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#### Nanoisozymes: The Origin behind Pristine CeO<sub>2</sub> as Enzyme-mimetics

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It is well-known that the interplay between molecules and active sites on the topmost surface of solid catalyst determines its activity in heterogeneous catalysis. The electron density of active site is believed to affect both adsorption and activation of reactant molecules at surface. Unfortunately, commercial X-ray photoelectron spectroscopy (XPS) often adopted for such characterization is not sensitive enough to catalyst' topmost surface. In our opinion, most researchers fail to acknowledge this point during their catalytic correlation, leading to different interpretations found in literatures over the past decades. Recent studies on pristine Cu<sub>2</sub>O (*Nat. Catal.* **2019**, *2*, 889; *Nat. Energy* **2019**, *4*, 957) have clearly suggested that the electron density of surface Cu is facet dependent and plays a key role in CO<sub>2</sub> reduction. Herein, we show that pristine CeO<sub>2</sub> can reach 2506%/1133% increase in phosphatase-/peroxidase-like activity once the exposed surface is wisely selected. Using nuclear magnetic resonance (NMR) with surface probe, the electron density of surface Ce (i.e. the active site) is found facet-dependent and the key factor dictating their enzyme mimicking activities. Most importantly, the surface area of those CeO<sub>2</sub> morphologies was demonstrated to become a factor only when their surface Ce can activate the adsorbed reactant molecules.

#### **1. Introduction**

Phosphatase is an essential enzyme widely found in living organisms that removes a phosphate group ( $PO_4^{3-}$ ) from phosphorylated substrates by the hydrolysis of the phosphate ester bond (Scheme 1a). The phosphate ester bond of many biologically important substrates has been demonstrated to regulate protein activity, DNA/RNA stability and cellular signaling<sup>[1-3]</sup>. For example, protein phosphatases can dephosphorylate an amino acid residue of its protein substrate, which is crucial to regular intracellular signaling with protein kinases. Nucleotidases can catalyze the hydrolysis of a nucleotide into a nucleoside and a phosphate, which is essential to maintain a balanced nucleotides and nucleosides with nucleotide kinases (cellular homeostasis). Despite Enzyme Commission identifies over one hundred distinct phosphatase families, all phosphatases basically catalyze the same hydrolysis reaction (Scheme 1a). Recently, CeO<sub>2</sub> has been demonstrated a promising material to mimic phosphatase via the interaction between surface Ce and substrates' phosphoryl oxygen (P=O) for the subsequent ester hydrolysis<sup>[4,5]</sup>. A surface Ce<sup>4+</sup>-mediated pathway was firstly suggested by Tan et al. using para-nitrophenyl phosphate (p-NPP) as a substrate<sup>[6]</sup>. However, Baker and colleagues later found the mimicking activity was inhibited once surface Ce<sup>3+</sup> is oxidized to  $Ce^{4+[7]}$ . A balanced surface  $Ce^{4+}/Ce^{3+}$  ratio<sup>[8]</sup> and surface oxygen vacancy<sup>[9,10]</sup> have also been proposed recently the decisive factor affecting CeO<sub>2</sub>-catalyzed dephosphorylation. Accordingly, the role of surface Ce in this reaction still remains unclear for pristine CeO<sub>2</sub> not to mention for their derivatives (i.e. CeO<sub>2</sub>-based composites/supports)<sup>[6-11]</sup>.

In addition to phosphatase, the redox property of  $CeO_2$  has been also reported can mimic various peroxidases by oxidizing corresponding substrates in the presence of  $H_2O_2$ (Scheme 1b). For example, the reduction of  $H_2O_2$  catalyzed by  $CeO_2$  can subsequently oxidize substrates such as tetramethylbenzidine (TMB) of horseradish peroxidase (HRP), nicotinamide adenine dinucleotide (NADH) of NADH peroxidase and glutathione of

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glutathione peroxidase  $(GP_x)^{[4,5]}$ . As H<sub>2</sub>O<sub>2</sub> plays a critical role in redox biology and cell signalling, those peroxidases are known to maintain the level of H<sub>2</sub>O<sub>2</sub> in the cell below the toxic threshold, protecting biologically important molecules from oxidative damage induced by excessive H<sub>2</sub>O<sub>2</sub>. The mis-regulation of H<sub>2</sub>O<sub>2</sub>, in the long term, could lead to various cardiovascular diseases, cancer and the disorder of neurodegeneration. Qu's group attributed the peroxidase-like activity of CeO<sub>2</sub> to surface Ce<sup>3+</sup> species<sup>[12]</sup>. However, Yang et al. later showed that CeO<sub>2</sub> morphologies with similar level of Ce<sup>3+</sup>/Ce<sup>4+</sup> ratio and oxygen vacancies can display different peroxidase-like activities<sup>[13]</sup>. Despite the sensing of H<sub>2</sub>O<sub>2</sub> by the surface Ce is believed the key step affecting its mimicking activity<sup>[12-15]</sup>, an in-depth study of the interplay between them is still lacking nowadays.

