ChemComm

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: X. Wang, A. Gong, W. Luo, H. Wang, C. Lin, X. Liu and Y. Lin, *Chem. Commun.*, 2018, DOI: 10.1039/C8CC04279A.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/chemcomm

View Article Online
DOI: 10.1039/C8CC04279A

Journal Name

COMMUNICATION

Remote Activation of Nanoparticulate Biomimetic Activity by Light Triggered pH-Jump

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

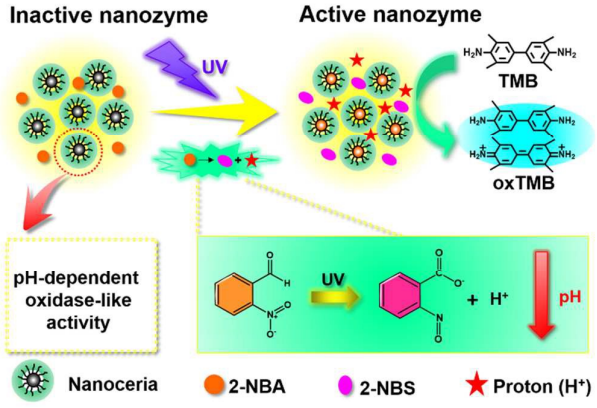
www.rsc.org/

Xiaopei Wang,[a] Ao Gong,[a] Wenhao Luo,[a] Haiqing Wang,[a] Changxu Lin,[a] Xiang Yang Liu[b, a] and Youhui Lin[a*]

Herein, we report a facile, efficient and versatile method for photo-regulation of pH-dependent activities of artificial enzymes by incorporating flash photolysis reagents. Under light excitation, a persistent pH shift is achieved by proton release from photosensitive 2-nitrobenzaldehyde. Following such change, controlled activation of oxidase-like activity of nanoceria is successfully demonstrated.

Recently, due to the merging of nanotechnology with biology, design and development of functional nanomaterials with intrinsic enzyme mimetic activities has attracted enormous research interest.[1] As promising candidates of natural enzymes, these nanomaterial-based artificial enzymes, termed as ''nanozymes'', have already been extensively utilized in a wide variety of applications, including biosensor, environmental chemistry, and therapeutics.[1] Among these, cerium oxide nanoparticle (CeO_2 NP or nanoceria) is one of the typical nanozymes, which has been discovered to possess multiple enzyme-like activities.[1f, 2] For example, nanoceria exhibits a specific pH-dependent oxidase-like activity,[2a] which can rapidly oxidize a variety of organic substrates without any oxidant, such as hydrogen peroxide. This activity likely comes from the reversible conversion of the oxidation state between Ce^{4+} and Ce^{3+}.[2a] On the other hand, control and especially controlled activation of biochemical events can offer many new opportunities for biotechnology and chemical industry.[3] Previous researchers have focused on the manipulation of enzyme function.[3] However, regulation of the activities of nanozymes has rarely been investigated.[3] Therefore, it would be highly desirable to develop new approaches for controlling these nanoparticulate biomimetic activities.

Over the past few decades, the capacity of flash photolysis reagents to induce an enduring change in pH during light exposure has been widely used for spatial and temporal control of acid-catalyzed and pH-sensitive processes.[3c, 3d, 4] A majority of these reagents possess a 2-nitrobenzyl group, which serves as an essential role in the photo-labile precursor. For instance, a commercial molecule 2-nitrobenzaldehyde (2-NBA) exhibits inherently pKa shift ability by photo-irradiation. Under the excitation of ultraviolet light, its permanent weak acidic-form, nitrosobenzoic acid (2-NBS), and proton can be produced quickly by the intramolecular proton-transfer reaction.[4a] The reaction mechanism about such photo-conversion as well as the kinetics have also been thoroughly studied.[5] Inspired by this light-triggered pH shift and the phenomenon that many enzyme-mimicking activities of nanozymes are highly dependent on pH, we develop a simple and very efficient approach for photo-mediated control of oxidase-like activity of nanoceria by incorporating photosensitive 2-NBA. This strategy is not only restricted to nanoceria-based artificial enzymes and can also be readily applied to all other kinds of nanozymes.



