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# An Unusual Two-Step Hydrolysis of Nerve Agents by a Nanozyme

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Organophosphate-based nerve agents irreversibly inhibit acetylcholinesterase enzyme, leading to respiratory failure, paralysis and death. Several organophosphorus hydrolases are capable of degrading nerve agents including pesticides and insecticides. Development of stable artificial enzymes capable of hydrolysing nerve agents is important for the degradation of environmentally toxic organophosphates. Herein, we describe a Zrincorporated CeO<sub>2</sub> nanocatalyst that can be used for an efficient capture and hydrolysis of nerve agents such as methyl paraoxon to less toxic monoesters. This unusual sequential degradation pathway involves a covalently linked nanocatalyst-phosphodiester intermediate.

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Organophosphate-based nerve agents such as tabun (1), sarin (2), VX (3) (Figure 1a) were used as chemical warfare agents since World War II.<sup>[1]</sup> These compounds and other highly toxic organophosphate-based pesticides/insecticides such as methyl paraoxon (4) and its analogues (5-7), dichlorvos (8), methyl chlorpyrifos (9) affect the nervous system, leading to paralysis, convulsions, and death by asphyxiation.<sup>[1-3]</sup> This is due to the irreversible inhibition of the enzyme acetylcholinesterase (AChE), which is responsible for the breakdown of the neurotransmitter acetylcholine (Figure S1).<sup>[1c]</sup> It is known that soil bacteria survive in extremely toxic conditions because they produce several enzymes that can detoxify nerve agents by hydrolysis.<sup>[4]</sup> The importance of nerve agent detoxification related to environment and human health led to the development of catalysts that can efficiently hydrolyse the phosphotriester bonds, which include metal complexes, organic polymers, metal-organic frameworks (MOFs) and nanomaterials. Initial studies on the hydrolysis of organophosphates were mainly focused on metal complexes having mono and binuclear Zn(II) and Cu(II) centres.<sup>[5]</sup> Farha, Hupp, and coworkers as well as Barea, Navarro, and co-workers reported the design and synthesis of a series of MOFs capable of hydrolysing nerve agents such as methyl paraoxon, soman and VX, under catalytic conditions.<sup>[6,7]</sup> Also, clay-based catalytic systems have emerged as promising candidates for the oxidative abatement of nerve agent simulants.<sup>[8]</sup> Recently, nanozyme-mediated catalysis attracted significant research interest.<sup>[9]</sup> We reported

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**Figure 1.** a) Chemical structures of some common nerve agents. b, c) TEM image and SAED pattern (inset) of VEC, 1ZC nanoparticles respectively. d) HRTEM image of 1ZC showing (111) planes. e) FT-Raman spectra of 1ZC showing characteristic peak for Ce–O and oxygen vacancies (inset). f) XRD pattern of VEC and 1ZC. \*Represents the characteristic shift in 20 (from 59.8° to 58.7°) of (222) plane upon Zr-doping. g) Quantification of vacancies on the surface of different nanoceria by XPS. h) Representation of surface vacancy (purple) on Zr-incorporated nanoceria.

that a vacancy-engineered nanoceria (VEC) functionally mimics the phosphotriesterase (PTE) activity and can convert paraoxon to diethylphosphate.<sup>[9g]</sup> While a rapid hydrolysis of phosphotriesters to diesters is important, it is equally important to convert the diesters further to monoester and phosphate as phosphodiesters are very persistent in the environment, and they retain significant cholinesterase inhibitory effect.<sup>[10]</sup> However, the diesters produced by a single-step hydrolysis of the triesters often have unactivated ester bonds that resist further hydrolysis. Furthermore, the diesters generally act as catalyst poisons, leading to an incomplete decontamination of the nerve agents.<sup>[Sc,6a]</sup> In this paper, we show that a simple modification in the vacancy-engineered nanoceria (VEC) by Zr



incorporation not only alters the substrate preference, but also facilitates the hydrolysis of diesters to monoesters through an unusual two-step process. This study represents the first example of a nanozyme-mediated two-step hydrolysis of a nerve agent, leading to the formation of a monoester.

