Supporting Information (ESI)

Porous nanozymes: peroxidase-mimetic activity of mesoporous iron oxide for colorimetric and electrochemical detection of global DNA methylation

Ripon Bhattacharjee,^a Shunsuke Tanaka,^b Sofia Moriam,^a Mostafa Kamal Masud,^{ac} Jianjian Lin,^b Saad M. Alshehri,^d Tansir Ahamad,^d Rahul R. Salunkhe,^e Nam-Trung Nguyen,^a Yusuke Yamauchi,^{cfg*} Md. Shahriar A. Hossain,^{ch*} and Muhammad J. A. Shiddiky^{a*}

- ^a School of Environment and Science & Queensland Micro- and Nanotechnology Centre (QMNC), Griffith University, Nathan Campus, Nathan, QLD 4111, Australia
- ^b College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China and Australian Institute for Innovative Materials (AIIM), University of Wollongong, North Wollongong, NSW 2500, Australia
- ^c Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Brisbane, QLD 4072, Australia

^d Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia.

^e International Centre for Materials Nanoarchitectonics (MANA), National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan.

^f School of Chemical Engineering, The University of Queensland, Brisbane, QLD 4072, Australia

^g Department of Plant & Environmental New Resources, Kyung Hee University, 1732 Deogyeong-daero, Giheung-gu, Yongin-si, Gyeonggi-do 446-701, South Korea

^h School of Mechanical and Mining Engineering, The University of Queensland, Brisbane, QLD 4072, Australia

*Corresponding author e-mails:

y.yamauchi@uq.edu.au; md.hossain@uq.edu.au; m.shiddiky@griffith.edu.au

Catalyst	Substrate	Km	V _{max} / 10 ⁻⁸	Reference
		mM	M s ⁻¹	
Fe ₂ O ₃	H_2O_2	146.7	6.37	This assay
Mesoporous	TMB	0.298	7.36	
HRP	H_2O_2	3.70	10	S1
	TMB	0.434	8.71	
Prussian Blue-Fe ₂ O ₃	H_2O_2	323.6	117	S2
(Nanoparticle)	TMB	0.307	106	
ZnFe ₂ O ₄	H_2O_2	1.66	7.74	S 3
	TMB	0.85	13.31	
CuZnFeS NCs	H_2O_2	0.07	0.56	S 4
	TMB	2.2	39	

Table S1 Comparison of different NPs and their kinetics parameter for TMB/H_2O_2 substrate.

1. Supplementary Figures



Figure S1 (A) XPS survey spectrum, (B) and (C) high-resolution C and O 1s XPS spectrum, respectively and (D) high-resolution Fe 2p XPS spectrum of the mesoporous iron oxide.



Figure S2. UV-vis absorption spectra for Fe_2O_3 (black), dextran-(red) and dextran/5mC antibody-modified (blue) mesoporous Fe_2O_3 .



Figure S3 Optimization of mesoporous Fe₂O₃ nanoparticle quantity. (A) naked eye evaluation and (B) mean values of corresponding samples. In the experiment, different quantity (10 μ g to 100 ng) of mesoporous Fe₂O₃ nanoparticle were used. For all experiments, required amount of MIO added to 60 μ L (0.2 M NaAc buffer, pH 3.5) containing 700 mM of H₂O₂ and 800 μ M of TMB. Incubation was 10 min. Error bars represent standard deviation of three independent experiments.



Figure S4 Optimization of 5mC antibody concentration and incubation time. Mean response for the Fe₂O₃-mC antibody concentrations of 10-100 ng (fixed 1µg of Fe₂O₃ and 50 ng methylated DNA input) absorbed on the SPGE for 30 min for (A) absorbance and (B) amperometric current density. (C) and (D) represent corresponding absorbance and amperometric current density for different incubation time of NPS-mC antibody from 10-60 min (fixed 1µg of Fe₂O₃, 50 ng methylated DNA input and 75 ng of Fe₂O₃-mC antibody). Error bars represent standard deviation of three independent experiments.



Figure S5. UV-vis (A) and electrochemical (B) data obtained for the synthetic heterogenious samples containing 5% and 10% methylation. Assays were peromed using both the HRP/H₂O₂ and MIO/H₂O₂ systems.

Reference

- S1 L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett, X. Yan, *Nat. Nanotechnol.*, 2007, 2(9), 577-583.
- S2 X. -Q. Zhang, S. -W. Gong, Y. Zhang, T. Yang, C. -Y., Wang, N. Gu, *Mater. Chem.*, 2010, **20**(24), 5110.
- S3 L. Su, J. Feng, X. Zhou, C. Ren, H. Li, X. Chen, Anal. Chem., 2012, 84(13), 5753-5758.
- S4 A. Dalui, B. Pradhan, U. Thupakula, A. H. Khan, G. S. Kumar, T. Ghosh, B. Satpati, S. Acharya, *Nanoscale*, 2015, 7(19), 9062-9074.