Electronic Supplementary Material (ESI) for RSC Advances


# Regulation of hemin peroxidase catalytic activity by arsenic-binding aptamers for the colorimetric detection of arsenic(III)

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# Electronic Supplementary Information

(Including Experimental details, Supplementary figures and table)

## Experimental details:

### (1) Reagents and chemicals

The sequence of Ars-3 aptamer is reference to previous literatures,[1] and was synthesized by Invitrogen China Limited/Applied Biosystems China Limited (Shanghai, China). Its sequence is 5′-GGTAATACGACTCACTATAGGGA GATACCAGCTTATTCAATTTTACAGAACAACCAACGTCGCTCCGGGTACTTCTTCATCGAGATAGTAAGTGCAATCT-3′. Before use, the Ars-3 aptamer was dissolved in 50 mM N-(2-hydroxyethyl) piperazine-N-2-ethanesulfonic acid (HEPES) buffer solution of pH 7.2. Hemin was obtained from Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). 3,3',5,5'-tetramethylbenzidine (TMB) was obtained from Sangon Biotechnology Inc. (Shanghai, China). Hydrogen peroxide ($H_2O_2$, 30%) and dimethyl sulphoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 96-well microplate was purchased from Thermo Fisher Scientific Inc. (Nunclon, Denmark). Unless otherwise mention, all other reagents were analytical grade and used without further purification or treatment. Ultrapure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout.

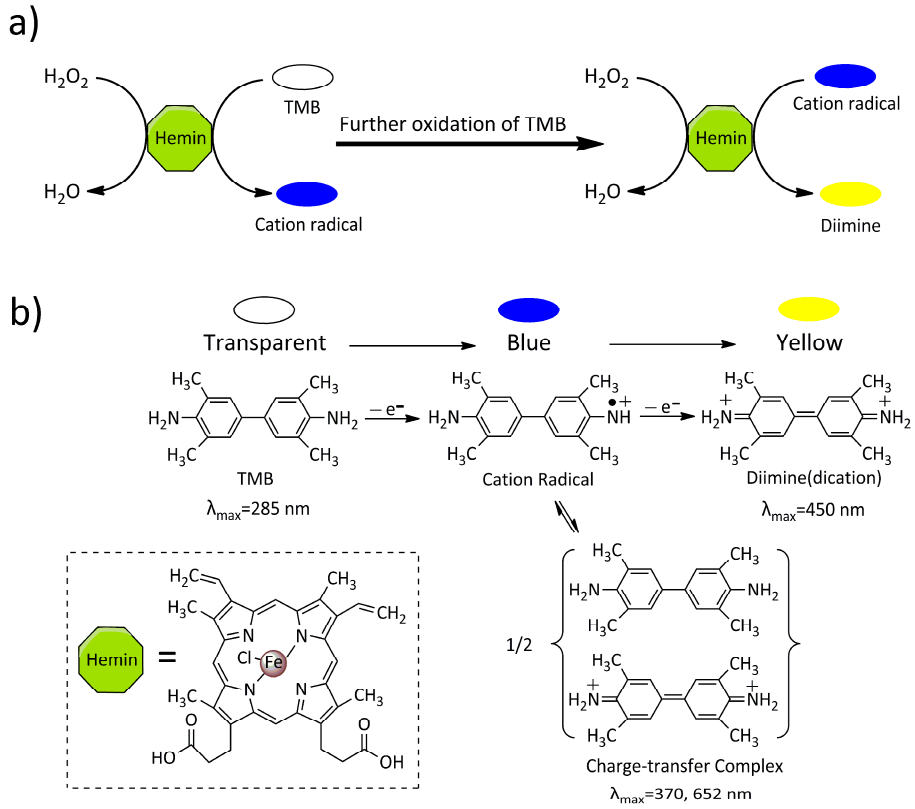
### (2) Peroxidase activity measurements

The absorption intensity at 422 nm and spectra of TMB in 10 mM HEPES buffer (pH 7.2) oxidized by hemin were determined to evaluate the peroxidase activity of hemin. In brief, an appropriate volume of hemin stock solution (5 mM in DMSO) was mixed with 100 μL $H_2O_2$ (200 mM) in a 2 mL plastic tube, and then some HEPES buffer (pH 7.2) was added to give a final volume of 450 μL in the presence of 0.05% (w/v) Triton X-100. After the addition of another 50 μL TMB stock solution (5 mM in DMSO), the absorption intensity at 422 nm and spectra of reaction mixtures were recorded by Microplate Spectrophotometer M200 Pro (Tecan Group Ltd, Switzerland).

