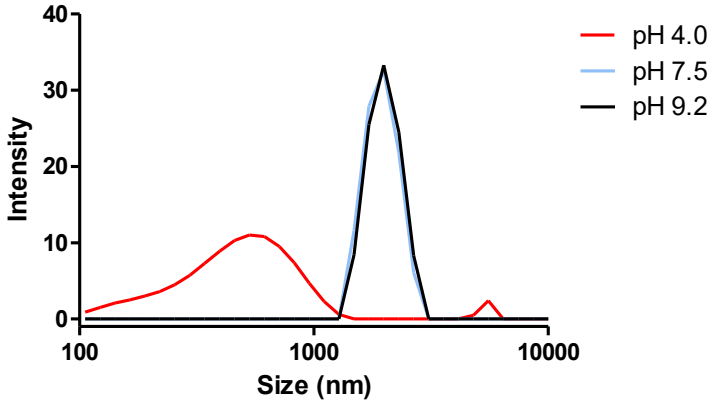

Figure S3. Hydrodynamic size measurement of NiO nanoparticles using dynamic light scattering. The *y*-axis is the scattering intensity. The size at pH 4.0 was smaller likely due to its higher positive charge for better electrostatic stabilization. The individual NiO particles was around 20 nm based on TEM, and thus the sample was extensively aggregated due to a lack of strong surface capping ligands.

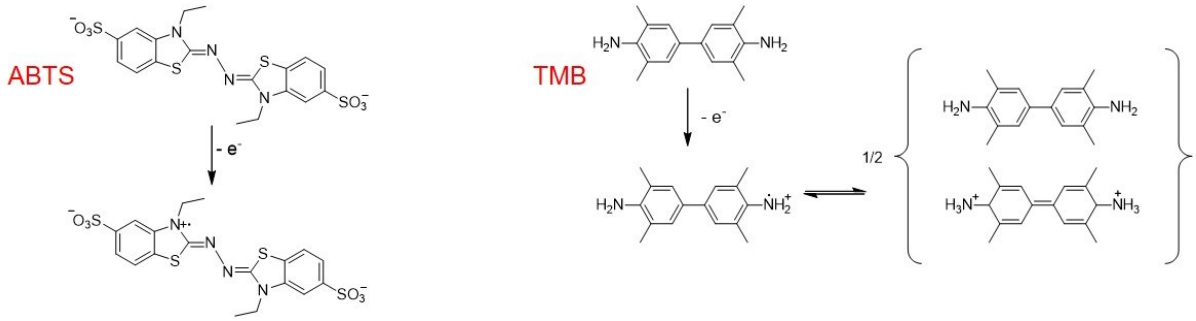

Figure S4. Reaction schemes of oxidation of ABTS and TMB.

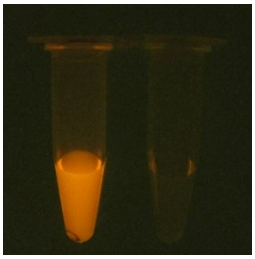

Figure S5. Photographs of the supernatant of a NiO solution after centrifugation (right), and the re-dispersed precipitant (left) after reacting with AR. This data indicated that the activity was from NiO instead of dissolved species.

Table S1. A comparison of the kcat value of our NiO nanozyme for AR and some other enzymes and nanozymes. Assuming a diameter of 20 nm for our NiO nanozyme (based on TEM), the molar concentration of NiO at 0.2 mg/mL is 12 nM. Due to a lack of literature data on oxidase nanozyme for AR, peroxidase data are also presented.

Catalyst	**Substrate**	k_{cat} **(s^{-1})**	k_{cat} **($\mu M\ s^{-1}\ L\ \mu g^{-1}$)**	**Reaction type**	**Reference**
HRP	AR		6.61×10^{-3}	Peroxidase	S1
CoO_xH-GO	AR		1.55×10^{-6}	Peroxidase	S1
Bi-AuNP	AR	150		Peroxidase	S2
CeO_2	TMB	0.16		Oxidase	S3
CeO_2	ABTS	2.8		Oxidase	S3
NiO	AR	0.14	$8.10\ X\ 10^{-9}$	Oxidase	This work

Additional References.

S1. C.-W. Lien, B. Unnikrishnan, S. G. Harroun, C.-M. Wang, J.-Y. Chang, H.-T. Chang and C.-C. Huang, *Biosens. Bioelectron.*, 2018, **102**, 510-517.

S2. C.-W. Lien, C.-C. Huang and H.-T. Chang, *Chem. Commun.*, 2012, **48**, 7952-7954.

S3. B. Liu, Z. Huang and J. Liu, *Nanoscale*, 2016, **8**, 13562-13567.