

Efficient Phosphodiester Cleaving Nanozymes Resulting from Multivalency and Local Medium Polarity Control

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S Supporting Information

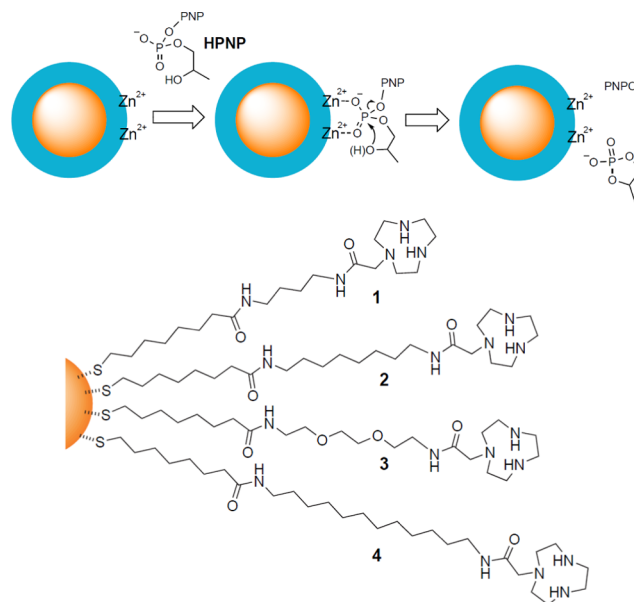
ABSTRACT: The self-organization of Zn(II) complexes on the surface of 1.6-nm diameter gold nanoparticles (nanozymes) allows the spontaneous formation of multiple bimetallic catalytic sites capable to promote the cleavage of a RNA model substrate. We show that by tuning the structure of the nanoparticle-coating monolayer, it is possible to decrease the polarity of the reaction site, and this in turn generates remarkable increments of the cleavage efficiency.

The obtaining of synthetic agents capable of reproducing the reactivity of phosphate ester cleaving enzymes has attracted considerable efforts over the years.¹ Indeed, such systems not only would be suitable for many important applications in the biomedical field, but also represent a stimulating intellectual challenge. In fact, nucleases and related enzymes, often containing metal ions in their active sites,² are among the most efficient enzymes known, producing accelerations up to 16 orders of magnitude of the background reaction.³ Activity of different metal ions and simple monometallic complexes in promoting the hydrolytic cleavage of phosphate esters, mainly by Lewis acid catalysis, has been known for decades, but the accelerations obtained are modest.⁴ The problem has been addressed over the years by rationally designing metal complexes where more than one (usually two) metal ions and/or appropriate organic groups are preorganized to achieve optimal cooperation.¹ However, even the most effective synthetic agents produced in this way are far from reaching enzymes' activity. Somehow, it appears that there is an intrinsic limit to the acceleration that can be achieved using small molecule catalysts in water.⁵ On the other hand, the investigations performed by Brown's group reveal that when the reaction is performed in low polarity solvents, which reproduce the dielectric constant of enzymes active site,⁶ impressive accelerations may be reached.⁷ Under these conditions, even poorly preorganized bimetallic complexes produce rate accelerations of 12 orders of magnitude, approaching those achieved by enzymes. These observations suggest that key elements of nuclease catalysis may be not only the precise preorganization of the functional groups participating in the reaction, but also the ability to place the negatively charged transition state in a low polarity environment, where the electrostatic stabilization exerted by the positively charged metal ions is maximized.

We previously showed that the assembly of Zn(II) complexes on the surface of monolayer-passivated nanoparticles leads to

remarkable cleavage efficiency of phosphate esters thanks to the cooperation between several identical active species ("nanozymes").⁸ In this communication we report how by taking full advantage of the modular structure of a nanozyme, which allows the easy modification of the organic coating monolayer, we were able to obtain a nanosystem capable of accelerating *in water* the cleavage of a RNA model substrate (2-hydroxypropyl-*p*-nitrophenyl phosphate, HPNP, Chart 1) more efficiently than any other artificial hydrolytic agent so far reported in this solvent.

Chart 1. (Top) HPNP Cleavage by Nanozyme Mechanism and (Bottom) Metal Chelating Thiols Used in This Work, Represented in Their Nanoparticle-Bound Form



Thiol **1** (Chart 1), bearing the 1,4,7-triazacyclononane metal chelating moiety, was used previously to prepare efficient nanoparticles for the cleavage of HPNP in the presence of Zn(II).^{8d} As in micellar catalysis, the cleavage reaction occurs at the interface between the bulk aqueous solution and the pseudophase formed by the nanoparticle coating monolayer.⁹ It occurred to us that the structure of thiol **1** allows its easy modification by the insertion of different diamine spacers between the metal chelating and the surface anchoring

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portions, which should consent the tuning of the properties of the nanoparticle-coating monolayer. With this in mind, we prepared¹⁰ gold nanoparticles AuNp1–4 (1.6-nm diameter of the metal core) coated by thiols 1–4 and investigated their ability to promote HPNP cleavage in the presence of Zn(II) ions.