In our opinion, different interpretation found in literatures above is due to the heavy reliance on X-ray photoelectron spectroscopy (XPS) for active site characterization<sup>[16,17]</sup>. The substrate molecules of these two enzyme reactions, *p*-NPP and H<sub>2</sub>O<sub>2</sub>, interact only with Ce atoms on the topmost surface of CeO<sub>2</sub> (also for most CeO<sub>2</sub>-catalyzed reactions<sup>[16,17]</sup>). However, the XPS often collects and averages signals > 10 atomic layers from the surface due to the long escaping depth of photoelectrons<sup>[18]</sup>. For example, the penetration depth for Ce<sub>3d</sub> photoelectrons with 550 eV kinetic energy can be up to 7 nm from CeO<sub>2</sub> surface using commercial XPS<sup>[19]</sup>. Since the electron density of surface Ce is believed different from their counterparts in subsurface/bulk, it makes no sense to correlate XPS results with the catalytic reaction only involves Ce on the topmost surface. Unfortunately, this point has been ignored by most researchers over the past decades<sup>[4-17]</sup>.

Herein, probe-assisted NMR was employed to investigate the electron density of surface Ce among CeO<sub>2</sub> morphologies. With supports from calculation and a wide range of techniques, the electron density of surface Ce was found facet-dependent and significantly affects the activation of adsorbed p-NPP/H<sub>2</sub>O<sub>2</sub>. We demonstrated that more than 2506% and

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1133% increase in phosphatase-/peroxidase-like activity can be easily achieved on pristine CeO<sub>2</sub> once its exposed surface is wisely chosen for future engineering.

#### 2. Results and Discussion

#### 2.1. Characterization and Mimicking Testing of CeO2 Morphologies

Octahedron, rod and cube shaped CeO<sub>2</sub> crystallites were firstly prepared according to literatures<sup>[10,20]</sup>. **Figure 1** shows the corresponding TEM and HRTEM images. The octahedron is about 20 nm in size (**Figure 1a**) and enclosed by (111) facets as evidenced by the subsurface lattice spacing of 0.32 nm (**Figure 1d**). The rod-shaped CeO<sub>2</sub> with length up to 200 nm has a uniform diameter around 10 nm (**Figure 1b**). Even though three lattice spacings of (111), (110) and (100) can be identified on rod (**Figure 1e**), we assume herein the majority of rod surface is terminated by (110) facet as often assigned<sup>[10,17,20]</sup>. CeO<sub>2</sub> cube (**Figure 1c**) has a wider size distribution ranging from 15 to 60 nm with surface mainly enclosed by (100) facet (**Figure 1f**). Scanning electron microscopy and the corresponding elemental analysis (**Figures S1**) suggest high shape homogeneity with cerium (Ce) to oxygen (O) ratio around 0.5 for all morphologies. Their crystallographic nature was confirmed by powder X-ray diffraction (XRD, **Figure S2**). Commercial XPS was also attempted to gain insight into the chemical state of surface Ce (**Figure S3**). Unfortunately, no apparent difference in XPS Ce<sub>3d</sub> spectra can be observed between morphologies.

Phosphatase-like activity of CeO<sub>2</sub> morphologies at 25°C were then evaluated using *p*-NPP as substrate in **Figure 2a-c**. Given that *p*-NPP and its hydrolyzed form, para-nitrophenol (*p*-NP), show different absorption at 310 nm and 405 nm, UV–vis spectroscopy was adopted to evaluate the corresponding activity (**Figure 2a**). **Figure S4** shows the time-dependent UV–vis spectra of their conversion over CeO<sub>2</sub> morphologies at 25 °C. As shown in **Figure 2b**, octahedron outperforms the other two morphologies with the yield of *p*-NP (91%) followed by rod (69%) and cube (3%) by 8 hours. The first order k constant was calculated

