Electronic supplementary information

Comparison of the peroxidase activities of iron oxide nanozyme with DNAzyme and horseradish peroxidase

Chang Lu[1, 2], Mohamad Zandieh[2], Jinkai Zheng[1*] and Juewen Liu[2*]

1. Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China

Email: zhengjinkai@caas.cn

2. Department of Chemistry, Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, Ontario, Canada

Email: liujw@uwaterloo.ca

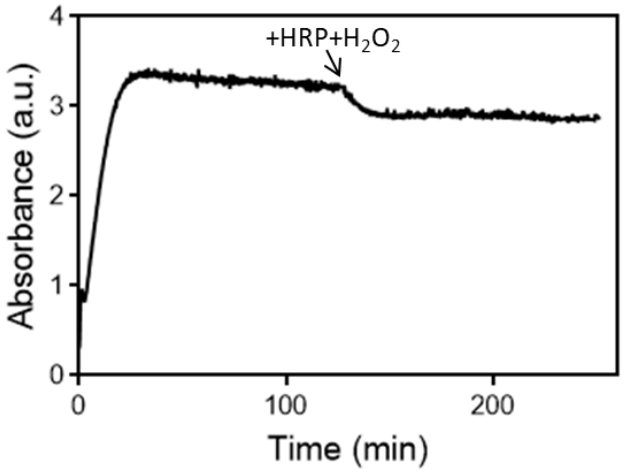

Figure S1. Stability of HRP after one catalysis of TMB and then adding more HRP and H_2O_2 in pH 4 acetate buffer. The absorbance did not increase, suggesting that the substrate TMB was consumed.

Calculations #1: Turnover Rates of G4, HRP and Fe_3O_4 NPs (50 nm)

Length of path of light (100 μL sample, 0.6 cm diameter of the well):

$$l = \frac{V}{A} = \frac{0.1}{\pi \times 0.3^2} = \frac{0.1\ cm^3}{0.283\ cm^2} = 0.35\ cm$$

Concentration of oxidized TMB:

(A) $G4$: $$[ox\_TMB] = \frac{Abs_{652}}{\varepsilon_{652} \times l} = \frac{1.39-0.26}{3.9\times10^4 \times 0.35} = 82.8\ \mu M$$

(B) HRP: $$[ox\_TMB] = \frac{Abs_{652}}{\varepsilon_{652} \times l} = \frac{3.29-0.22}{3.9\times10^4 \times 0.35} = 225\ \mu M$$

(C) Fe_3O_4: $$[ox\_TMB] = \frac{Abs_{652}}{\varepsilon_{652} \times l} = \frac{0.42-0.14}{3.9\times10^4 \times 0.35} = 20.5\ \mu M$$

Catalytic turnovers during 20 min:

(A) $G4$: $$n = \frac{Conc.\ of\ ox-TMB}{Conc.of\ G4} = \frac{82.8\times10^{-6}\ M}{0.5\times10^{-6}\ M} = 166\ catalytic\ turnovers$$

(B) HRP: $$n = \frac{Conc.\ of\ ox-TMB}{Conc.of\ HRP} = \frac{225\times10^{-6}\ M}{0.5\times10^{-9}\ M} = 4.5\times10^5\ catalytic\ turnovers$$

(C) Fe_3O_4: $$n = \frac{Conc.\ of\ ox-TMB}{Conc.of\ Fe_3O_4 NPs} = \frac{20.5\times10^{-6}\ M}{0.5\times10^{-9}\ M} = 4.1\times10^4\ catalytic\ turnovers$$

$$n = \frac{Conc.\ of\ ox-TMB}{Conc.of\ surface\ Fe\ atoms} = \frac{20.5\times10^{-6}\ M}{28\times10^{-6}\ M} = 0.73\ catalytic\ turnovers$$

Turnover rate:

(A) $G4$: $$T = \frac{166}{1200\ s} = 0.14\ s^{-1}$$

(B) HRP: $$T = \frac{4.5\times10^5}{1200\ s} = 375\ s^{-1}$$

(C) Fe_3O_4: $$T = \frac{4.1\times10^4}{1200\ s} = 34.2\ s^{-1}$$ (each NP= 1 active site)

$$T = \frac{0.73}{1200\ s} = 6.1\times10^{-4}\ s^{-1}$$ (each surface Fe= 1 active site)

Calculations #2: Rates Normalized to Mass Concentration of G4, HRP and Fe_3O_4 NPs (50 nm)

(A) $G4$: $r = \frac{slope\ of\ the\ kinetics\ curve}{mass\ concentration} = \frac{9.2\times10^{-4}\ s^{-1}}{3.18\ mg.L^{-1}} = 2.9 \times 10^{-4}\ L.mg^{-1}.s^{-1}$

(B) HRP: $r = \frac{slope\ of\ the\ kinetics\ curve}{mass\ concentration} = \frac{2.5\times10^{-3}\ s^{-1}}{0.02\ mg.L^{-1}} = 0.125\ L.mg^{-1}.s^{-1}$

(C) Fe_3O_4: $r = \frac{slope\ of\ the\ kinetics\ curve}{mass\ concentration} = \frac{2.2\times10^{-4}\ s^{-1}}{110\ mg.L^{-1}} = 2 \times 10^{-6}\ L.mg^{-1}.s^{-1}$

Calculations #3: Molar Concentration of Fe_3O_4 NPs (50 nm) and the Surface Fe Atoms

Surface area of each Fe_3O_4 NP: $A_{NP} = 4\pi \times 25^2 = 7854\ nm^2$

Surface area of each hexagonal surface unit cell: $A_{unit\ cell} = 2 \times \frac{\sqrt{3}}{4} \times (0.594)^2 = 0.305\ nm^2$

Number of surface unit cells per NP: $N_{unit\ cell} = \frac{A_{NP}}{A_{unit\ cell}} = \frac{7854}{0.305} = 25751$

Number of surface Fe atoms per NP: $N_{surface\ Fe} = 25751 \times 2 = 51502$ (*)

Mass of each NP: $mass = D \times V = 5.17\ {}^{g}/_{cm^3} \times \frac{4}{3}\ \pi\ (25 \times 10^{-7})^3 = 3.38 \times 10^{-16}\ g$

Number of NPs per 110 mg Fe_3O_4 NP: $N_{NPs} = \frac{110\times10^{-3}\ g}{3.38\times10^{-16}\ g} = 3.25 \times 10^{14}\ nanoparticles$ (**)

Molar concentration of Fe_3O_4 NP: $N_{NPs} = 3.25 \times 10^{14} \times \frac{1\ mol}{6.022\times10^{23}} = 0.5\ nmol$

Using (*) and (**) ↘

Number of total surface Fe atoms: $N_{total\ surface\ Fe} = 51502 \times 3.25 \times 10^{14} = 1.67 \times 10^{19}$

Concentration of total surface Fe atoms (catalytic active sites):

$$[E] = 1.67 \times 10^{19} \times \frac{1\ mol}{6.022\times10^{23}} = 28\ \mu mol$$