### (3) Procedure of As(III) determination

An appropriate volume of 500 nM Ars-3 aptamer solution and 5 μL $NaAsO_2$ solution [As(III)] with varying concentration were mixed thoroughly in a 2 mL plastic tube, and then diluted to 100 μL with HEPES buffer (pH 7.2) and incubated at 25 °C for 15 min. The blank sample was added 5 μL $ddH_2O$ instead As(III) solution. Subsequently, the optimal amount of hemin was added into the above mixed solutions and incubated at 25 °C for another 15 min. Finally, 100 μL $H_2O_2$ (200 mM), 50 μL TMB stock solution (5 mM in DMSO) and appropriate volume of HEPES buffer (pH 7.2) were added to give a final volume of 500 μL in the presence of 0.05% (w/v) Triton X-100. After incubation at 25 °C for a suitable time, 200 μL of above samples were immediately moved into a 96-well microplate for colorimetric assay. The absorption spectra (the wavelength range from 300 nm to 800 nm) and intensity at 442 nm were measured by Microplate Spectro-photometer M200 Pro. The absorbance in sample solutions ($A$) and the blank solution without arsenic ($A_0$) were recorded. The value of $\Delta A=A-A_0$ was calculated to evaluate As(III) detection throughout. To test the selectivity of present method, other metal salts including $Na_2HAsO_4$ [As(V)], $Pb(NO_3)_2$, $Hg(NO_3)_2$, $Cd(NO_3)_2$, $AgNO_3$, $CaCl_2$, $ZnCl_2$, $MgSO_4$, $MnSO_4$, $NiSO_4$, $CuSO_4$ and $FeSO_4$ were used.

## Supplementary figures and table:




Scheme S1. Illustration of hemin with peroxidase-like activity and its application for TMB oxidation. (a) Oxidation of TMB by hemin in the presence of $H_2O_2$. (b) Chemical structures and the colour changes of TMB in the different stages of oxidation. Inset: Chemical structure of hemin.

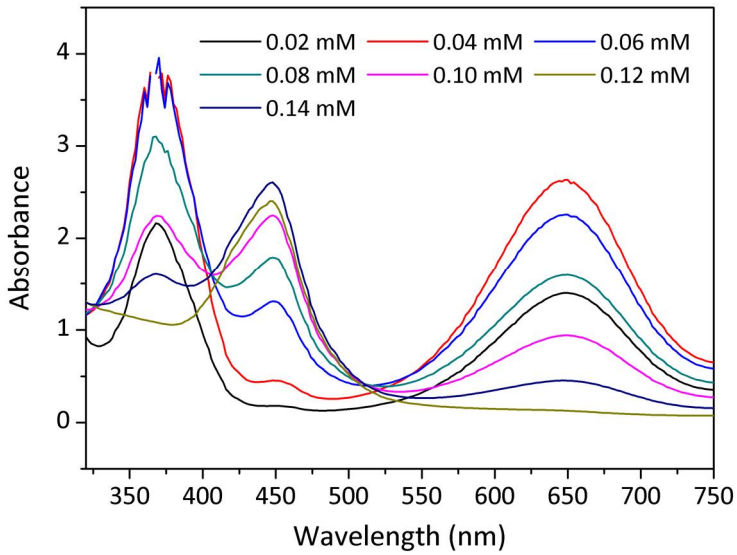



Fig. S1 The absorption spectra of TMB in 10 mM HEPES buffer (pH 7.2) oxidized by varying concentrations of hemin in 30 min.

