

# Phosphate Triesters Cleavage by Gold Nanozymes <sup>†</sup>

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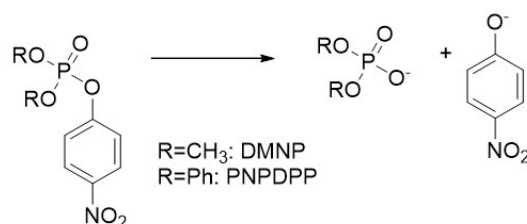
**Abstract:** Phosphate triesters are cleaved by gold nanoparticles functionalized with metal complexes (Zn(II), Cu(II), Co(II), Co(III), Eu(III), Yt(III), Zr(IV)) of triazacyclononane and cyclen ligands with a mononuclear mechanism with impressive rate accelerations with respect to the uncatalyzed processes, constituting a remarkable example of nerve agents hydrolyzing nanozymes.

**Keywords:** phosphate triesters; gold nanoparticles; nanozymes; nerve agents

## 1. Introduction

Phosphate triesters share with phosphate diesters one of the most important bonds (P-OR) present in nature for its relevance in the chemistry of life. To the class of phosphate triesters belong nerve agents that are among the most noxious compounds known to man, with lethal effects even at very low doses [1]. Some of these chemicals were developed for use in agriculture for pest control [2]. They operate by irreversibly reacting with the enzyme acetylcholinesterase which is involved in controlling neuronal signaling [3,4]. Although they are banned by the Chemical Weapons Convention (1997) enforced by the Organisation for the Prohibition of Chemical Weapons [5], they are still available and have been used in regional wars, terroristic attacks or for other criminal purposes. Defined protocols exist for the destruction of large stockpiles of nerve agents, mostly relying on their hydrolysis under strongly basic conditions. Mild methods for the hydrolysis of these compounds are still required and actively sought particularly for use when civilians or military personnel are exposed to them [6–8].

We have reported in the past that gold nanoparticles (AuNPs) functionalized with metal ion complexes are powerful catalysts of the cleavage of phosphate diesters including DNA [9–11]. We thought they were excellent candidates as catalysts for the cleavage of phosphate triesters and nerve agents, in particular. Typically, catalysts developed for this purpose are not tested with the real nerve agents for their toxicity and simulants, far less toxic, are used. We report here the results for the development of AuNPs-based catalysts for the cleavage of nerve agent simulants *p*-nitrophenyl diphenyl phosphate (PNPDPP) and dimethyl *p*-nitrophenyl phosphate (DMNP, methyl paraoxon) shown in Figure 1.



**Figure 1.** Cleavage of nerve agent simulants PNPDP and DMNP (methyl paraoxon) by gold nanoparticles (AuNPs).

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## 2. Experimental Details

The details of the synthesis and characterization of the ligands reported in Figure 2 and the gold nanoparticles obtained by passivation of ca. 2 nm gold clusters will be reported elsewhere.

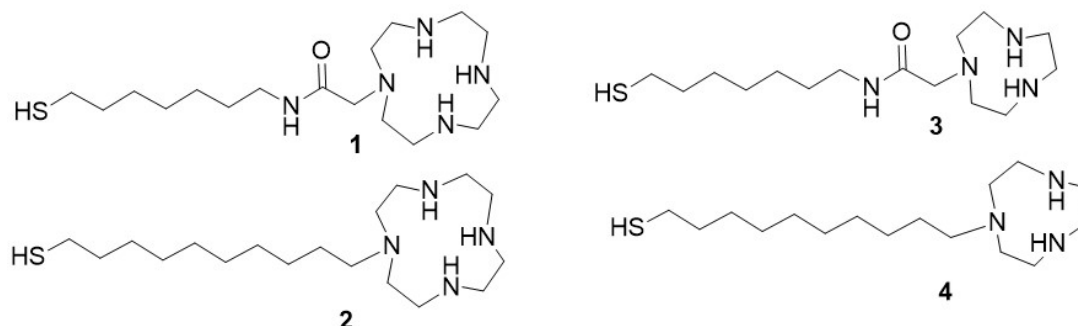


Figure 2. Ligands used for the passivation of the AuNPs.