(hour<sup>-1</sup>) 0.2706 for octahedron, 0.1927 for rod and 0.0108 for cube (Figure 2c & Figure S5a). Surface area (Table S1) clearly plays a neglected role that the rod (95  $m^2/g$ ) possesses more than twice of the octahedron (42  $m^2/g$ ) while exhibits lower activity. Moreover, ~70% more surface area of the octahedron compared to 25  $m^2/g$  of the cube cannot explain the 2506% increase in the corresponding k constant. The peroxidase-like property of those  $CeO_2$ morphologies was then examined in Figure 2d-f. UV-vis spectroscopy was also adopted here because the reduction of  $H_2O_2$  to water by the surface Ce can initiate TMB oxidation to generate a color change of solution at 652 nm (i.e. the formation of  $TMB_{(ox)}$ , Figure 2d). In stark contrast to the phosphatase-mimicking results (Figure 2b), the octahedron is nearly inactive as peroxidase mimetics while the cube shows very high mimicking activity (Figure **2e**). The cube thus exhibits largest k constant  $(10^{-4} \text{ min}^{-1})$  of 7.0005 followed by rod (2.1509) and octahedron (0.6177) (Figure 2f & Figure S5b). Notice that the very active octahedron in dephosphorylation is now 1133% less active than the cube and their surface area is clear not the decisive factor (Table S1). Such extreme phosphatase-/peroxidase-like activities obtained among CeO<sub>2</sub> morphologies suggest that the reactivity of surface Ce is closely associated with their host facets.

Literatures have often relied on commercial XPS as the key surface tool for both qualitative and quantitative correlation of facet activity. However, Ce<sub>3d</sub> photoelectrons excited by Al source (1486.6 eV) with kinetic energy 550 eV its escaping depth can be up to 7 nm from the surface<sup>[19]</sup>. Noted that this value (i.e. 7 nm) is even larger than the size of ultra-small CeO<sub>2</sub> catalysts used in many literatures<sup>[16,17]</sup>. The signal of Ce atoms on the topmost CeO<sub>2</sub> surface is thus averaged by subsurface Ce atoms without involving in catalysis, leading to similar XPS Ce<sub>3d</sub> pattern between morphologies (**Figure S3**). Unfortunately, most researchers fail to acknowledge this point and even try to establish catalytic correlation based on the deconvoluted XPS. Same method was adopted here to show how unconvincing the obtained

correlation could be (**Figure S3**). The Ce<sup>4+</sup>/Ce<sup>3+</sup> ratio was then calculated 10.03 for octahedron > 5.07 for rod > 3.94 for cube (**Table S1**). This order seems can rationalize their phosphatase-like activity (**Figure 2c**) since Ce<sup>4+</sup> is more acidic (cf. Ce<sup>3+</sup>) to catalyze the ester hydrolysis and hence the octahedron with higher Ce<sup>4+</sup>/Ce<sup>3+</sup> ratio exhibits better performance. However, no literature<sup>[6-11]</sup> can explain why the Ce<sup>4+</sup> portion (90.93%) of octahedron is very active in dephosphorylation while it becomes inactive for cube with only 10% less of Ce<sup>4+</sup> (79.76%) (**Figure 2a-c**). On the other hand, it is believed that Ce<sup>3+</sup> requires lower overpotential for H<sub>2</sub>O<sub>2</sub> reduction (cf. Ce<sup>4+</sup>) and hence the cube with the lowest Ce<sup>4+</sup>/Ce<sup>3+</sup> ratio provides higher peroxidase-like activity. However, literatures<sup>[12-15]</sup> again failed to argue why the Ce<sup>3+</sup> portion of octahedron (9.07%) is inactive here while it suddenly became active for cube with doubled Ce<sup>3+</sup> portion (20.24%) (**Figure 2d-f**). Above discussion clearly suggests that XPS not only has the detection depth issue for catalytic correlation the provided "discrete" oxidation state (e.g. 3+ and 4+ for Ce) is also not a proper descriptor.

#### 2.2. Surface Structure Analysis and Probe-assisted NMR

From our point of view, the extreme behavior of CeO<sub>2</sub> morphologies observed in **Figure 2** should associate with their surface physiochemical properties and hence the interaction with reactants. Structural analysis in **Figure 3a-c** shows that the Ce atom on CeO<sub>2</sub>(111) surface (**Figure 3a**) has only one coordination unsaturated site (cf. 8-coordinated Ce in bulk) while it has two such sites on that of CeO<sub>2</sub>(110) (**Figure 3b**) and CeO<sub>2</sub>(100) (**Figure 3c**) surfaces. Since the electron density of surface Ce is inversely proportional to its coordination number, the 6-coordinated Ce atom on the CeO<sub>2</sub>(110) indeed exhibits higher electron density than its 7-coordinated counterpart on the CeO<sub>2</sub>(111) surface. While for surfaces with 6-coordinated Ce, the CeO<sub>2</sub>(100) can accommodate higher electron density than the CeO<sub>2</sub>(110) presumably due to the difference in their surface energy<sup>[16,17]</sup>. According to above structural analysis, the electron density of surface Ce should change "continuously" Chemistry - A European Journal

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with their host surfaces in the order of (100) > (110) > (111) (cf. 3+ or 4+ "discretely" provided by XPS). This facet-dependent property of surface Ce is believed to affect the corresponding adsorption/activation energy of reactants, leading to the extreme CeO<sub>2</sub> activities observed in **Figure 2**.