Scheme 1. The working principle of controllable oxidase-like activity of dextran-decorated nanoceria based on photo-induced proton release from pH-jump 2-NBA.

The working principle of controllable oxidase-like activity of dextran-decorated cerium oxide nanoparticles by photo-induced pH-jump 2-NBA is illustrated in Scheme 1. Firstly, dextran-decorated cerium oxide nanoparticles were

[a] Research Institute for Biomimetics and Soft Matter, Fujian Provincial Key Laboratory for Soft Functional Materials Research, College of Physical Science and Technology & College of Materials, Xiamen University, Xiamen 361005, China.
[b.] Department of Physics, National University of Singapore, 2 Science Drive 3, 117542 Singapore.

† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C8CC04279A

synthesized based on a previously reported procedure.[6] Dextran can serve as an excellent stabilizer based on strong coordination interactions between hydroxyl groups of dextran and rare earth ions.[7] The as-prepared cerium oxide nanoparticles were characterized by transmission electron microscopy (TEM). The HRTEM image indicated the presence of discrete nanocrystals with an average size of 4 nm as well as good monodispersity (Fig. 1A). Our obtained cerium oxide nanoparticles with small sizes are expected to exhibit intriguing catalytic performance.[8] Moreover, the preferred plane (111) (d = 0.314 nm) on the surface was observed (Fig. 1B).[9] Also, the formation of a face-centered-cubic nanoceria core was identified by selected-area electron diffraction (SAED) and X-ray powder diffraction (XRD) pattern (Fig. 1C and 1D).[9]

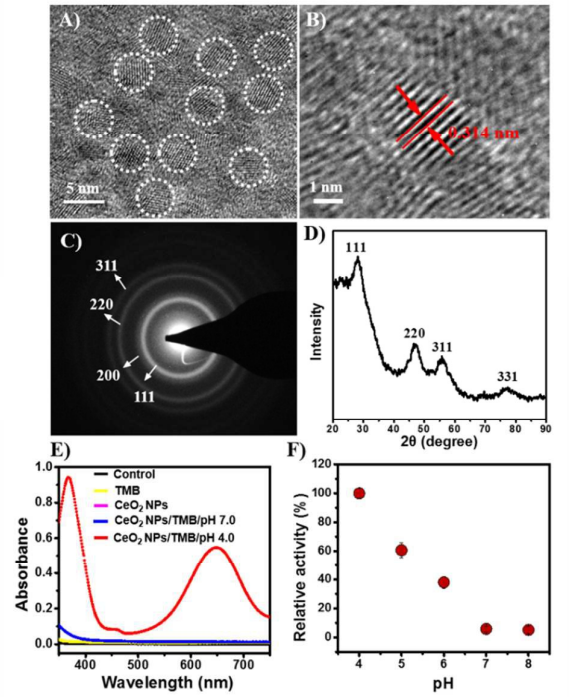


Fig. 1 (A, B) TEM images of as-prepared nanoceria. (C) The corresponding SAED pattern. (D) Wide-angle powder XRD pattern of nanoceria. (E) The absorbance changes of different samples as a result of the formation of oxTMB. (F) The pH-dependent catalytic activity of nanoceria.

To evaluate the oxidase-like activity of cerium oxide nanoparticles, 3,3',5,5'-tetramethylbenzidine (TMB) is selected for an oxidase substrate, which can yield a product (oxTMB) upon oxidation. In the absence of hydrogen peroxide, cerium oxide nanoparticles could catalyze the rapid oxidation of substrate TMB to oxTMB and produced a blue color at pH 4.0 with central absorbance peaks at 370 nm and 652 nm (Fig. 1E and Fig. S1). Meanwhile, with the increasing concentrations of nanoceria, the more oxTMB product was generated (Fig. S2). We selected the absorbance changes at 652 nm for monitoring the catalytic reaction to avoid possible interferences from 2-NBA and 2-NBS. To further confirm this oxidase-like activity, 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), is also utilized as an oxidase substrate. Similarly, nanoceria could also catalyze the oxidation of ABTS to generate a green color product with main absorbance peaks at 417 nm (Fig. S3). Very interestingly, no apparent oxidation of TMB was observed under physiological condition, which could be confirmed by the absorbance change of TMB (Fig. 1E). Furthermore, pH-dependent studies showed that the ability of cerium oxide nanoparticles to oxidize TMB decreased as the pH value of the solution increased from 4.0 to 8.0 (Fig. 1F). The above results indicated that the as-prepared nanoceria as an oxidation catalyst behaved in a pH-dependent manner with strong catalytic activity under acidic environment.[2a, 10] We expect such characteristic pH-dependent oxidase-like activity could be controlled by light if the combination of photosensitive pH-Jump reagents.