## 3. Cleavage of *p*-Nitrophenyl Diphenyl Phosphate (PNPDPP) by AuNP1–4 and Metal Ions

Due to its faster reactivity, PNPDPP was used for a quick screening of a small 28-member library constituted by AuNP1–4 in the presence of metal ions Zn(II), Cu(II), Co(II), Co(III), Eu(III), Yb(III) and Zr(IV). The ease of passivation of AuNPs renders the preparation of such a library a relatively simple task and highlights the versatility of AuNPs for rapid nanocatalyst screening. The size of the nanoparticles studied was slightly lower than 2 nm (diameter). This means that each of them is passivated with ca. slightly less than 70 ligands. In analyzing the rate of cleavage of the substrates, we always used the concentrations of the ligands and not that of the nanoparticles. The results of this quick screening are reported in Table 1. The structure of the four ligands studied is reported in Figure 2.

Table 1. Observed rate constant ( $k_{\text{obs}}$ ) obtained for the 28-member library of AuNPs for the cleavage of PNPDPP (25 °C, pH = 8) <sup>a</sup>.

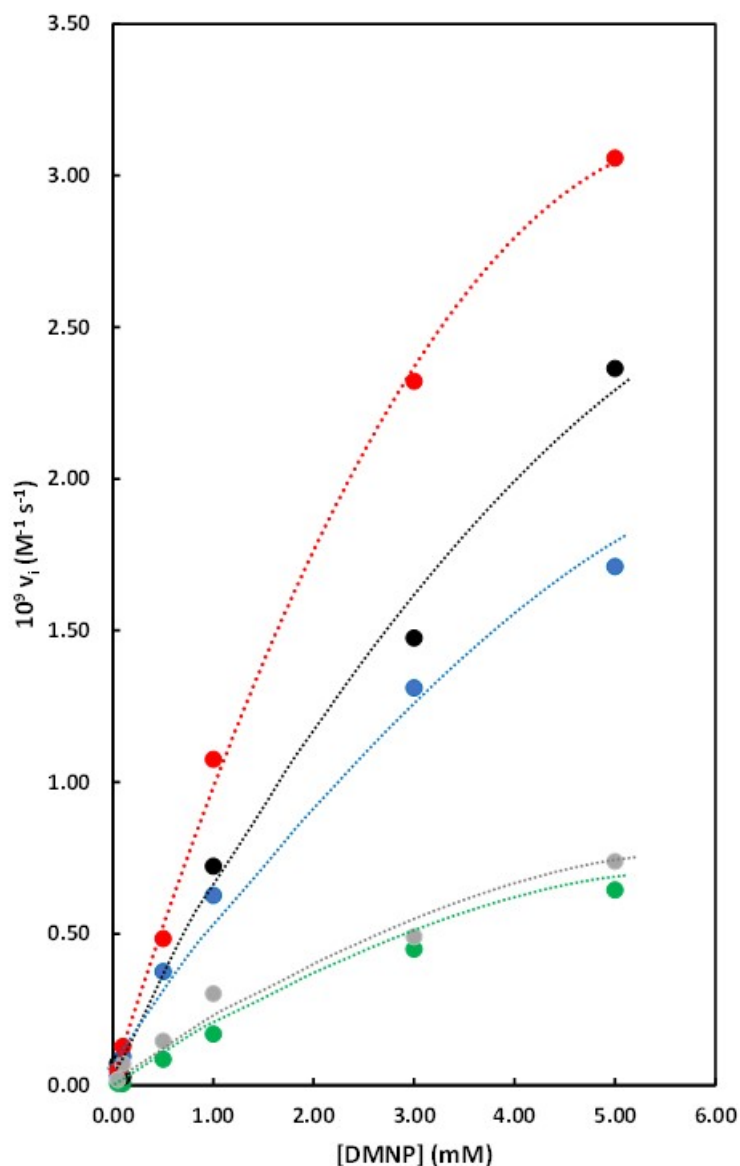
AuNP	Metal Ion	$10^4 k_{\text{obs}}, \text{s}^{-1}$	AuNP	Metal Ion	$10^4 k_{\text{obs}}, \text{s}^{-1}$
AuNP1	Zn(II)	6.03	AuNP3	Zn(II)	6.49
AuNP1	Cu(II)	0.55	AuNP3	Cu(II)	6.37
AuNP1	Co(II)	1.01	AuNP3	Co(II)	0.51
AuNP1	Co(III)	0.61	AuNP3	Co(III)	0.33
AuNP1	Eu(III)	2.44	AuNP3	Eu(III)	0.96
AuNP1	Yb(III)	2.42	AuNP3	Yb(III)	1.00
AuNP1	Zr(IV)	1.47	AuNP3	Zr(IV)	0.74
AuNP2	Zn(II)	20.00	AuNP4	Zn(II)	8.28
AuNP2	Cu(II)	4.94	AuNP4	Cu(II)	8.53
AuNP2	Co(II)	6.17	AuNP4	Co(II)	5.09
AuNP2	Co(III)	4.92	AuNP4	Co(III)	2.76
AuNP2	Eu(III)	10.30	AuNP4	Eu(III)	10.30
AuNP2	Yb(III)	7.79	AuNP4	Yb(III)	1.00
AuNP2	Zr(IV)	5.49	AuNP4	Zr(IV)	4.72