In the CeO<sub>2</sub>-mediated decontamination of nerve agents, it is important to generate Ce<sup>3+</sup> on the surface in addition to the existing Ce<sup>4+</sup> ions.<sup>[9g]</sup> This can be achieved by creating oxygen vacancies (OVs) as surface defects in the material. It has been shown theoretically that the introduction of  $Zr^{4+}$  ions to CeO<sub>2</sub> not only can increase the oxygen release, but also can enhance the thermal stability of its surface vacancies.<sup>[11]</sup> Furthermore, the OVs created near the Zr<sup>4+</sup> sites are expected to be less mobile than that of the surface having only cerium ions.<sup>[11c]</sup> The Zrincorporated CeO<sub>2</sub> nanomaterials (ZCs) required for this study were prepared by co-precipitation method by changing the Zr/ Ce molar ratio at 1%, 5% and 10% to give 1ZC, 5ZC and 10ZC, respectively. The X-ray diffraction (XRD) pattern (Figure 1f and Figure S2a) showed all three Zr-doped CeO<sub>2</sub> nanoparticles have a cubic fluorite structure similar to the previously reported CeO<sub>2</sub> (JCPDS no. 01-0800) and Zr-doped CeO<sub>2</sub> (JCPDS no. 02-1311). Transmission electron microscopy (TEM) images show spherical morphologies of all the three ZCs with particle diameter in the range of 4.0 to 5.0 nm, which is in good agreement with the crystallite size obtained from XRD (Figures 1b, c, S3-S4 and S2b-d). High-resolution TEM (HRTEM) images indicate that there is no major morphological variation upon Zr-doping (Figure 1d and S5). The presence and distribution of Ce, Zr and O were confirmed by energy dispersive spectroscopy (EDS) and X-ray mapping respectively (Figures S6 and S7). In FT-Raman spectra, the characteristic peak for Ce-O vibration appears at 460 cm<sup>-1</sup> in ZCs and bulk CeO<sub>2</sub> (Figure 1e and S8).<sup>[12]</sup> With an increase in the Zr content, the peak position is shifted slightly to higher wavenumber with respect to VEC, indicating that the introduction of Zr<sup>4+</sup> strengthens the Ce-O bond and thereby enhances the thermal stability (Table S2). In contrast to bulk CeO<sub>2</sub>, a broad peak around 600 cm<sup>-1</sup>, characteristic of OVs in the CeO<sub>2</sub> lattice, was observed for all nanoceria.

The presence of OVs on nanoceria surface was also confirmed by X-ray photoelectron spectroscopy (XPS) and the increase in the OVs upon Zr doping was quantified by measuring the ratio of  $Ce^{3+}/Ce^{4+}$  from the deconvolution of Ce 3d spectra (Figure 1g, S9, Table S3 and S4).<sup>[12]</sup> The removal of oxygen from CeO<sub>2</sub> lattice during the formation of vacancy leads to an electronically dense environment by the reduction of  $Ce^{4+}$  to  $Ce^{3+}$  as well as a partial reduction of  $Zr^{4+}$  to  $Zr^{(4-x)+}$ .<sup>[12b]</sup> This suggests that Zr ions are localized in the vicinity of the vacancies on the surface of nanoceria as proven by XPS analysis and shown in Figure 1h. The Zr/Ce ratio on the surface of different ZCs as calculated from the XPS was found to correlate well with the amount determined by inductively-coupled plasma mass-spectrometry (ICP-MS) (Table S5).