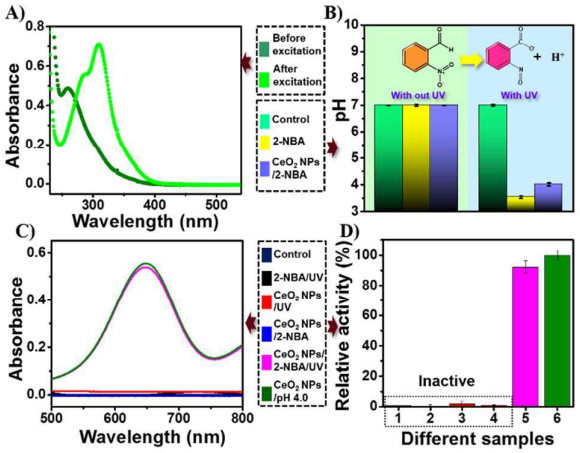


Fig. 2 (A) Absorption spectra of 2-NBA solution prior and after UV excitation (10 min). (B) pH changes of different samples determined by pH meter prior and after UV excitation (10 min). (C) Absorption spectra and (D) the relative catalytic activities of TMB reaction solutions under different conditions. 0 unless otherwise stated. [Nanoceria] = 0.1 mg/mL, [TMB] = 1 mM, [2-NBA] = 4 mM, [Phosphate buffer] = 0.5 mM.

Before investigating whether light could switch the oxidase-like activity of nanoceria in the presence of 2-NBA, the UV responsive properties of 2-NBA and 2-NBA/CeO_2 hybrid solutions was taken into consideration. Upon optical excitation toward 2-NBA, its acidic-form 2-NBS was promptly generated as a result of the intramolecular proton-transfer reaction,[3c, 5] accompanied with the absorption change of 2-NBA (Fig. 2a and Fig. S4). During the formation of the nitronate anion, proton was released simultaneously, which could dramatically decrease the pH in aqueous solution. To detect the changes in the solution pH, the acid-base indicator methyl red, which presents red in pH below 4.4 and yellow in pH over 6.2, was selected (Fig. S5). Before UV exposure, the indicator displayed a yellow color in the phosphate buffer solution (0.5 mM, initial pH 7.0) containing 4 mM 2-NBA. While after excitation of ultraviolet light, a distinct yellow to red color change was observed, indicating the pH of the aqueous phase was decreased (Fig. S5). The solution pH value measured by pH meter further confirmed that the pH was changed from 7.0 down to 3.6 (Fig. 2B). Without 2-NBA, the excitation of UV light toward solution could not cause any pH change (Fig. S6). These experiments indicated that light-triggered proton release from 2-NBA induced the change in pH. Since the dextran-coated nanoceria has a large number of hydroxyl groups on the surface, these groups can serve as proton scavengers and

View Article Online
DOI: 10.1039/C8CC04279A

weaken the final pH-jump.[4g] For this reason; we also texted the pH change ability of 2-NBA in the presence of the suitable concentrations of nanoceria. Importantly, in the system containing 0.1 mg/mL nanoceria, the presence of 4 mM 2-NBA after UV irradiation could still cause a pH jump of approximately three units from 7.0 to 4.0 (Fig. 2B and Fig. S6). Although the pH change ability of 2-NBA/CeO_2 hybrid system was slightly weaker than that containing 2-NBA alone, such resulting pH shift was massive enough to change the oxidase-like activity of nanoceria remarkably.[2a]