Interestingly, modification of the spacer unit in thiols 1–4 produces remarkable effects on the nanoparticles reactivity as highlighted by Figure 1, which reports the dependence of the

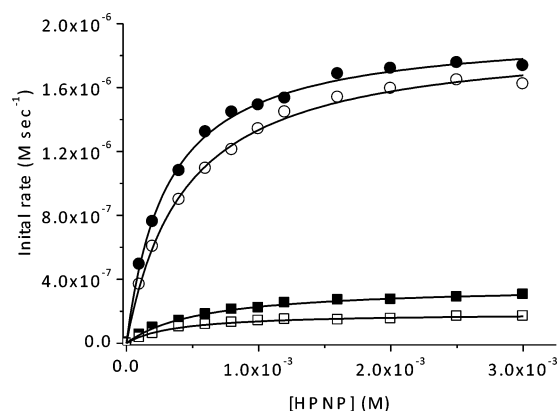


Figure 1. Rate of HPNP cleavage promoted by AuNp1–4 and Zn(II) as a function of the concentration of HPNP (●, AuNp4; ○, AuNp2; ■, AuNp1; □, AuNp3). Conditions: [AuNp, in thiol units] = 2.0×10^{-5} M, [Zn(II)] = 2.0×10^{-5} M, [buffer] = 1.0×10^{-2} M, pH = 7.5, 40 °C.

initial rate of HPNP cleavage (at a fixed AuNp-Zn(II) concentration) as a function of the substrate concentration. Inspection of the curves clearly reveals that the reactivity of the nanoparticles coated with the longer alkyl-spaced thiols 2 and 4 is substantially higher than that of 1-coated nanoparticles. Saturation profiles are followed by all nanoparticles diagnostic of a mechanism involving the formation of a complex between the nanosystem and the substrate before the cleavage reaction. As previously reported, at least two Zn(II) ions are involved in the substrate binding and cleavage,^{8a,d} and a pseudoternary complex is formed at the reaction site, which is likely placed at the interface between the coating monolayer and the bulk aqueous solvent. Accordingly, experiments performed at different Zn(II)/nanoparticle ratios show sigmoidal reactivity profiles, clearly diagnostic of the cooperation of two metal ions in the reaction (see Supporting Information).

Fitting of the reactivity profiles reported in Figure 1 with the Michaelis–Menten equation provides the kinetic parameters reported in Table 1, which reveal several interesting aspects of nanoparticles reactivity. First of all K_M values are similar for all nanoparticles. This indicates that elongation and modification of the metal chelating thiols, while potentially introducing a greater conformational flexibility, are not affecting substantially the binding site preorganization. This is true even for the nanoparticles coated with the highly flexible thiol 3 featuring an oligo(oxyethylene) spacer.

The different activities of the four nanozymes hence arise only from the different reactivity of the fully formed substrate-catalyst complex. In other words, being the structure of the reaction site similar for all nanoparticles, as witnessed by the similar K_M values, what differentiates the nanozymes is their ability to stabilize the dianionic transition state of the reaction. Such ability appears to arise from the properties of the

Table 1. Michaelis–Menten Parameters for HPNP Cleavage in the Presence of AuNp1–4 and Zn(II) in Water at pH 7.5 and 40 °C^a

np	k_{cat}^b (s ⁻¹)	K_M^b (mM)	k_2^c (M ⁻¹ s ⁻¹)	k_{rel}^d	k_{NBIC}^e (s ⁻¹)
1	0.036	0.58	62	1.8×10^5	3.0×10^{-5}
2	0.212	0.40	437	1.1×10^6	1.1×10^{-4}
3	0.019	0.38	50	9.5×10^4	6.8×10^{-5}
4	0.196	0.30	638	9.8×10^5	3.0×10^{-4}

^aErrors are within $\pm 5\%$. ^bNormalized for the theoretical concentration of bimetallic sites, i.e., [Zn(II)]/2. ^c $k_2 = k_{cat}/K_M$. ^d $k_{rel} = k_{cat}/k_{uncat}$. ^eFirst order rate for the NBIC spontaneous decarboxylation in the presence of AuNp1–4 and Zn(II) in water at pH 7.5 and 25 °C, extrapolated at [NBIC] = 0. (Conditions: [NP, in thiol units] = 2.0×10^{-5} M, [Zn(II)] = 2.0×10^{-5} M, [buffer] = 1.0×10^{-2} M).

pseudophase formed by the nanoparticle coating monolayer, where the reaction occurs. In line with Brown's observations,⁷ the lower is the polarity of the monolayer pseudophase, the higher is the nanozyme reactivity. This means that, in this reaction, the stabilization of the transition state is higher than that of the ground state as polarity decreases.¹¹ A strong point in support of such a hypothesis is the comparison of the reactivity of AuNp2 and AuNp3 nanoparticles. Here the length of the coating thiols is identical, with the only difference being the presence of two ether oxygens instead of two methylenes in the structure of thiol 3. As already stressed, such a difference, which should bring about both a higher conformation flexibility and a higher polarity of the nanoparticle coating monolayer, has no effect on the nanoparticle ability to bind the substrate but produces a 10-fold difference on the reactivity.