The nerve agent degradation by structurally stable ZCs was studied using the common pesticides/insecticides, dimethyl pnitrophenyl phosphate (DMNP) also known as methyl paraoxon (4) and its thio-analogue, methyl parathion (6) (Figure 2). The

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**Figure 2.** a) Reaction scheme of the hydrolysis of **4** by nanocatalyst. b) Comparison of the conversion of **4** by 5 mol% of 1ZC, 5ZC and 10ZC and c) Conversion of **6** by different mol% of 1ZC determined using UV-Vis spectroscopy. d) <sup>31</sup>P NMR spectra of products formed after 24 h of hydrolysis of **4**: (I) without catalyst, 0.035 mol% of nanocatalysts (II) VEC, (III) 0.5ZC, (IV) 1ZC, (V) 5ZC and (VI) 10ZC. Conditions: nanocatalyst, organophosphate, 0.9 M MM in water, 45 °C.

hydrolysis was performed by using 5 mol% of 1ZC in the presence of 0.9 M N-methyl morpholine (NMM) as a general base in water at 45 °C. When the progress of the hydrolysis of 4 was followed by UV-Vis spectroscopy, a peak at 405 nm indicated the formation of *p*-nitrophenol (Figure S11). It was observed that the amount of Zr present in the nanozymes has a significant effect on the activity, which follows the order: 1ZC > 5ZC > 10ZC (Figure 2b and c). Interestingly, 1ZC catalyses an unusual two-step hydrolysis of **4** and **6** at faster rate  $(t_{1/2})$ value of 1.2 min and 3.5 min for methyl paraoxon and methyl parathion hydrolysis, respectively) as compared to the one-step hydrolysis by other materials reported earlier (Table 1). When the hydrolysis of **4** was followed by <sup>31</sup>P NMR, a rapid decrease in the peak corresponding to 4 at -4.3 ppm was observed with two new peaks appearing at 2.5 ppm and 4.5 ppm corresponding to the diester 10 (DMP) and monoester 11 (MMP), respectively (Figure 2d). While no hydrolysis was observed in the presence of bulk CeO<sub>2</sub> or ZrO<sub>2</sub> nanomaterial even after 24 h (Figure S12), the formation of monoester 11 produced in the presence of 5ZC and 10ZC was relatively lower than that of 1ZC.

While higher amount (%) of unreacted methyl paraoxon was detected in the presence of 5ZC and 10ZC, no change in the concentration of intermediate **10** was observed (Figure 3a). The rate of the formation of MMP (**11**) was also found to be lower for 5ZC and 10ZC as compared to that of 1ZC (Figure S15c). This is probably due to the substitution of  $Ce^{4+}$  by  $Zr^{4+}$  which can decrease the number of catalytically active  $Ce^{4+}$  on the surface of nanoceria. It should be noted that optimum



Table 1.	Comparison	of t <sub>1/2</sub>	and	product	formed	after	hydrolysis	of	nerve
agents by Zr-doped nanoceria with previous studies.									

Catalyst	Cat. [mol%]	Substrate	Hydrolysis ter <sup>[d]</sup>	Formation of mono-		
	[]		Product <sup>[c]</sup>	t <sub>1/2</sub> [min]	ester	
UiO-66	6.0	4	DMP	45 <sup>[6a]</sup>	not ob-	
NU-1000	6.0	4 DMP 1		15 <sup>[6b]</sup>	served not ob- served	
UiO-66-NH <sub>2</sub>	6.0	4	DMP	1 <sup>[6f]</sup>	not ob-	
MIL-101(Cr)- DAAP <sup>[b]</sup>	-	5	DEP	300 <sup>[13]</sup>	not ob- served	
VEC	-	5	DEP	10 <sup>[9g]</sup>	observed <sup>[e]</sup>	
1ZC	5.0 <sup>[a]</sup>	4	MMP	1.2	observed <sup>[e]</sup>	
1ZC	5.0 <sup>[a]</sup>	6	MMTP	3.5	observed <sup>[e]</sup>	
5ZC	5.0 <sup>[a]</sup>	4	MMP	10.2	observed <sup>[e]</sup>	
10ZC	5.0 <sup>[a]</sup>	4	MMP	14.5	observed <sup>[e]</sup>	