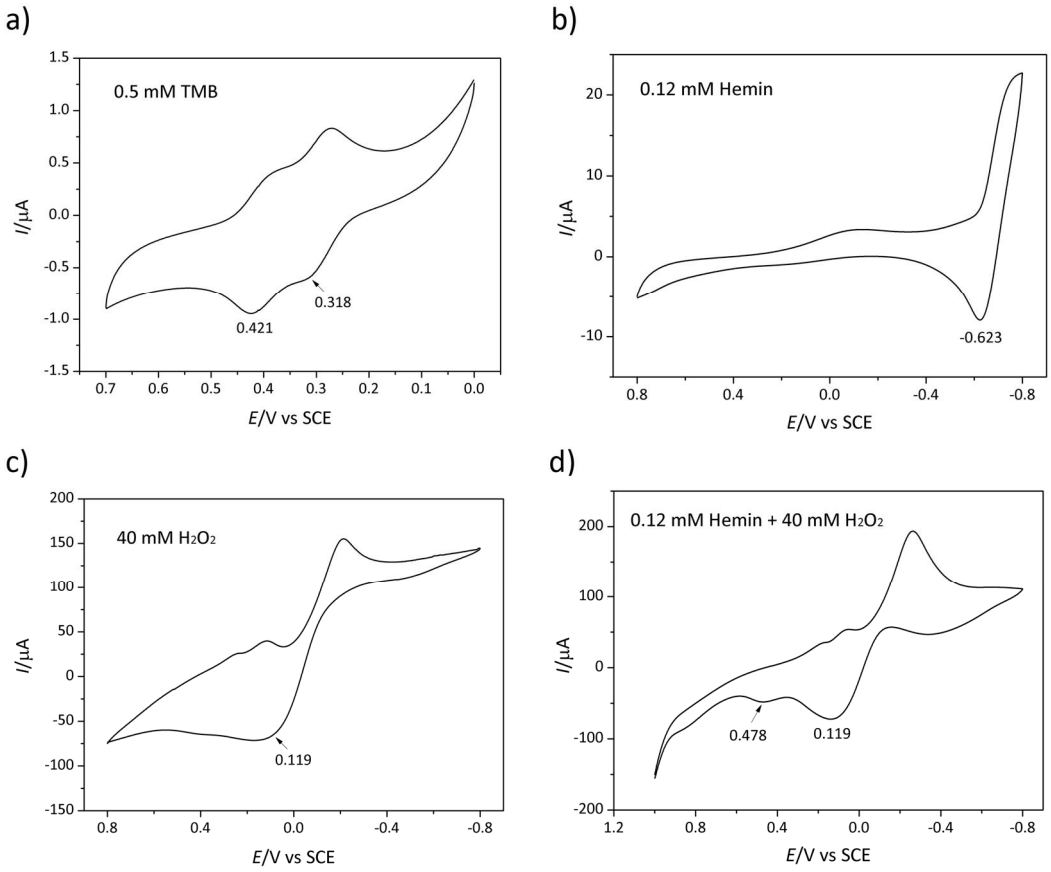



Fig. S2 Cyclic voltammograms of 0.5 mM TMB (a), 0.12 mM hemin (b), 40 m$MH_2O_2$ (c) and 0.12 mM hemin+40 m$MH_2O_2$ (d) in 10 mM HEPES buffer (pH 7.2) + 0.1 M KCl. Experimental conditions: starting potentials were 0 V (a), 0.8 V (b and c) and 1.0 V (d), respectively; potential scan rate was 50 mV $s^{-1}$.

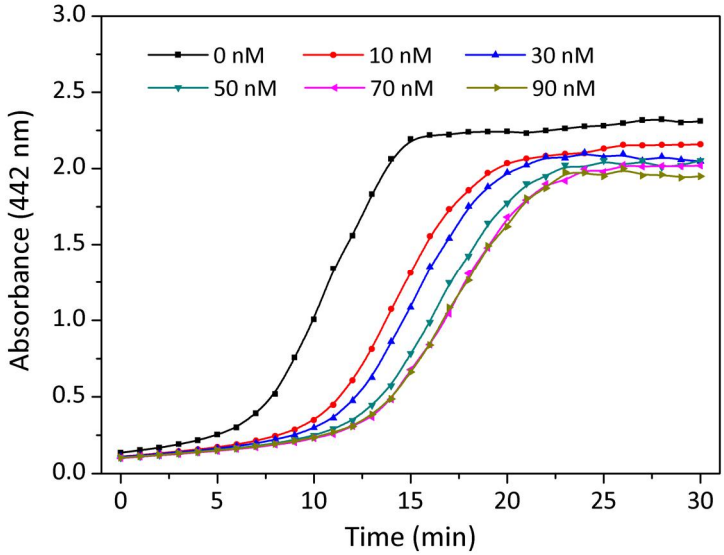



Fig. S3 The absorbance value of TMB in 10 mM HEPES buffer (pH 7.2) oxidized by hemin treated with varying concentration of Ars-3 aptamers in different time.

