Supplementary Information

Optimization of Fe_3O_4 nanozyme activity via single amino acid modification mimicking an enzyme active site

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Experimental Section

Materials: Chemicals and materials were supplied by Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

Synthesis and characterization of Fe_3O_4 nanozymes: Histidine modified Fe_3O_4 nanozymes and alanine modified Fe_3O_4 nanozymes were synthesized according to the solvothermal method[1, 2] with some modifications. Briefly, 0.82 g $FeCl_3$•$6H_2O$ was dissolved in 40 mL ethylene glycol. When the solution became clear, 3.6 g NaAc and different concentrations of histidine (0.1 g, 0.5 g, 1 g) or alanine were added with continuous vigorous stirring for 30 min. The mixture was sonicated for 10 min and then transferred into a 50 mL Teflon-lined stainless steel autoclave and reacted at 200 °C for 12 h. After the reaction was completed, the autoclave was cooled to room temperature.

The products were washed several times with ethanol and dried at 60 °C.

As a comparison, naked Fe_3O_4 nanozyme were also synthesized using the same procedure but without the presence of histidine or alanine in the reaction system.

Morphology and structure images of Fe_3O_4 nanozymes were characterized with a transmission electron microscope (TEM, JEOL JEM-1400 120 kV) and a scanning electron microscope (SEM, Hitachi S-4800) respectively. X-ray Photoelectron Spectroscopy (XPS) technique has been used for the characterization of histidine and state of elements present in the investigated Fe_3O_4 nanozymes. XPS spectra were recorded on a Thermo ESCALAB 250 spectrometer using an Al Kα X-ray source. Thermo-gravimetric analysis (TGA) was performed to determine the amount of histidine on Fe_3O_4 nanozymes with a heating rate of 10^{o}C/min using a Perkin-Elmer Pyris TGA in synthetic N_2 atmosphere from 30^{o}C to 800^{o}C.

Kinetic analysis of Fe_3O_4 nanozymes as a peroxidase: The kinetic parameters of different Fe_3O_4 nanozymes and HRP were determined by monitoring the absorbance change at 652 nm on a iMark™ Microplate Reader (Bio-Rad, USA) in time course mode at room temperature. The kinetic assays were carried out using 0.2 μg Fe_3O_4 nanozymes or 0.1 ng HRP in 100 μL of reaction buffer (0.2 M NaAc buffer, pH 4.5) in the presence of H_2O_2 and TMB. The kinetic analysis of Fe_3O_4 with H_2O_2 as the substrate was performed by varying the concentrations of H_2O_2 with 0.8 mM TMB, and vice versa. The absorbance (652 nm) changes were calculated to molar concentration changes of TMB by using a molar absorption coefficient of 39000 M^{-1} cm^{-1} for TMB-derived oxidation products according to the Beer-Lambert Law[3]. All the measurements were performed at least in triplicate, and the values were then averaged. Results were given as mean ± the standard deviation (SD). The Michaelis–Menten constant was calculated using Lineweaver–Burk plots of the double reciprocal of the Michaelis−Menten equation, $\nu=V_{max}\times[S]/(K_M+[S])$ by GraphPad Prism 6.02 (GraphPad Software), where ν is the initial velocity, V_{max} is the maximal reaction velocity, [S] is the concentration of substrate and K_M is the Michaelis–Menten constant. The values of k_{cat} were calculated according to the formula: $k_{cat} = V_{max}/[S]$.