Using <sup>31</sup>P NMR with <sup>31</sup>P-containing molecules as surface probe has been shown a promising method for the surface study of metal oxides<sup>[21-27]</sup>. Trimethylphosphine oxide (TMPO) was selected here not only for above purpose but also to investigate the interaction between surface Ce and the P=O of *p*-NPP by <sup>31</sup>P NMR (**Figure 3d**). The P=O group of TMPO can specifically bind to surface Ce cation (Lewis acid, LA) through Lewis acid-base interaction (i.e. P=O→Ce). Since the surface Ce with higher acidity (i.e. lower electron density) generates stronger bond with TMPO and pushes  $\delta^{31}$ P to positive ppm, the  $\delta^{31}$ P can be used to evaluate the electron density of surface Ce among CeO<sub>2</sub> facets<sup>[18]</sup>. As shown in **Figure 3e**, the  $\delta^{31}$ P (ppm) varies with CeO<sub>2</sub> morphologies from 50.2 for octahedron, 47.2 for rod to 45.1 for cube, suggesting that the electron density of surface Ce is facet-dependent (100) > (110) > (111) as predicted in **Figure 3a-c**. This result can be evidenced by a recent STEM-EELS study showing that the oxidation state of Ce atoms on (111) facet is Ce<sup>4+</sup>-like (more acidic) while they are Ce<sup>3+</sup>-like (less acidic) on (100) facet<sup>[28]</sup>.

#### 2.3. The Origin of Pristine CeO2 as Phosphatase Mimetics

As shown in **Figure 4a**, CeO<sub>2</sub> octahedron with (111) terminal surfaces shows a strong STEM-EELS signal of phosphorus from the adsorbed *p*-NPP while only a weak signal can be observed for that of CeO<sub>2</sub> cube terminated by (100) surfaces (see experimental section for details). This result suggests that the electron density of surface Ce can significantly affect *p*-NPP adsorption among CeO<sub>2</sub> morphologies. Since the dephosphorylation of *p*-NPP is activated by the surface Ce, TMPO was further adopted to investigate the interplay between P=O group and surface Ce among morphologies by X-ray absorption spectroscopy (XAS) Chemistry - A European Journal

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(Figure 4b and Figure S6a). Figure 4c summarizes the corresponding curve-fitting results. The decrease in the percentage of coordination unsaturated Ce (Ce<sub>un</sub>) upon TMPO adsorption suggests the chelating of P=O group to surface Ce<sub>un</sub>. A significant drop of Ce<sub>un</sub> percentage can be observed for both octahedron and rod after TMPO adsorption while not for cube, implying that the Ce atoms on (111) and (110) surfaces can bind strongly with *p*-NPP (via P=O $\rightarrow$ Ce) and hence activate subsequent ester hydrolysis (Figure 2a-c). This observation can be further supported by the corresponding local structure analysis (Figure S6b). The averaged Ce coordination number indeed increases from 6 to around 7.5 for octahedron and rod in the presence of TMPO, while it remains almost the same for cube (Table S2).

The strong adsorption of *p*-NPP on CeO<sub>2</sub> surface is believed to lower its activation energy for subsequent ester hydrolysis. To confirm this, we further carried out this reaction at different temperatures over CeO<sub>2</sub> morphologies for kinetic study (**Figure S7 and Table S3**). The activation energy (kJ/mol) was obtained 44.24 for octahedron, 50.81 for rod and 83.69 for cube (**Figure S7d**), indicating that the surface Ce with lower electron density can indeed activate ester hydrolysis easily and hence accelerate this reaction. Surface area plays a very minor role here since no enhanced activity can be obtained for cube ( $25 \text{ m}^2/\text{g}$ ) when its testing amount was doubled (i.e. 8 mg) or even quadrupled (i.e. 16 mg) in this reaction (**Figure S8**). The reaction energy profile of *p*-NPP dephosphorylation on CeO<sub>2</sub>(111) facet was recently calculated by Xu's group.<sup>[29]</sup>. The desorption of phosphate from CeO<sub>2</sub>(111) was found with the highest energy barrier (~115 kJ/mol) and hence the rate-limiting step instead of the P=O bond activation concluded above. However, this value deviates significantly from the activation energy of our (111) enclosed octahedron (i.e. 44.24 kJ/mol). The deviation can be attributed to the solvation effect in aqueous solution which can facilitate the removal of phosphate from CeO<sub>2</sub> surfaces and hence free the active site for next catalytic cycle<sup>[30]</sup>.