[a] Number of moles of catalyst in 1 mL = (number of catalyst particles / NA), NA = Avogadro's number. [b] DAAP: dialkylaminopyridine. [c] DMP: dimethyl phosphate, MMP: monomethyl phosphate, MMTP: monomethyl thiophosphate, DEP: diethyl phosphate. [d] Hydrolysis monitored through *p*-nitrophenol formation,  $\lambda_{max}$ =405 nm, 45 °C, 0.9 M NMM, pH 10 by UV-Vis spectroscopy. [e] Products confirmed by <sup>31</sup>P NMR spectroscopy.



**Figure 3.** a) Amount (%) of DMP (**10**) and MMP (**11**) formed after hydrolysis of methyl paraoxon (**4**) by different nanocatalysts. b) Effect of [NMM] on the relative ratio of [MMP] to [DMP] in the reaction mixture formed after hydrolysis of methyl paraoxon by 1ZC. c) Time-dependent <sup>31</sup>P NMR spectroscopy analysis to monitor hydrolysis of **4** by 1ZC up to 24 h. d–e) Speciation of reaction mixture during hydrolysis of **4** by VEC and 1ZC respectively. Conditions: Nanocatalyst (0.035 mol%), methyl paraoxon (**4**) (27.0 mM), 0.9 M NMM in water, 45 °C, total reaction time = 24 h.

amount of Ce<sup>4+</sup> ions is important for the hydrolysis of organophosphate triester to diester, i.e., the first step of the catalysis.<sup>[9g]</sup> In contrast, when the Zr content was decreased to

0.5% in the nanoceria (Figure S10), a complete hydrolysis of 4 was observed. However, in this case, there was a significant decrease in the amount of monoester 11 produced in the reaction. Interestingly, when VEC (0% Zr) was used as catalyst, the amount of monoester 11 produced in the reaction was found to decrease further such that it was almost identical to that of the diester 10 even after 24 h (Figures 3a, S15b). These observations suggest that an optimum Zr doping is important for the complete two-step degradation of methyl paraoxon 4 to monoester 11. Thus, among different ZCs, 1ZC was found to be the most effective catalyst for the two-step hydrolysis of nerve agent stimulants. The steady state kinetics studies at a fixed concentration of nanoparticles (0.035 mol%) indicated that the hydrolysis of 4 by the nanocatalysts leading to the formation of 11 follows a typical Michaelis-Menten kinetics (Figure S18 and Table S6).

To further understand the role of base in the two-step hydrolysis of **4**, experiments were performed with an increasing concentration of NMM. It was observed that the two-step hydrolysis is more favoured at higher concentrations of NMM (Figure 3b, S14 and S15a). A control reaction with NMM alone indicated that the hydrolysis of **4** in the absence of nanocatalyst is extremely slow. Similarly, no hydrolysis was observed in the presence of nanocatalyst when NMM was not used. These observations suggest that NMM plays a key role in generating a nucleophilic OH<sup>-</sup> species on the catalyst surface, which can attack at the electrophilic P(V) centre of the nerve agent.