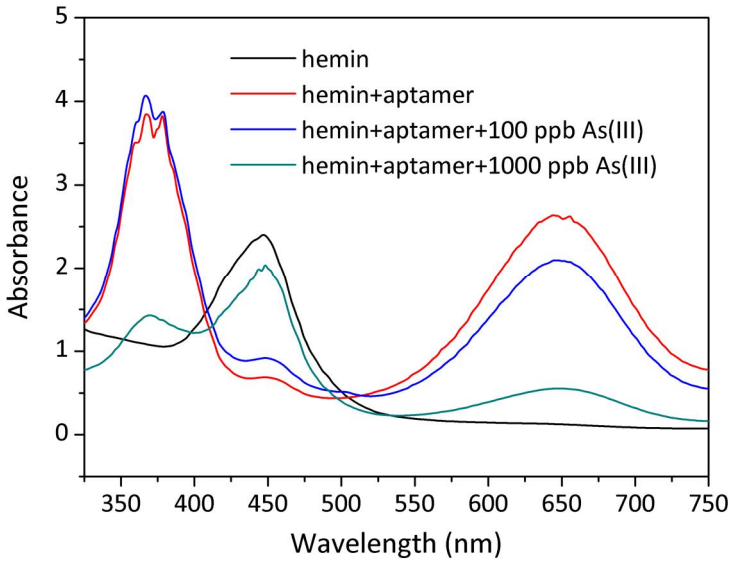



Fig. S4 Absorption spectra of TMB in 10 mM HEPES buffer (pH 7.2) oxidized by hemin in the presence of Ars-3 aptamer and As(III) in 15 min. The concentrations of hemin and aptamers were 0.12 mM and 70 nM.

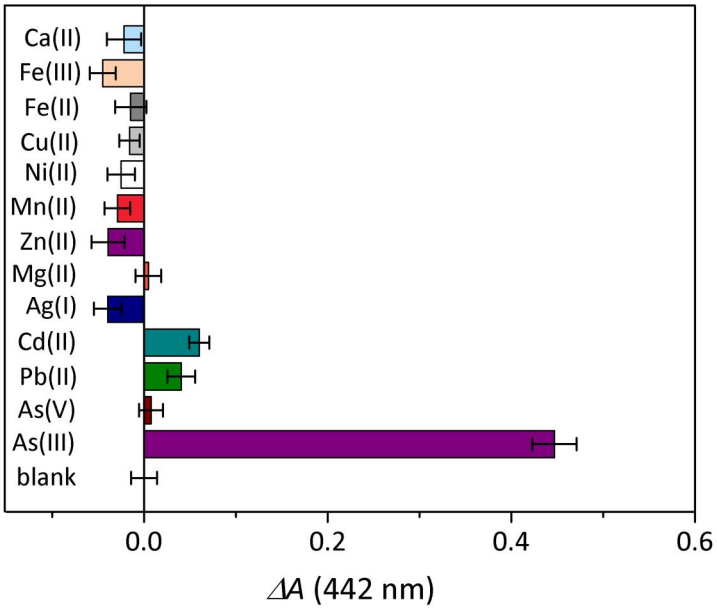



Fig. S5 Selectivity of the colorimetric detection of As(III). The concentrations of metal ions were all 200 ppb.

Table S1 Determination of As(III) in water samples

| Simples | Mean found (ppb) | Mean recovery (%) | RSD (%) |
|---|---|---|---|
| $As^{(III)}$ (50)†, $Fe^{(III)}$ (100), $Ca^{(II)}$ (50), $Mn^{(II)}$ (50), $Ni^{(II)}$ (50), $SO_4^{2-}$ (100) , $Cl^-$ (400) | 51 | 102 | 5.6 |
| $As^{(III)}$ (200), $As^{(V)}$ (100), $Zn^{(II)}$ (100), $Mg^{(II)}$ (200), $Ag^{(I)}$ (200), $NO_3^-$ (200), $SO_4^{2-}$ (200) , $Cl^-$ (200) | 187 | 93.5 | 10.3 |
| $As^{(III)}$ (400), $As^{(V)}$(300), $Ni^{(II)}$ (500), $Ag^{(I)}$ (400), $Cu^{(II)}$ (300), $NO_3^-$ (400), $SO_4^{2-}$ (800) | 453 | 113 | 8.7 |
| $As^{(III)}$ (600), $Fe^{(III)}$ (400), $Mg^{(II)}$ (500), $Zn^{(II)}$ (600) , $Ca^{(II)}$ (500), $SO_4^{2-}$ (500) , $Cl^-$ (2200) | 614 | 102 | 8.9 |

† Final concentration (ppb) of ions was added.

## Reference

1. M. Kim, H. J. Um, S. Bang, S. H. Lee, S. J. Oh, J. H. Han, K. W. Kim, J. Min and Y. H. Kim, *Environ Sci Technol.* 2009, 43, 9335-9340.