<sup>a</sup> [Catalyst] =  $2 \times 10^{-5}$  M.

The results indicate that AuNP2-Zn(II) and Eu(III) as well as AuNP4-Zn(II), Cu(II) and Eu(III) are the best performing catalysts.

#### 4. Cleavage of Dimethyl *p*-Nitrophenyl Phosphate (DMNP) by the Best Performing Catalysts

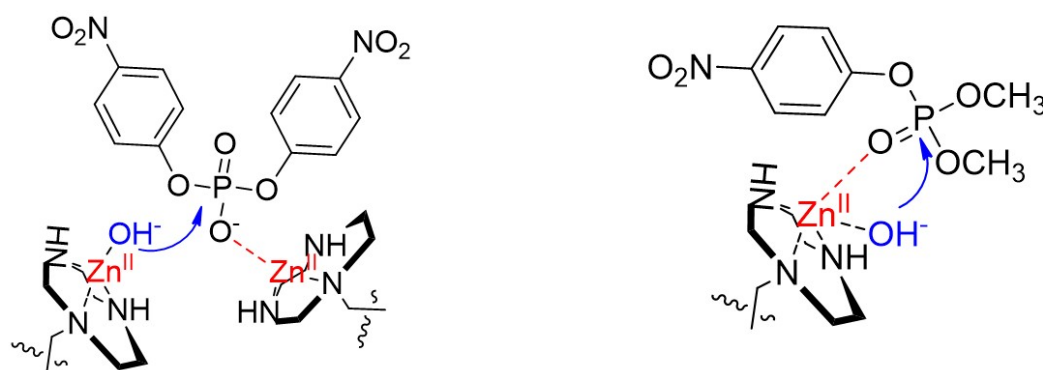
The above best performing catalysts were tested in the hydrolysis of DMNP. Apart from being much less reactive than PNPDP (more than two orders of magnitude) and, hence, more similar to the real nerve agents, DMNP is significantly more hydrophilic. The interaction with the monolayer of the nanoparticles is expected to be driven by a hydrophobic interaction and not by the very weak binding constants of a neutral phosphate with the metal ions. This means that DMNP, at the very low catalyst concentration we have used ( $2 \times 10^{-5}$  M), is mostly not bound to the nanoparticles. However, by performing kinetics under Michealis–Menten conditions, where they show saturation kinetics as for enzymes (hence they are nanozymes) [12,13], (Figure 3) we were able to obtain relevant activity parameters for these catalysts. The analysis allowed us to determine the half-lives for the best performing catalysts (AuNP2-Zn(II) and AuNP4-Cu(II)) that were 27 and 38 min, respectively, for the substrate fully bound to the nanoparticles, at pH = 8 and 25 °C.



**Figure 3.** Michaelis–Menten kinetics for the cleavage of DMNP by the five catalysts AuNP2-Zn(II) (black), AuNP2-Eu(III) (green), AuNP4-Zn(II) (blue), AuNP4-Cu(II) (red) and AuNP4-Eu(III) (gray) at 25 °C and pH = 8.