A time-dependent investigation of the hydrolysis of 4 by 1ZC and other nanocatalysts showed some interesting features. A careful analysis of the hydrolysis mediated by all ZCs by <sup>31</sup>P NMR indicates that small amount of 10 produced during the initial period of the reaction remains constant even after 24 h, indicating that the hydrolysis of 4 to 11 occurs in a single cycle without removal of 10 from the active site pocket of the nanozyme (Figures 3c-e, S17 and S16b-c). In other words, DMP generated from methyl paraoxon remains attached to the reaction sites throughout the catalytic cycle. These observations suggest the possibility of a covalent nanozyme catalysis, in which a covalent bond formation between the substrate and nanomaterial may favour the further hydrolysis of the diester to the corresponding monoester. In contrast, a significant increase in the amount of 10 was observed for VEC, suggesting that a covalent attachment of 10 at the active site pocket of VEC is not a favoured process (Figure 3d and S16a). The increase in the formation of monoester in the presence of ZCs can be ascribed to the Zr-doping in CeO<sub>2</sub> lattice, which can stabilize the covalent nanozyme-DMP complex. This is in agreement with the earlier report that Zr present on the catalyst surface acts as anchoring site for organophosphates.<sup>[7b]</sup> If the DMP gets released from the active site after the first step hydrolysis, the subsequent hydrolysis is not a favoured process as observed for VEC. This is in agreement with our earlier observation that greater amount of 10 is produced in the VEC catalysed hydrolysis of 4 (Figure 3a).

To study the versatility of 1ZC for the hydrolysis of nerve agents, we carried out the hydrolysis of extremely toxic pesticides and insecticides, methyl parathion (**6**), dichlorvos (**8**),



and methyl chlorpyrifos (9).<sup>[14]</sup> As observed for methyl paraoxon, its thio analogue, methyl parathion underwent a two-step hydrolysis to produce the monoester, *O*-methyl thiophosphate (17) (Figure S19–S20). Similarly, 1ZC was also able to hydrolyse other related phosphotriesters, dichlorvos (8) and methyl chlorpyrifos (9) to the corresponding monoester derivatives (Figure S20–21). While all the triesters underwent the two-step hydrolysis by 1ZC to form monoesters, the rate of conversion was found to be dependent on the nature of leaving group attached to the phosphorus centre as well as the P=X (X=O or S) moiety in the molecule (Figure S23).

To understand the unusual two-step hydrolysis of nerve agents by 1ZC in detail, we synthesized the mixed triester, ethylmethyl *p*-nitrophenyl phosphate (12) and the activated diester, methyl *p*-nitrophenyl phosphate (14) (Figure 4a). When



**Figure 4.** a) Chemical structures of substrates used in the reaction and their corresponding products (**12-15**). b) Hydrolysis of **12** (20.0 mM) by 1ZC as a function of time showing selective formation of **13**. c) Hydrolysis of **14** (20.0 mM) by 1ZC, showing an increase in the formation of **15** as a function of time. The reactions were monitored by <sup>31</sup>P NMR spectroscopy. d) Attempted hydrolysis of the unactivated ester DMP (**10**) (25.0 mM) by: (from top to bottom) VEC, 1ZC, 1ZC with 2.5 mM *p*-nitrophenol (2.5 mM DMP used in this case), 1ZC with ionic salt of DMP as substrate. Conditions: Nanocatalyst (2.5 mg mL<sup>-1</sup>), substrate, 0.9 M NMM in water, 45 °C, 24 h.

compound 12 was treated with 1ZC, an almost quantitative conversion to monoethyl phosphate (13, MEP) was observed (Figure 4b). The selective formation of 13 from 12 can be ascribed to the better leaving group ability of methoxy group as compared to that of an ethoxy substituent. If the reaction proceeds through the formation of a covalent bond between the product of the first step, i.e. a diester and the nanozyme, a direct hydrolysis of diester to phosphate must occur when an activated diester is used for the hydrolysis. As expected, methyl p-nitrophenyl phosphate (14) readily underwent hydrolysis by 1ZC to produce 15 (Figure 4c). In contrast, 10 did not undergo any hydrolysis by VEC, 1ZC (Figure 4d), 5ZC or 10ZC even after 48 h in the presence of higher concentrations of NMM (Figure S24a-b and d-e). The role of *p*-nitrophenol (eliminated in the first step) in the hydrolysis of 10 was ruled out as 10 did not undergo hydrolysis in the presence of externally added pnitrophenol. Furthermore, to verify the possibility of whether