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Accordingly, we believe the rate-limiting step for this reaction is the activation of P=O bond (by surface Ce) rather than the desorption of phosphate from CeO<sub>2</sub> surface.

**Figure 4d** summarizes the correlation between the Ce electron density (using  $\delta^{31}$ P as descriptor), activation energy (kJ/mol) and the resulting rate constant (hour<sup>-1</sup>) among CeO<sub>2</sub> morphologies for this reaction. In addition to the facilitated *p*-NPP adsorption, the increase of Ce acidity from 45.1 ppm of cube, 47.2 ppm of rod to 50.2 ppm of octahedron can lower the activation energy of *p*-NPP (green line) and hence increases the corresponding phosphatase-like activity (brown line). Since rod is active while cube is inactive at 25°C (**Figure 2b**), we believe the threshold of Ce electron density ( $\delta^{31}$ P) for catalyzing this reaction is between 47.2 ppm and 45.1 ppm.

#### 2.4. The Origin of Pristine CeO2 as Peroxidase Mimetics

For the peroxidase-mimicking reaction (**Figure 2d-f**), the oxidation of TMB (i.e. color indicator) is catalyzed by the reduction of H<sub>2</sub>O<sub>2</sub> to water in the presence of CeO<sub>2</sub>. The ratelimiting step of this reaction is believed the sensing of H<sub>2</sub>O<sub>2</sub> by the surface Ce<sup>[4,5]</sup>. To confirm this, steady-state Michaelis–Menten kinetics was adopted to study the affinity of H<sub>2</sub>O<sub>2</sub> and TMB to CeO<sub>2</sub> surfaces (**Figure S9**). A series of Michaelis–Menten constant (K<sub>m</sub>) and maximum initial rates (V<sub>max</sub>) were obtained in **Table 1** (see experimental section for details). In general, a lower K<sub>m</sub> value suggests a higher affinity of substrate to catalyst and hence higher enzymatic activity<sup>[4,5]</sup>. As expected, CeO<sub>2</sub> cube shows the lowest K<sub>m</sub> value towards H<sub>2</sub>O<sub>2</sub> followed by rod and octahedron, matching well with the order of their H<sub>2</sub>O<sub>2</sub> V<sub>max</sub> (**Table 1**) and k constant (**Figure 2f**) (i.e. cube > rod > octahedron). Given that octahedron and cube with comparable TMB K<sub>m</sub> (**Table 1**) while exhibit extreme activity as peroxidase mimetics, the sensing of H<sub>2</sub>O<sub>2</sub> by the surface Ce is thus confirmed the key step dictating their peroxidase-like activity.

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Since the adsorption behavior of H<sub>2</sub>O<sub>2</sub> on CeO<sub>2</sub> morphologies cannot be visualized by STEM-EELS (**Figure 4a**), density function theory (DFT) calculation was carried out to study its adsorption configuration/energy on CeO<sub>2</sub> surfaces (**Figure S10**). As shown in **Figure 5a**, a H<sub>2</sub>O<sub>2</sub> adsorption energy ( $E_{ad}$ ) of -0.70 eV can be obtained when the H<sub>2</sub>O<sub>2</sub> molecule uses one oxygen atom to bind to the 7-coordinated Ce on the (111) surface. A stronger  $E_{ad}$  of -1.33 eV can be obtained on the (110) surface with 6-coordinated Ce due to the formation of Ceperoxido species (**Figure 5b**). Similar Ce-peroxido species can be also found on the (100) surface with surface 6-coordinated Ce (**Figure 5c**). However, one oxygen atom of Ceperoxido species tends to refill the structural O vacancy and hence provides the most negative  $E_{ad}$  of -1.95 eV among three surfaces. The adsorption of H<sub>2</sub>O<sub>2</sub> on CeO<sub>2</sub> surfaces is thus facetdependent in the order of (100) > (110) > (111), matching well with the peroxidase-like activity of their morphologies (**Figure 2f**).

In additional to the facilitated  $H_2O_2$  adsorption on cube (100), the Ce atom with higher electron density on this surface (**Figure 3e**) is believed to reduce  $H_2O_2$  easier (i.e. the activation of O-O bond). Since Raman band associated with peroxide species on CeO<sub>2</sub> can be monitored at around 870 cm<sup>-1[31,32]</sup>, *in-situ* Raman spectra were thus performed to monitor the fate of surface adsorbed  $H_2O_2$  (**Figure S11**). **Figure 5d** shows the corresponding Raman spectra with enlarged range between 800 cm<sup>-1</sup> and 950 cm<sup>-1</sup>. This signal was further normalized by the intensity of bulk Ce-O signal at 463 cm<sup>-1</sup> as a function of time (**Figure 5e**). It is clear that the peak ratio of  $I_{872}/I_{463}$  for the cube returns to its origin in 15 mins after  $H_2O_2$ addition while this ratio almost remains the same for the octahedron over the reaction time. This result confirms that the O-O bond of  $H_2O_2$  can be activated (or weakened) upon its adsorption on cube (100) but not for octahedron (111).

