Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

Accelerating peroxidase mimicking nanozymes using DNA

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Materials and Methods

Chemicals. All of the DNA samples were from Integrated DNA Technologies (IDT, Coralville, IA, USA). Their sequences and modifications are shown in Table S1. Fe_3O_4 NPs (637106), nanoceria (catalog number: 289744, 20% dispersed in 2.5% acetic acid), 3,3',5,5'-tetramethylbenzidine (TMB), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 30 wt% H_2O_2 solution, polystyrene sulfonate (PSS, catalog number: 527483), polyacrylic acid, sodium salt (PAA, catalog number: 416037), and guanosine monophosphate (GMP) were purchased from Sigma-Aldrich. Fe_2O_3 NPs (Stock number: US3200) were purchased from US Research Nanomaterials (Houston, TX). Sodium acetate, sodium citrate, sodium phosphate, sodium chloride, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), 2-(N-morpholino) ethanesulfonic acid (MES) were from Mandel Scientific (Guelph, ON, Canada). Milli-Q water was used for all of the experiments.

Table S1. The sequences and modifications of DNA used in this work

DNA names	Sequences (from 5′ to 3′) and modifications
Alexa-DNA	TCA CAG ATG CGT-Alexa Fluoro 488
FAM-24 mer	FAM-ACG CAT CTG TGA AGA GAA CCT GGG
FAM-A_5	FAM-AAA AA
FAM-A_{15}	FAM-AAA AAA AAA AAA AAA
FAM-A_{30}	FAM-AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA
A_5	AAA AA
C_5	CCC CC
C_{10}	CCC CCC CCC C
A_{15}	AAA AAA AAA AAA AAA
T_{15}	TTT TTT TTT TTT TTT
C_{15}	CCC CCC CCC CCC CCC
G_{15}	GGG GGG GGG GGG GGG
C_{30}	CCC CCC CCC CCC CCC CCC CCC CCC CCC CCC

Modification of Iron Oxide NPs. To coat Fe_3O_4 NPs with polymers, PSS or PAA (final concentration 10 mg/mL) was mixed with 1 mg/mL of the NPs. After overnight stirring, the excess polymers were removed by centrifugation (10 000 rpm, 10 min) and the resulting conjugates were washed with Milli-Q water three times. PSS-modified Fe_2O_3 NPs were prepared in a similar way. Phosphate (1 mM), GMP (1 mM), and DNA (G_{15}, 500 nM) modified NPs were prepared by incubating them with designated concentrations of Fe_3O_4 NPs and capping agents in acetate buffer (pH 4) for at least 10 min without further purification.

Transmission Electron Microscopy (TEM) and UV-vis spectroscopy. The particle size and morphology of Fe_3O_4 NPs was studied using TEM (Philips CM10). The TEM sample was prepared by dropping Fe_3O_4 NPs dispersion (50 μg/mL) into a copper grid and was allowed to dry overnight at room temperature. The UV-vis spectra of Fe_3O_4 NPs, TMB and oxidized TMB were obtained after reacting H_2O_2 (20 mM) with TMB (0.5 mM) at different conditions for 15 min using a UV-vis spectrometer (Agilent 8453A). C_{30} DNA (500 nM) was used to modify Fe_3O_4 NPs. The concentration of Fe_3O_4 NPs was 50 μg/mL for most experiments unless otherwise specified. The visual images were taken by a digital camera.

ζ-potential measurement . The ζ-potential was measured by dynamic light scattering (DLS) using a Nanosizer ZS90 (Malvern). Effects of pH, buffer, and surface modification on the ζ-potential of Fe_3O_4 NPs and Fe_2O_3 NPs were tested and the reaction conditions were specified in Table S2. The temperature was maintained at 25 °C during the measurements.

Inhibition of DNA adsorption. To study the effect of H_2O_2 and TMB on DNA adsorption kinetics, Alexa-DNA (50 nM) was dissolved in a pH 4 acetate buffer (10 mM) with varying concentrations of H_2O_2 or TMB. The initial fluorescence of the free DNA (F_0) was monitored for 3 min (excitation at 485 nm, emission at 535 nm) using a microplate reader (Infinite F200Pro, Tecan). After a quick addition of Fe_3O_4 NP dispersion (final concentration 25 μg/mL), the fluorescence was monitored for another 30 min. The fluorescence was then normalized based on the initial intensity (F/F_0).