an ion-pair between 1ZC and **10** is responsible for the hydrolysis, we used 1,3-dimethylimidazolium salt of DMP as substrate and found that the formation of an ion-pair does not mediate the reaction (Figure 4d and S24c). Interestingly, externally added DMP did not inhibit the hydrolysis of **4**, indicating that DMP may not interact with the nanoceria catalyst surface, which is in contrast to a few other catalysts that are poisoned by DMP (Figure S25).<sup>[Sc,6a]</sup> These observations indicate that an activated phosphoester bond is essential for the first step to form a covalent intermediate. Once such an intermediate is formed, even an unactivated diester such as DMP can undergo hydrolysis to produce the corresponding monoester.

To understand the mode of binding between the nanozyme and *in-situ* generated DMP (**10**), FT-Raman spectroscopic analysis<sup>[15]</sup> was performed using the nanomaterial isolated from the reaction mixture at different time points. The spectra indicated the presence of two types of P–O bonds on Ce–O surface: P–O–C at 865 cm<sup>-1</sup> and P–O\*–Ce at 1190 cm<sup>-1</sup> for the phosphodiester bound to 1ZC during the hydrolysis of **4** and **6** (Figures 5a-b).<sup>[16]</sup> It was also observed that the intensity of the



**Figure 5.** a–d) FT-Raman spectra of catalyst-bound phosphodiester intermediates using nanocatalysts recovered at different time points from the reaction mixture containing nanocatalyst (2.0 mg mL<sup>-1</sup>), substrate (25.0 mM), 0.9 M NMM in water, 45 °C. a) Hydrolysis of **4** by 1ZC. b) Hydrolysis of **6** by 1ZC. c) Hydrolysis of **4** by 10ZC. d) Hydrolysis of **4** by VEC. e) FT-IR analysis of the reaction mixture during hydrolysis of **4** (25 mM) by 1ZC (2.0 mg mL<sup>-1</sup>), 0.9 M NMM in water, 45 °C. f) Representation of the phosphodiester intermediate bound to nanocatalyst. g) After the hydrolysis of **4** by VEC (top) and 1ZC (bottom), isolated nanocatalysts were treated with 10 M NaOH and analysed by <sup>31</sup>P NMR spectroscopy after a complete dissolution.



P-O\*-Ce peak at 1190 cm<sup>-1</sup> decreases during the course of the reaction, which is due to the dissociation of the diester from the catalyst surface, leading to the formation of monoester. The minor difference in the peak position for the phosphate-bound catalyst (P-O\*-Ce) upon changing the substrate from 4 to 6 suggests that both the substrates form similar covalent intermediates with the catalyst surface after the first step of hydrolysis (Figures 5a-b and S26a-b). Similar changes were observed for the intermediates isolated for 4 in the presence of VEC and 10ZC (Figures 5c-d and S26c-d). Furthermore, the FT-IR analysis of the reaction mixture at different time points showed a decrease in the intensity of the peak for the nanozyme-bound phosphodiester intermediate (P-O\*-Ce) at 1110 cm<sup>-1</sup> as the reaction proceeded towards completion due to dissociation of the bound intermediate, which is in agreement with the FT-Raman spectra discussed earlier (Figure 5e, S27-S28).<sup>[16]</sup> Interestingly, while the treatment of VEC-based intermediate with 10 M NaOH released phosphodiester also from the nanozyme, treatment of 1ZC-based intermediate released exclusively the monoester (Figure 5g and S29), indicating that the covalent bond between the diester and 1ZC is much stronger than that of VEC. This is consistent with the earlier observation that no major change in the percentage composition of products 10 and 11 was observed between 12 and 24 h during the hydrolysis of 4 by VEC. This observation indicates that the diester 10 produced in the reaction is resistant to further hydrolysis by VEC (Figure 3d and S16a). As 1ZC is expected to be structurally more stable than VEC, a Zr-O-Ce mixed surface may facilitate the formation of a covalent intermediate with the diester during the catalysis (Figure 5f). Together, the above observations suggest that just 1% Zr-doping is sufficient not only to alter the rate of the reaction, but also to remarkably change the mechanistic pathway.