According to above discussion, the high peroxidase-like activity of cube (100) can be attributed to (1) the facilitated  $H_2O_2$  adsorption and (2) the promoted  $H_2O_2$  activation by

surface electron-rich Ce species. Figure 5f summarizes the correlation between Ce electron density ( $\delta^{31}$ P), H<sub>2</sub>O<sub>2</sub> affinity (K<sub>m</sub>) and the resulting rate constant (min<sup>-1</sup>) for this reaction. The increasing Ce electron density ( $\delta^{31}$ P) from octahedron (111), rod (110) to cube (100) (green line), together with the enhanced  $H_2O_2$  affinity (i.e. lowered  $K_m$ ), can lower the energy barrier for  $H_2O_2$  reduction and hence synergistically increase peroxidase-like activity (brown line).

#### 2.5. Discussion

Since the first hydrothermal synthesis of  $CeO_2$  morphologies<sup>[20]</sup>, researchers have dedicated to study their facet-dependent catalysis in many important reactions<sup>[16,17]</sup>. However, most researchers simply correlate the observed activity of a catalyst using commercial XPS without acknowledging how representative the result could be. Even though the adsorption and activation of reactants on catalyst' surface are the two key steps in heterogeneous catalysis<sup>[18]</sup>, the interplay between them has been often ignored in literatures<sup>[4-17]</sup>. This explains why very limited enhancements (< 200%) in the phosphatase/peroxidase-like activity of CeO<sub>2</sub> was reached although lots of efforts have been put on their surface engineering over the past decades<sup>[11,12,14,15]</sup>. Herein, we demonstrate that 2506%/1133% increase in the corresponding k constant can be easily reached even on pristine  $CeO_2$  once its terminal surface is correctly selected.

#### **3.** Conclusion

Taking two CeO<sub>2</sub>-catalyzed reactions as an example, we demonstrated here that the adsorption/activation of reactants at catalyst' surface is highly relevant to the electron density of active sites even for catalysts with the same composition/crystal structure. As revealed by TMPO-<sup>31</sup>P NMR, the electron density of surface Ce among CeO<sub>2</sub> shapes is facet-dependent in the order of (100) > (110) > (111). For phosphatase-mimicking reaction, Ce atoms on the (111) surface with low electron density can effectively promote the adsorption and hydrolysis of *p*-NPP at room temperature. In stark contrast, the high peroxidase-like activity of CeO<sub>2</sub>

cube can be attributed to the facilitated adsorption of  $H_2O_2$  and its reduction by the electronrich Ce atoms on the (100) surface. This explains the extreme 2506%/1133% rate constant obtained for pristine CeO<sub>2</sub> as phosphatase-/peroxidase-mimetics. Most importantly, "surface area" was found only becomes an important factor when surface Ce can activate the adsorbed molecules in both reactions. This study thus provides some fundamental but critical insights into facet-dependent properties for the future design of CeO<sub>2</sub>-based nanozymes.

#### 4. Experimental Section

*Synthesis of CeO*<sub>2</sub> *Octahedron, Rod and Cube:* For octahedron, 10 mL of 0.25M hexamethylenetetramine (HMT) was mixed with 50 mL of 0.0185M Ce(NO<sub>3</sub>)<sub>3</sub> solution. The reaction was carried out at 75 °C (water bath) for 3 h. Both rod and cube were prepared by hydrothermally heating the solution of Ce(NO<sub>3</sub>)<sub>3</sub> and NaOH at 100°C and 180°C for 24 hours. 0.744g of Ce(NO<sub>3</sub>)<sub>3</sub>.6H<sub>2</sub>O was mixed with 30 mL of 6 M NaOH solution for rod, while 0.6513g of Ce(NO<sub>3</sub>)<sub>3</sub>.6H<sub>2</sub>O was mixed with 30 mL of 3M NaOH solution for cube. All asprepared CeO<sub>2</sub> samples were washed three times by DI water and dried in oven at 200 °C for 12 h before use.

*Phosphatase-mimic Activity:* This reaction was carried out at 25 °C by mixing 4 mg CeO<sub>2</sub> and 10 mL of *p*-NPP solution (0.2 mg/mL). At each time point, 0.5 mL of the reaction solution was sampled and diluted to 1 mL with ethanol. CeO<sub>2</sub> in the collected solution was further removed by centrifugation. The supernatants were then evaluated by UV-Vis adsorption spectra (UV-Vis). The concentration of *p*-NPP (reactant) and *p*-NP (product) can be determined by the absorbance at 310 nm and 405 nm. For kinetic study, this reaction was further carried out at 0 °C (ice bath), 50 °C and 75 °C (oil bath) during reaction.