Peroxidase activity assays. In a typical assay, 1 μL of TMB in DMSO solution (50 mM) was added into 100 μL of Fe_3O_4 NPs (final concentration 50 μg/mL) with or without DNA at pH 4 (acetate buffer, 10 mM), followed by a quick mixing to avoid TMB precipitation. The absorbance at 652 nm was recorded in the kinetic mode using the microplate reader. Afterwards, H_2O_2 (10 mM) was added to initiate the reaction and the absorbance was monitored for another 30 min. The effect of DNA sequence was studied using homo poly DNAs with different bases (A_{15}, T_{15}, C_{15}, or G_{15}, 500 nM each). Poly C DNAs (5, 10, 15, or 30 mer) were used to investigate the DNA length effect. The total concentration of nucleotides of cytosine was set at 3 μM. For the DNA concentration and pH effect studies, C_{30} was used as the capping agent. The solution pH was controlled by using designated buffers (acetate buffer for pH 4 and 5, MES for pH 6, and HEPES for pH 7 and 8). The oxidation kinetics of ABTS at various salt concentrations were studied in a similar way except that the final concentration of ABTS was 1 mM. The absorbance at 420 nm was recorded. NaCl was used to adjust the ionic strength of the reaction.

Activity of CeO_2 NPs. DNA (A_5, 5 μM) was incubated with CeO_2 NPs (0.1 mg/mL) for 15 min before adding TMB (1 mM). To study the DNA effect on the peroxidase activity of CeO_2, a mixture of TMB and H_2O_2 solution was added into CeO_2 or DNA-CeO_2. The final concentration of H_2O_2 was 10 mM. All the reactions were performed in a pH 4 acetate buffer (10 mM). The photographs were taken after 30 min reaction time.

Gel electrophoresis. The conjugate was prepared by mixing FAM-24 mer (200 nM) and Fe_3O_4 NPs (25 μg/mL) in acetate buffer (pH 4.0, 10 mM) and 10 mM H_2O_2 was added. As a control, 50 μM of Fe^{2+} was used to cleave DNA at the same reaction condition. For denaturing gels, DNA-Fe_3O_4 NPs in the absence or the presence of H_2O_2 was loaded onto 15% polyacrylamide gel with 8 M urea. The gels were then imaged using blue LED epi excitation (Bio-Rad, Chemidoc MP).

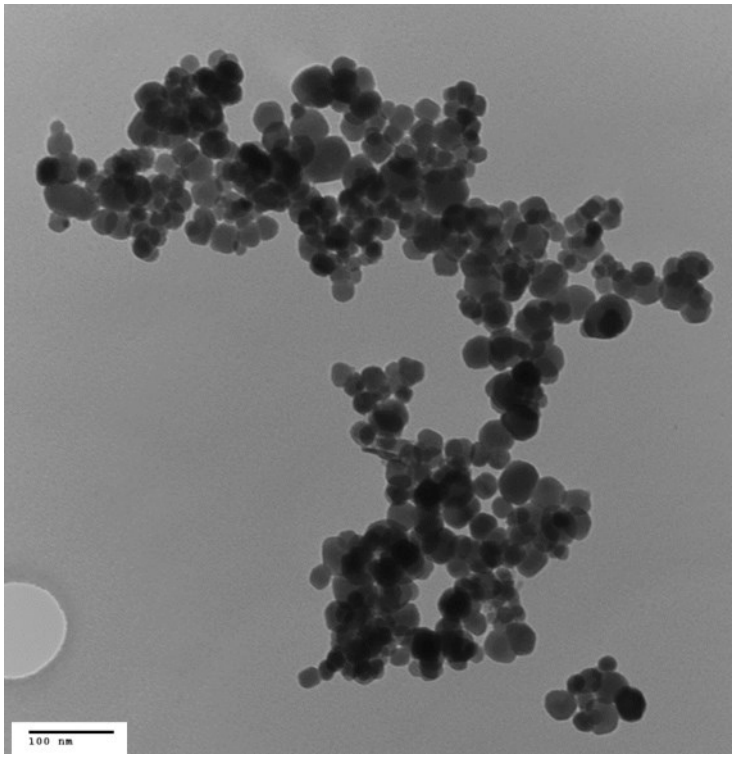

Figure S1. A TEM micrograph of Fe_3O_4 NPs used in this work.