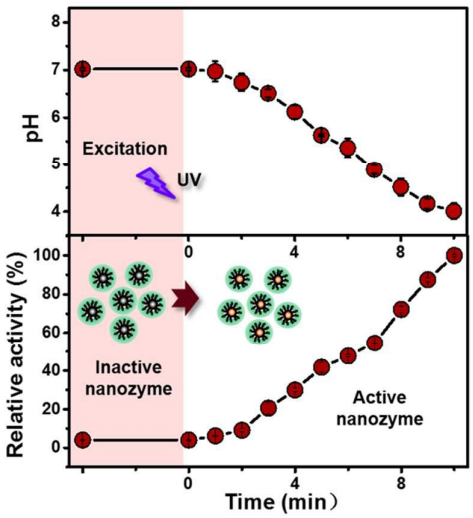


Fig. 3 The pH change of 2-NBA/CeO_2 hybrid system under different illuminating time and the corresponding catalytical activity under different illuminating time. [Intial pH] = 7.0, [Nanoceria] = 0.1 mg/mL, [TMB] = 1 mM, [2-NBA] = 4 mM, [Phosphate buffer] = 0.5 mM.

Next, we addressed the possibility of applying UV light to switch the oxidase-like activity of cerium oxide nanoparticles in the presence of flash photolysis reagents. Without the excitation of ultraviolet light, no distinct activity was observed for all samples in 0.5 mM phosphate buffer (pH 7.0), even the presence of both 2-NBA and nanoceria (Fig. 2C and Fig. 2D). However, the reaction system containing 2-NBA and CeO_2 NPs after UV exposure showed strong ability toward the catalytic oxidation of TMB to product oxTMB (Fig. 2C). We noted that to avoid the oxidation of TMB by UV, TMB was added in the above system after UV exposure. Moreover, the catalytic ability of activated cerium oxide nanoparticles reached approximately 97.1% of that at pH 4.0 (Fig. 2D). Above experiments indicated that the resulting low pH from photosensitive 2-NBA could almost entirely activate the oxidase-like activity of nanoceria, and there was no significant impact on the oxidase-like activity of nanoceria by 2-NBA, 2-NBS and photoexcitation. In contrast, under the same condition, neither nanoceria nor 2-NBA alone could catalyze the oxidation TMB efficiently (Fig. 2C). It meant that without 2-NBA, only ligh could not activate the activity of cerium oxide nanoparticles. Also there was no significant impact on the oxidation state of nanoceria upon the photoexcitation (Fig. S7). Furthermore, our photo-activation method toward ABTS-based substrate, citrate-based buffer and pH-dependent V_2O_5 peroxidase mimic, has also been demonstrated (Fig. S8-S10). Overall, we have demonstrated the feasibility of the above scheme that we proposed.

More importantly, the pH change of the system containing 2-NBA and cerium oxide nanoparticles was highly depended on the activated time of ultraviolet light. With the increasing of excitation time, the pH value decreased from 7.0 down to around 4 gradually (Fig. 3). Meanwhile, the corresponding catalytic activity of such system (using TMB as a substrate) could be tuned easily by adjusting the illuminating time (Fig. 3 and Fig. S11). Similar result was also observed for ABTS-based substrate (Fig. S12). Above results sufficiently proved the activation efficiency of nanozymes are highly tunable.

In summary, this study provides a first example of using flash photolysis reagents for controlled activation of pH-dependent activity of nanoceria. This strategy presented herein is simple in design, label-free, non-invasive, and suitable for almost all different nanozymes. Moreover, the activation efficiency can be easily tailored. Since control of nanozymes' activity can modulate their selectivity and catalytic efficiency and avoid some potential problems,[11] our work may open a range of potential applications in future biomedical, industrial, and environmental fields.

This work was supported by National Nature Science Foundation (Nos. 21771150, 21401154, U1405226), 111 project (B16029), the Fundamental Research Funds for the Central Universities of China (Nos. 20720170011, 20720140528), and Doctoral Fund of the Ministry of Education (20130121110018).