On the basis of above observations, a catalytic cycle involving a covalent intermediate is proposed for the hydrolysis of organophosphate nerve agents (Figure 6). According to this



Figure 6. Mechanism of organophosphate ester hydrolysis by 1ZC.

mechanism (shown for methyl paraoxon (4) and methyl parathion (6), a  $Ce^{3+}$ -bound oxygen nucleophile, generated by a proton abstraction by NMM, attacks at the phosphorus centre of a Ce<sup>4+</sup>-bound phosphotriester to form a covalent bond, with the elimination of *p*-nitrophenol. The coordination of P=X (X=O or S) to Ce<sup>4+</sup> ions polarises the P=X bond, making the phosphorus centre electron deficient. As the phosphodiester bound to the nanozyme is insoluble, we were unable to detect this intermediate by <sup>31</sup>P NMR spectroscopy under given reaction conditions. However, we were able to confirm the formation of a covalent intermediate by FT-Raman and FT-IR spectroscopy. In the second step, the nucleophilic attack of another hydroxyl group at the metal-bound diester leads to the cleavage of P-O bond to produce the corresponding monoester and methanol. Once the monoester is formed, it is cleaved from the active site by a base-mediated hydrolysis, leading to the regeneration of reaction sites. As shown in Figure S30, no changes in the crystal structure, arrangement of planes or morphology were observed, indicating that the catalytically active surface of the nanozyme is very stable under the reaction conditions. The ICP-MS analysis also confirmed that there was no leaching of Ce and Zr ions during the hydrolysis (Table S7).

To understand the ability of the ZC to act as a catalyst for the purification of contaminated water, a small column was designed using 1ZC nanomaterial. The treatment of 1% methyl paraoxon-containing water (56.0 mM, i.e.10<sup>5</sup> times greater concentration than usually found in waste water) was carried out. When methyl paraoxon solution was passed through the column packed with 1ZC, the <sup>31</sup>P NMR spectroscopy analysis of the eluted pesticide showed that more than 90% methyl paraoxon has been converted to monomethyl phosphate. Thus, such covalent catalysis mediated hydrolysis by Zr-doped nanoceria (1ZC) has the potential for the removal of pesticide/ insecticide residues from drinking water. (Figure S31).

In conclusion, we showed for the first time that a simple Zrincorporation in CeO<sub>2</sub>-based nanozymes can facilitate the twostep hydrolysis of nerve agents, leading to the formation of less toxic monoesters. The mechanism of the degradation involves an unusual formation of a covalent intermediate with the nanomaterial, which makes it possible for the cleavage of an unactivated diester bond. The efficiency of two-step covalent catalysis depends not only on the nature of leaving group, but also on the groups attached to the phosphorus centre as well as on the nature of the P=X (X=O or S) moiety in the molecule. While the vacancies in CeO<sub>2</sub> can act as reactive sites for nerve agent degradation, Zr doping can fine-tune the activity as well as the selectivity of the nanozymes.

**Caution:** The organophosphates described in this paper are highly hazardous in nature and necessary precautions should be taken at all stages when working with these compounds.

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#### **Conflict of Interest**

The authors declare no conflict of interest.

**Keywords:** catalysis • enzyme models • nanozymes • nerve agents • phosphotriesterase

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## COMMUNICATIONS

The first two steps! Zr-incorporation in nanoceria facilitates the two-step hydrolysis of nerve agents, by which unactivated diesters can be hydrolysed to monoesters. This study represents the first example of a nanozyme-mediated two-step hydrolysis of a nerve agent.



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An Unusual Two-Step Hydrolysis of Nerve Agents by a Nanozyme