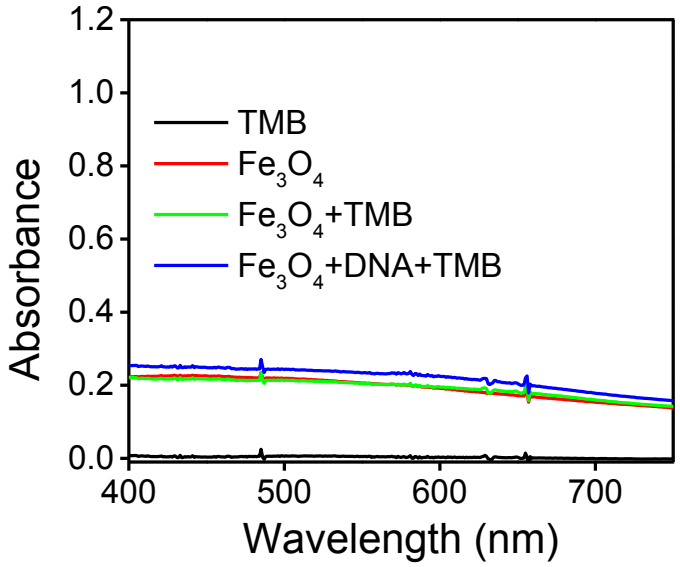

Figure S2. UV-vis absorbance spectra of non-oxidized TMB, Fe_3O_4 NPs, Fe_3O_4 NPs-TMB, Fe_3O_4 NPs-DNA with TMB. The final concentration of TMB is 0.5 mM. DNA (C_{30}, 500 nM) modified Fe_3O_4 NPs (50 μg/mL) were prepared as mentioned above. The spectra were collected after adding H_2O_2 (20 mM) for 15 min. Fe_3O_4 NPs do not show any specific absorption peak in the visible range (400 nm-750 nm). Neither Fe_3O_4 NPs nor DNA modified Fe_3O_4 NPs cause TMB oxidation in the absence of H_2O_2.