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 a) L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett and X. Yan, *Nat Nanotechnol.,* 2007, **2**, 577-583; b) H. Wei and E. Wang, *Chem. Soc. Rev.*, 2013, **42**, 6060-6093; c) Y. Lin, J. Ren and X. Qu, *Acc. Chem. Res.*, 2014, **47**, 1097-1105; d) W. Zhang, S. Hu, J.-J. Yin, W. He, W. Lu, M. Ma, N. Gu and Y. Zhang, *J. Am. Chem. Soc.*, 2016, **138**, 5860-5865; de) Z. Chen, J.-J. Yin, Y.-T. Zhou, Y. Zhang, L. Song, M. Song, S. Hu and N. Gu, *ACS Nano*, 2012, **6**, 4001-4012.f) C. Xu and X. Qu, *Npg Asia Mater.*, 2014, **6**, e90; g) J. Wu, S. Li and H. Wei, *Chem. Commun.*, 2018, DOI: 10.1039/C8CC01202D; h) K. Fan, J. Xi, L. Fan, P. Wang, C. Zhu, Y. Tang, X. Xu, M. Liang, B. Jiang, X. Yan and L. Gao, *Nat. Commun.*, 2018, **9**, 1440, i) H. Cheng, Y. Liu, Y. Hu, Y. Ding, S. Lin, W. Cao, Q. Wang, J. Wu, F. Muhammad, X. Zhao, D. Zhao, Z. Li, H. Xing and H. Wei, *Anal. Chem.*, 2017, **89**, 11552-11559.

2 a) A. Asati, S. Santra, C. Kaittanis, S. Nath and J. M. Perez, *Angew. Chem. Int. Ed.,* 2009, **48**, 2308-2312; b) E. G. Heckert, A. S. Karakoti, S. Seal and W. T. Self, *Biomaterials*, 2008, **29**, 2705-2709; c) T. Pirmohamed, J. M. Dowding, S. Singh, B. Wasserman, E. Heckert, A. S. Karakoti, J. E. S. King, S. Seal and W. T. Self, *Chem. Commun.*, 2010, **46**, 2736-2738; d) C. Kaittanis, S. Santra, A. Asati and J. M. Perez, *Nanoscale*, 2012, **4**, 2117-2123; e) A. Asati, C. Kaittanis, S. Santra and J.

View Article Online
DOI: 10.1039/C8CC04279A

M. Perez, *Anal. Chem.*, 2011, **83**, 2547-2553; f) C. Korsvik, S. Patil, S. Seal and W. T. Self, *Chem. Commun.*, 2007, 1056-1058.

3 a) V. Blanco, D. A. Leigh and V. Marcos, *Chem. Soc. Rev.*, 2015, **44**, 5341-5370; b) Y.-T. Kao, C. Saxena, L. Wang, A. Sancar and D. Zhong, *Cell Biochem. and Biophys.*, 2007, **48**, 32-44; c) S. Kohse, A. Neubauer, A. Pazidis, S. Lochbrunner and U. Kragl, *J. Am. Chem. Soc.*, 2013, **135**, 9407-9411; d) K. Kamada, T. Nakamura and S. Tsukahara, *Chem. Mater.*, 2011, **23**, 2968-2972; e) S. Kumar and P. Nahar, *Talanta*, 2007, **71**, 1438-1440; f) C. Xu, W. Bing, F. Wang, J. Ren and X. Qu, *ACS nano*, 2017, **11**, 7770-7780; g) G. Grossi, M. Dalgaard Ebbesen Jepsen, J. Kjems and E. S. Andersen, *Nat. Commun.*, 2017, **8**, 992; h) T. Tian, Y. Song, J. Wang, B. Fu, Z. He, X. Xu, A. Li, X. Zhou, S. Wang and X. Zhou, *J. Am. Chem. Soc.*, 2016, **138**, 955-961.