*Peroxidase-mimic Activity and Michaelis–Menten Kinetic Study:* 1 mg of CeO<sub>2</sub> morphologies was firstly dispersed in 1 mL of acetate buffer (50 mM). The reaction can be initiated by mixing 25 µL of this solution with 0.1 mL of TMB solution (5 mM), 2.8 mL of acetate buffer

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and 0.1 mL of  $H_2O_2$  (1.5 M). The final concentration of  $H_2O_2$  is thus 0.5 M. The reaction was carried out at 25°C and monitored at 652 nm (TMB<sub>ox</sub>) every 12 seconds. For Michaelis– Menten kinetic study, different concentration of  $H_2O_2$  and TMB were adopted to evaluate the corresponding affinity to CeO<sub>2</sub> morphologies. For Michaelis–Menten kinetic study, the K<sub>m</sub> (Michaelis constant) and the maximal reaction velocity (V<sub>max</sub>) can be obtained by the equation below:

$$V_0 = \frac{V_{\text{max}} \times [C]}{K_m + [C]} \tag{1}$$

Where [C] is the concentration of substrate ( $H_2O_2$  or TMB) and  $V_0$  is the corresponding initial rate calculated from the first 3 min of the reaction.

XPS, EPR and CV Measurements: Ce<sub>3d</sub> XPS spectra were recorded by a Thermo XPS equipped Al as X-ray source. The X-band CW electron paramagnetic resonance (EPR) spectrometer (Bruker EMX) was used for collecting the EPR spectra of CeO<sub>2</sub> morphologies. Cyclic voltammetry (CV) measurement of CeO<sub>2</sub> morphologies were carried out by CHI650E Electrochemistry Workstation using Ag/AgCl (3M KCl) as reference electrode and a Pt wire as counter electrode.

STEM-EELS Experiment for p-NPP Adsorption: To visualize the adsorption over CeO<sub>2</sub> surface, scanning transmission electron microscopy-electron energy loss spectroscopy (STEM-EELS) mappings of phosphorus and cerium were carried out by JEOL 2100F under STEM mode. Before conducting the mapping, CeO<sub>2</sub> cube and octahedron were pretreated in p-NPP solution. Briefly, 1 mg of ceria was dispersed in 1 mL of DI water. After that, 0.1 mL of ceria suspension was mixed with 0.9 mL of p-NPP ethanol solution (0.2 mg/mL). The mixture was kept in ice bath (0°C) for 5 min to avoid the dephosphorylation of the p-NPP. Finally, the pretreated samples were analyzed by STEM-EELS mapping.

In-situ Raman for H<sub>2</sub>O<sub>2</sub> Activation: Raman measurements were carried out by BaySpec's Agility<sup>™</sup> Raman spectrometer using an excitation laser wavelength of 785 nm. The H<sub>2</sub>O<sub>2</sub> solution (6mM) was prepared using acetate buffer (pH=4.0, 50 mM) and 30% H<sub>2</sub>O<sub>2</sub>. 3 mg of CeO<sub>2</sub> (cube/octahedron) was loaded on a clean glass slide in 2 mm\*2 mm area. Raman measurements were carried out by BaySpec's Agility<sup>™</sup> Raman spectrometer using an excitation laser wavelength of 785 nm. Before adding H<sub>2</sub>O<sub>2</sub> solution, the Raman spectrum obtained from raw ceria was set as 0 min. After recoding the spectrum of raw ceria, 10 µL of H<sub>2</sub>O<sub>2</sub> solution (6mM) was added gently on ceria. The spectra were recorded at 1, 4, 10, 15 and 30 min after the addition of H<sub>2</sub>O<sub>2</sub> solution.

Density functional theory (DFT) calculation: Geometry optimizations, total energies and charge analysis calculations were performed using the Vienna ab initio simulation package, with projector augmented wave pseudopotential and the PBE-type exchange-correlation functional. See supporting information (SI) for details.

X-ray absorption spectroscopy (XAS): Ce L<sub>3</sub>-edge XAS studies on CeO<sub>2</sub> morphologies were carried out at the National Synchrotron Radiation Research Centre in Taiwan.