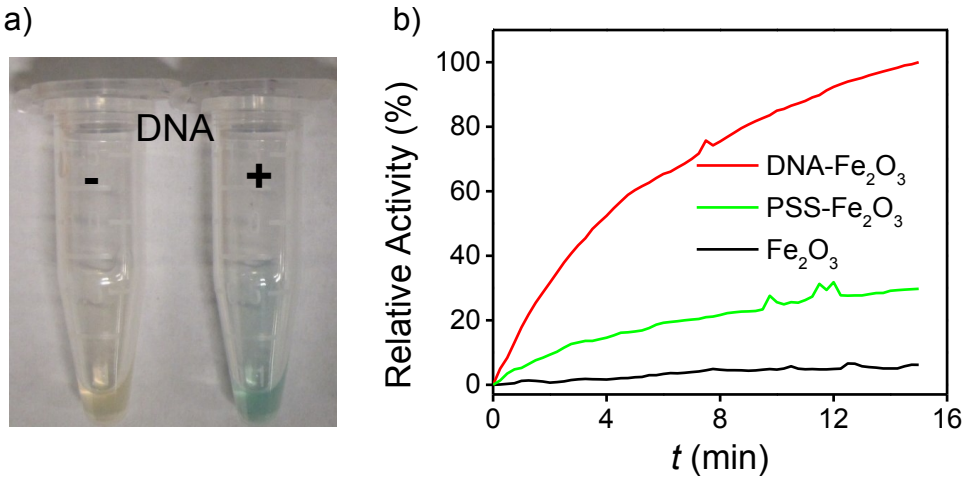

Figure S3. DNA enhanced peroxidase activity with Fe_2O_3 NPs. (a) Photograph shows the colour change of TMB (0.5 mM) catalysed by (-) bare NPs without DNA and (+) DNA modified Fe_2O_3 NPs. (b) Kinetic traces of TMB (0.5 mM) oxidation catalysed by bare Fe_2O_3, PSS modified Fe_2O_3, and DNA modified Fe_2O_3. The concentration of Fe_2O_3 NPs is 50 µg/mL and that of DNA (C_{30}) is 500 nM. The highest absorbance at 15 min is set as 100% activity. PSS-Fe_2O_3 conjugates were prepared by incubating PSS (1 wt %) with Fe_2O_3 NPs for overnight, followed by centrifugation, and washing three times. Finally, the conjugates were dispersed in Milli-Q water. All reactions were performed in pH 4 buffer (acetate, 10 mM).

Table S2. ζ-potential of iron oxides at various conditions: pH, buffer, surface, and substrate.

pH	Nanoparticles	Buffer	Surface Modification	Substrate	ζ-potential (mV)
4.0	Fe_3O_4	Acetate	No	No	12.91 ± 3.66
4.0	Fe_3O_4	Acetate	No	TMB	28.70 ± 0.98
4.0	Fe_3O_4	Acetate	DNA	No	-36.68 ± 1.71
4.0	Fe_3O_4	Acetate	DNA	TMB	-25.30 ± 0.14
4.0	Fe_3O_4	Acetate	PAA	No	-34.80 ± 1.17
4.0	Fe_3O_4	Acetate	PSS	No	-34.80 ± 1.28
4.0	Fe_3O_4	Phosphate	No	No	-20.37 ± 0.23
7.6	Fe_3O_4	HEPES	No	No	-27.40 ± 0.61
7.6	Fe_3O_4	Phosphate	No	No	-47.60 ± 1.15
4.0	Fe_2O_3	Acetate	No	No	12.33 ± 0.67
4.0	Fe_2O_3	Acetate	DNA	No	-34.17 ± 0.51

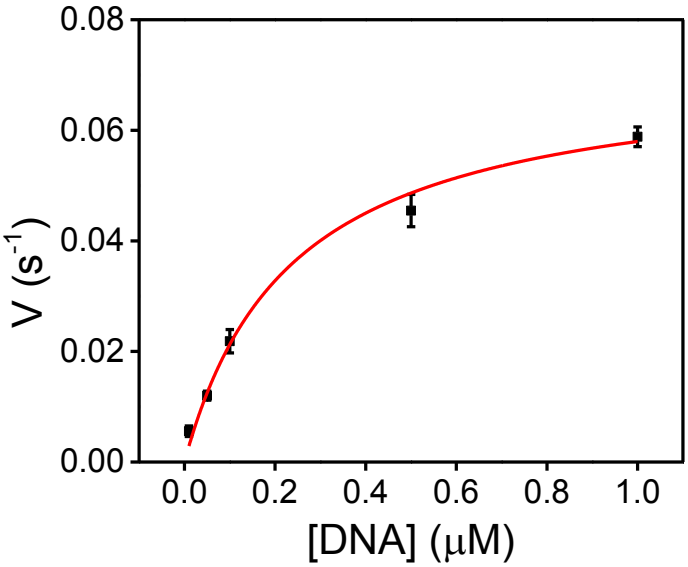

Figure S4. DNA concentration dependent acceleration of TMB oxidation. V is the initial reaction velocity. The error bars represent three measurements. Fe_3O_4 NPs were incubated with different concentrations of C_{30} (10, 50, 100, 500, 1000 nM) at pH 4 acetate buffer for 10 min, followed by adding TMB (0.5 mM) and H_2O_2 (10 mM). The initial reaction velocity is fitted to the equation: $V = \frac{Vmax*[DNA]}{K+[DNA]}$. V_{max} is saturated at 0.072 s^{-1}, indicating DNA adsorption reached saturation as well.