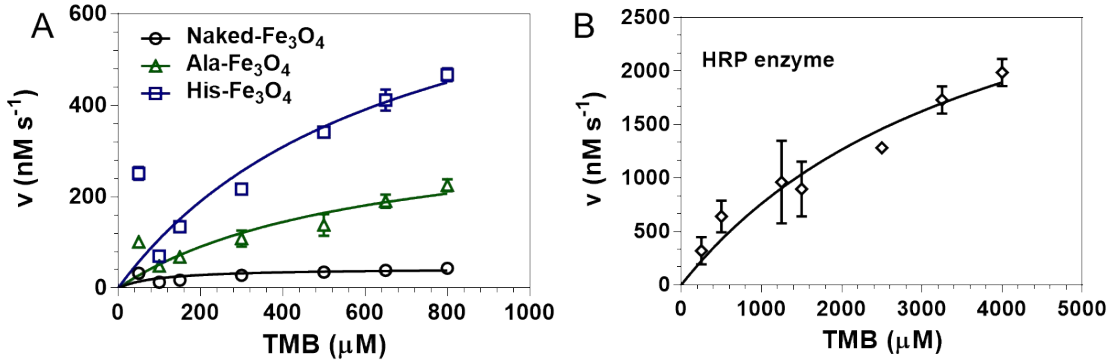
Kinetic analysis of Fe_3O_4 nanozymes as a catalase: The catalase-like activity assays of Fe_3O_4 were carried out at room temperature by measuring the generated oxygen

using a specific oxygen electrode on Multi-Parameter Analyzer (JPSJ-606L, Leici, China). The kinetic assays were carried out using 75 μg Fe_3O_4 nanozyme in 3 ml of 0.2 M NaAc buffer (pH 7.0) with different concentrations of H_2O_2. The velocity was calculated based on the generated O_2 (unit: mg/L) under a certain time. All the measurements were performed at least in triplicate, and the values were then averaged. Results were given as mean ± the standard deviation (SD). The Michaelis–Menten constant was calculated using the same method as mentioned above.

Supplementary Table. Comparing the influence of 20 standard amino acids modification on the affinity (K_M) of Fe_3O_4 nanozyme for H_2O_2.

Modifications	K_M **(mM)** (H_2O_2)
Naked*	458.9±29.1
Histidine	37.99±7.6
Alanine	226.6±18.3
Phenylalanine	1198.5±376.9
Glycine	578.8±150.8
Leucine	1132.8±338.6
Isoleucine	887.5±189.1
Valine	346.7±71.1
Serine	315.6±73.6
Threonine	768.6±174.7
Proline	236.5±42.7
Tyrosine	554.2±90.5
Tryptophan	2951.1±1348.2
Glutarnine	648.6±154.1
Glutamic acid	376.2±71.3
Asparagine	949.6±210.3
Aspartic acid	390.6±72.5
Lysine	340.1±50.9
Arginine	262.9±68.5
Cystine	354.3±104.8
Methionine	2528.4±921.1

Note: *, naked means no modification.



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Michaelis-Menten	Naked-Fe_3O_4	Ala-Fe_3O_4	His-Fe_3O_4	HRP enzyme
[E] (M)	4.9×10^{-14}	4.9×10^{-14}	4.9×10^{-14}	2.3×10^{-13}
V_{max} (nM s^{-1})	43.27±5.49	830.7±266.1	361.5±95.73	3765±794.1
K_M (mM)	101.4±48.29	676±388.8	598±297.6	3956±1406
k_{cat} (10^6 s^{-1})	0.884±0.012	17±5.43	7.38±1.95	14.4±3.45
k_{cat}/K_M (10^{10} s^{-1} M^{-1})	0.87±0.018	2.5±0.81	12±0.32	0.36±0.09

Supplementary Figure. Histidine modification increased the activity of Fe_3O_4 nanozyme for TMB substrate. A. Kinetics for Fe_3O_4 nanozymes; B Kinetics for HRP; C, Comparison of the parameters.

Theoretical calculation. The Fe_3O_4 (111) surface was chosen to investigate the Fe_3O_4 nanozyme[4, 5] and the adsorption of H_2O_2 on which was studied using (2×2) supercell model. All calculations were performed using VASP with plane-wave pseudopotential method.[6, 7] The electronic exchange and correlation effects were described by the Perdew-Burke-Ernzerhof (PBE) function[8] with the generalized gradient approximation (GGA) and the core electrons were described by the full-potential projector augmented wave (PAW) method.[9, 10] An energy cutoff of 550 eV for the plane-wave expansion was used and the forces on the relaxed atoms were less than -0.03 eV/Å. The spin-polarization was taken into account in all calculations. The Brillouin zone was sampled by (5×5×1) k-point grid generated within the Monkhorst–Pack scheme.

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