<sup>31</sup>P NMR measurement: The surface of CeO<sub>2</sub> sample (100 mg) was firstly cleaned at 373 K under vacuum ( $10^{-1}$  Pa) before trimethylphosphine oxide (TMPO) adsorption. This sample was then mixed with CH<sub>2</sub>Cl<sub>2</sub> solution containing 0.1M TMPO for probe adsorption. The TMPO-adsorbed CeO<sub>2</sub> sample was dried under vacuum before <sup>31</sup>P NMR measurement at room temperature (Bruker Avance III 400WB). The <sup>31</sup>P chemical shifts were reported relative to 85% aqueous solution of H<sub>3</sub>PO<sub>4</sub>. The quantitative analysis of adsorbed TMPO molecules was then calculated according to the <sup>31</sup>P calibration line established in our previous literatures<sup>[21-25]</sup>.

#### **Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.



#### **Conflict of Interest**

The authors declare no competing financial interest.

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Z.T., J.Z. and Y.K.P. carried out all experiments from particle synthesis, characterization, catalytic testing to data analysis and wrote the manuscript. All DFT calculations were performed by Y.C.C., J.P.C. and A. H., Y.K.P. supervised this project.

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Scheme 1. Schematic illustration of (a) dephosphorylation of substrates by phosphatases in the presence of  $H_2O$  and (b) oxidation of substrates by peroxidases in the presence of  $H_2O_2$  (TMB: tetramethylbenzidin; NADH: nicotinamide adenine dinucleotide; GSH: glutathione).



**Figure 1.** Transmission electron microscope (TEM) images of CeO<sub>2</sub> (a) octahedron, (b) rod, (c) cube and (d-f) the corresponding high-resolution TEM (HRTEM) images.



**Figure 2.** (a-c) Phosphatase-mimicking reaction and (d-f) peroxidase-mimicking reaction over CeO<sub>2</sub> morphologies. The corresponding (b, e) enzyme-like activity as a function of time and (c, f) rate constant k at room temperature.



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**Figure 3.** (a-c) Surface crystallographic analysis of CeO<sub>2</sub> surfaces showing the difference in electron density between saturated Ce in bulk (8 coordination) and surface unsaturated Ce. Noted that the electron density of 7-coordinated Ce on CeO<sub>2</sub>(111) was set at 0.0000 for comparison. (d) Schematic illustration of the characterization of surface Ce by TMPO-<sup>31</sup>P NMR and (e) the corresponding spectra of CeO<sub>2</sub> morphologies.



**Figure 4.** (a) STEM-EELS Ce/O/P mapping of CeO<sub>2</sub> octahedron and cube after *p*-NPP adsorption. (b) Schematic illustration of the characterization of surface Ce by TMPO-XAS. (c) The change of coordination unsaturated/saturated Ce w/o TMPO adsorption among CeO<sub>2</sub> morphologies. (d) The correlation of Ce electron density with the corresponding k constant (hour<sup>-1</sup>) and activation energy (kJ/mol) of CeO<sub>2</sub> morphologies.



**Figure 5.** The calculated adsorption configuration and energy of  $H_2O_2$  on CeO<sub>2</sub> (a) (111), (b) (110), (c) (100) surfaces (also see **Figure S10**). (d) The time-dependent in-situ Raman results of CeO<sub>2</sub> octahedron and cube in the presence of  $H_2O_2$  and (e) the corresponding change of peak ratio  $I_{872}/I_{463}$ . (d) The correlation of Ce electron density with the corresponding k constant (hour<sup>-1</sup>) and  $H_2O_2$  K<sub>m</sub> (M) of CeO<sub>2</sub> morphologies.

**Table 1.** The summary of the Michaelis–Menten constant ( $K_m$ ) and the maximum reaction rate ( $V_{max}$ ) of  $H_2O_2$  and TMB among CeO<sub>2</sub> morphologies.

	H <sub>2</sub> O <sub>2</sub>		TMB	
	$K_{m}\left(M ight)$	$V_{max}$ ( $\mu$ M/s)	$K_{m}\left(mM ight)$	$V_{max} \left( \mu M / s \right)$
Octahedron	2.2614	0.0135	0.1019	0.0078
Rod	1.1328	0.0198	0.2439	0.0157
Cube	0.1236	0.0284	0.1021	0.0460

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The electron density of surface Ce is revealed facet-dependent among CeO<sub>2</sub> morphologies in the order of cube (100) > rod (110) > octahedron (111), which significantly affects their enzyme mimicking activities. It is concluded for pristine CeO<sub>2</sub> that the Ce atoms on octahedron (111) with lower electron density can facilitate its phosphatase-like activity, while its counterpart on cube (100) is the key for high peroxidase-like activity.



Nanoisozyme, Ceria, Facet-dependent electron density, Enzyme-mimetic activity Zicong Tan, Yu-Cheng Chen, Jieru Zhang, Jyh-Pin Chou, Alice Hu, and Yung-Kang Peng\* Nanoisozymes: The Origin behind Pristine CeO<sub>2</sub> as Enzyme-mimetics