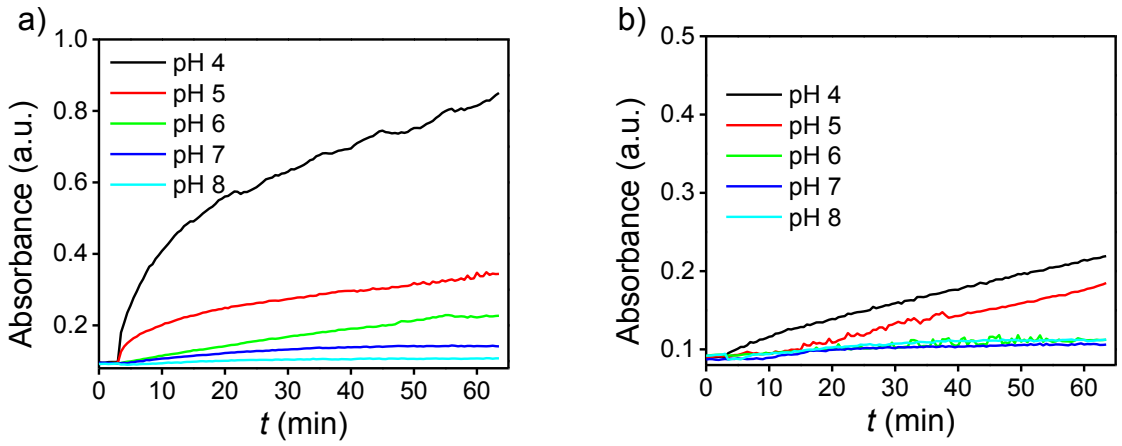

Figure S5. Kinetics of TMB oxidation catalyzed by (a) DNA-Fe_3O_4 NPs and (b) bare Fe_3O_4 NPs at various pH values. Fe_3O_4 NPs (50 μg/mL) were incubated with DNA (C_{30}, 500 nM) at different buffers (acetate buffer for pH 4 and 5, MES for pH 6, and HEPES for pH 7 and 8, 10 mM for all buffers) for 10 min. TMB (0.5 mM) and H_2O_2 (10 mM) were then subsequently added and the absorbance at 652 nm was monitored for another 60 min.

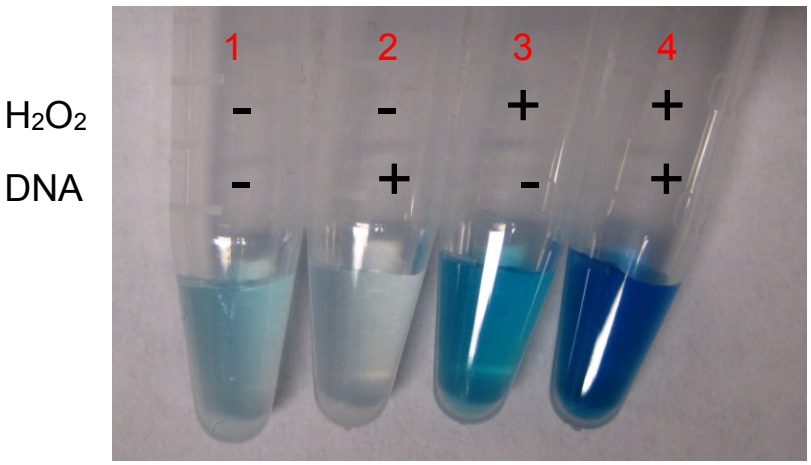

Figure S6. Effect of DNA on the (tubes 1 and 2) oxidase and (tubes 3 and 4) peroxidase activity of CeO_2 NPs. The concentration of CeO_2 is 0.1 mg/mL and that of TMB is 1 mM. H_2O_2 (10 mM) was added in tube 3 and 4 to mimic the peroxidase activity. The image was taken after 30 min reaction by digital camera. The addition of A_5 DNA (5 μM) inhibits TMB oxidation in the absence of H_2O_2, indicated by the weaker color of tube 2 compared to tube 1. This inhibition is consistent with our previous report.[S1] Interestingly, DNA can enhance TMB oxidation in the presence of H_2O_2, suggested by the stronger blue color of tube 4 compared to tube 3. Taken together, DNA can decrease the oxidase activity and increase the peroxidase activity of CeO_2.

Additional references

S1. Pautler, R.; Kelly, E. Y.; Huang, P.-J. J.; Cao, J.; Liu, B.; Liu, J., Attaching DNA to Nanoceria: Regulating Oxidase Activity and Fluorescence Quenching. *ACS Appl. Mater. Interfaces* **2013,** *5* (15), 6820-6825.