4 a) S. Abbruzzetti, S. Sottini, C. Viappiani and J. E. T. Corrie, *Photochem. Photobiol.*, 2006, **5**, 621-628; b) S. Abbruzzetti, E. Crema, L. Masino, A. Vecli, C. Viappiani, J. R. Small, L. J. Libertini and E. W. Small, *Biophys. J.*, 2000, **78**, 405-415; c) M. Gutman, D. Huppert and E. Pines, *J. Am. Chem. Soc.*, 1981, **103**, 3709-3713; d) J. Choi, N. Hirota and M. Terazima, *J. Phys. Chem. A, 2*001, **105**, 12-18; e) R. M. D. Nunes, M. Pineiro and L. G. Arnaut, *J. Am. Chem. Soc.*, 2009, **131**, 9456-9462; f) J. Choi and M. Terazima, *J. Phys. Chem. B*, 2003, **107**, 9552-9557; g) S. Kohse, A. Neubauer, A. Pazidis, S. Lochbrunner and U. Kragl, *J. Am. Chem. Soc.*, 2013, **135**, 9407-9411; h) S. Neri, S. Garcia Martin, C. Pezzato and L. J. Prins, *J. Am. Chem. Soc.*, 2017, **139**, 1794-1797; i) S. Laimgruber, W. J. Schreier, T. Schrader, F. Koller, W. Zinth and P. Gilch, *Angew. Chem. Int. Ed.,* 2005, **44**, 7901-7904; j) M. V. George and J. C. Scaiano, *J. Phys. Chem.*, 1980, **84**, 492-495.

5 a) S. Laimgruber, T. Schmierer, P. Gilch, K. Kiewisch and J. Neugebauer, *Phys. Chem. Chem. Phys.*, 2008, **10**, 3872-3882; b) S. Abbruzzetti, C. Viappiani, F. Sinibaldi and R. Santucci, *Protein J.*, 2004, **23**, 519-527.

6 P. J. Manuel, A. Atul, N. Sudip and K. Charalambos, *Small*, 2008, **4**, 552-556.

7 A. Kajimura, J. Sumaoka and M. Komiyama, *Carbohydr. Res.,* 1998, **309**, 345-351.

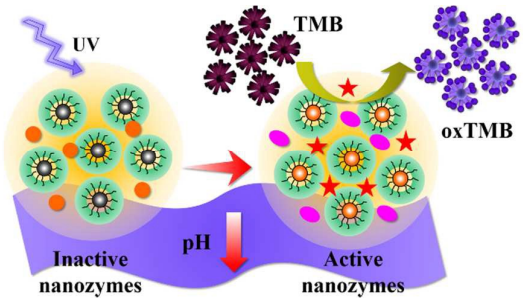
8 M. Valden, X. Lai and D. W. Goodman, *Science*, 1998, **281**, 1647-1650.

9 Y. Zhai, K. Zhou, Y. Xue, F. Qin, L. Yang and X. Yao, *RSC Adv.*, 2013, **3**, 6833-6838.

10 H. Cheng, S. Lin, F. Muhammad, Y.-W. Lin and H. Wei, *ACS Sensors*, 2016, **1**, 1336-1343.

11 W. Faming, J. Enguo, G. Yijia, R. Jinsong and Q. Xiaogang, *Small*, 2017, **13**, 1603051;

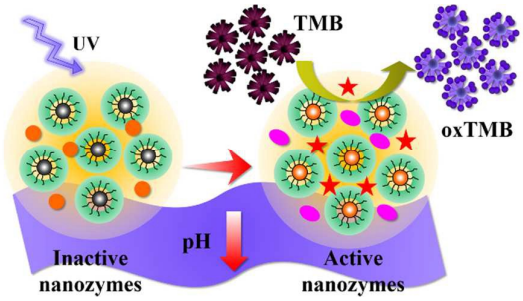
View Article Online
DOI: 10.1039/C8CC04279A



TOC

By incorporating flash photolysis reagents, a facile and versatile method for photo-regulation of pH-dependent activities of artificial enzymes is presented.

View Article Online
DOI: 10.1039/C8CC04279A



TOC

By incorporating flash photolysis reagents, a facile and versatile method for photo-regulation of pH-dependent activities of artificial enzymes is presented.