## 5. Conclusions

The experimental evidence gathered for the cleavage of DMNP allowed us to conclude that the picture is quite different from that observed with the hydrolysis of phosphate diesters. With phosphate diesters, two metal ions cooperate in the cleavage process (Figure 4, left). One coordinates the anionic phosphate oxygen while the other one delivers the nucleophilic OH<sup>-</sup>. In the case of phosphate triesters, only a single metal ion appears to be involved in the catalytic site (Figure 4, right). This metal ion delivers both the nucleophilic species and coordinates the phosphate as the negative charge is forming going towards the transition state. In comparison to other reported catalysts able to cleave nerve agents, the gold nanoparticles perform only slightly worse than the best performing ones, placing them among the most attractive systems working close to physiological pH.



**Figure 4.** Proposed mechanism of cleavage of the phosphate diester bis-*p*-nitrophenyl phosphate (BNP left) and phosphate triester DMNP (right) by gold nanozymes.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Detailed experimental procedures have been published and can be found at: <https://doi.org/10.1016/j.jhazmat.2021.125644> (accessed on 10 November 2020).

## References

- Costanzi, S.; Machado, J.-H.; Mitchell, M. Nerve agents: what they are, how they work, how to counter them *ACS Chem. Neurosci.* **2018**, *9*, 873–885.
- Everts, E. The Nazi origins of deadly nerve gases. *Chem. Eng. News* **2016**, *94*, 26–28.
- Worek, F.; Jenners, J.; Thierman, H. (Eds.) *Rice in Chemical Warfare Toxicology: Volume 1: Fundamental Aspects*; Chapter 3; Toxicology of Organophosphorus Nerve Agents; Royal Society of Chemistry: London, UK, 2016; pp. 81–116.
- Wiener, S.W.; Hoffman, R.S. Nerve agents: a comprehensive review *J. Intensive Care Med.* **2004**, *19*, 22–37.
- Available online: <https://www.opcw.org/chemical-weapons-convention> (accessed on 10 November 2020).
- Kirlikovali, K.O.; Chen, Z.; Islamoglu, T.; Hupp, J.T.; Farha, O.K. Zirconium-based Metal–organic frameworks for the catalytic hydrolysis of organophosphorus nerve agents *ACS Appl. Mater. Inter.* **2020**, *12*, 14702–14720.
- Bobbitt, N.S.; Mendonca, M.L.; Howarth, A.J.; Islamoglu, T.; Hupp, J.T.; Farha, O.K.; Snurr, R.Q. Metal–organic frameworks for the removal of toxic industrial chemicals and chemical warfare agents *Chem. Soc. Rev.* **2017**, *46*, 3357–3385.
- Jiang, L.; Sun, Y.; Chen, Y.; Nan, P. From DNA to nerve agents – the biomimetic catalysts for the hydrolysis of phosphate esters, *ChemistrySelect* **2020**, *5*, 9492–9516.
- Diez-Castellnou, M.; Martinez, A.; Mancin, F. Phosphate ester hydrolysis: the path from mechanistic investigation to the realization of artificial enzymes, *Adv. Phys. Org. Chem.* **2017**, *51*, 129–186.
- Czescik, J.; Zamolo, S.; Darbre, T.; Mancin, F.; Scrimin, P. Factors influencing the activity of nanozymes in the cleavage of an rna model substrate, *Molecules* **2019**, *24*, 2814.
- Czescik, J.; Zamolo, S.; Darbre, T.; Rigo, R.; Sissi, C.; Pecina, A.; Riccardi, L.; De Vivo, M.; Mancin, F.; Scrimin, P. A gold nanoparticle nanonuclease relying on a Zn(II) mononuclear complex *Angew. Chem. Int. Ed.* **2020** doi:10.1002/anie.202012513.
- Gabrielli, L.; Prins, L.J.; Rastrelli, F.; Mancin, F.; Scrimin, P. Hydrolytic nanozymes, *Eur. J. Org. Chem.* **2020**, 5044–5505.
- Manea, F.; Houillon, F.B.; Pasquato, L.; Scrimin, P. Nanozymes: Gold-nanoparticle-based transphosphorylation catalysts, *Angew. Chem. Int. Ed. Engl.* **2004**, *43*, 6165–6169.