To get more clues on such a behavior we investigated the temperature dependence of the reaction rate. Figure 2 summarizes the ΔH^\ddagger and ΔS^\ddagger values obtained from the Eyring plots for the k_{cat} values measured in the interval 30–52 °C.¹² Even though the differences are small, as expected for rate accelerations spanning 1 order of magnitude, Figure 2 appears

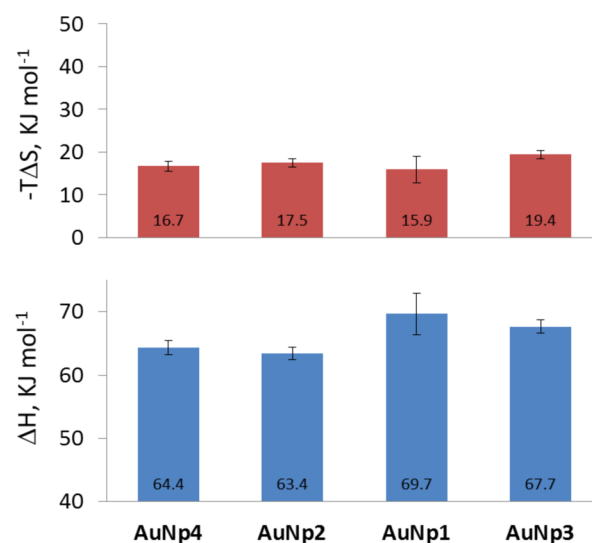


Figure 2. ΔH^\ddagger (blue) and $-T\Delta S^\ddagger$ (red, $T = 318$ K) values obtained from the Eyring plots of k_{cat} values measured in the interval 25–50 °C for AuNp1–4 coated nanoparticles and Zn(II). Conditions: [AuNp, in thiol units] = 2.0×10^{-5} M, [Zn(II)] = 2.0×10^{-5} M, [buffer] = 1.0×10^{-2} M, pH = 7.5.

to indicate that the larger reactivity of **AuNp2** and **AuNp4** has a prevalent enthalpic origin, the activation entropy being quite similar in all cases. These results suggest that in **AuNp2** and **AuNp4** there is a stronger interaction between the transition state and the metal complexes (and, consequently, an higher stabilization), as one would expect in a low polarity environment.

To independently assess the different monolayer polarity of the nanoparticles investigated we used the well-known 6-nitrobenzisoazole-3-carboxylate (NBIC) probe.¹³ The rate of the spontaneous decarboxylation of NBIC is very sensitive to solvent polarity, and this reaction has been used to assess the medium effect in several systems, including micelles, polymers and macrocyclic hosts.¹³ We measured the rate of NBIC decarboxylation in the presence of the different nanozymes and the first order rate values, extrapolated at zero NBIC concentration,¹⁴ are reported in Table 1. Remarkably, the reaction rate in the presence of **AuNp2** and **AuNp4** is sensibly larger than that measured in the presence of **AuNp1** and **AuNp3**. Moreover, the latter values are very similar to those measured in buffer only, indicating that in these nanoparticles the polarity at the monolayer-water interface is very similar to that of bulk water.

Taken all together, the results here reported provide an insightful picture of the potential of monolayer-coated nanoparticles as enzyme mimetic systems. On one hand they allow the self-assembly of the bimetallic catalytic site, while on the other, the simple modification of the structure of the coating thiol by the insertion of longer alkyl chains leads to the obtainment of a microenvironment with a polarity lower than that of bulk water. In such conditions the ability of the metal ions to stabilize, likely by electrostatic interaction (coordination), the dianionic transition state formed during the HPNP cleavage is increased, and consequently, the reaction is faster. Indeed, to the best of our knowledge, k_{cat} values for its cleavage corresponding to $t_{1/2}$ less than 20 s, like those we observe with **AuNp4**, have never been reported previously for Zn(II)-based catalysts working in water.

The reactivity data for the best performing bimetallic systems so far reported and for the nanozymes here described are summarized in Figure 3 (note that 25 °C rates for nanozymes **AuNp1** and **AuNp4** are reported to allow the comparison with the literature data). Richard and Morrow bimetallic complex **5** is based on the same triazacyclonane metal chelating unit we used for our nanozymes.¹⁵ When its reactivity is compared with that of **AuNp1**, it appears evident that the higher activity of the nanoparticles arises substantially from higher substrate affinity. As recently demonstrated by Prins, such a decrease in K_M values is due to the multivalency of the nanoparticles.^{8d,16} The number of potential bimetallic binding sites is statistically increased and this causes an apparent increase in binding affinity.

Beside increased affinity, the intrinsic reactivity of the two systems is similar indicating that in both the cases the optimal (or the best possible) reactive site organization is reached and that the reaction occurs in a similar environment. It should be noted that such an optimally preorganized active site is reached in the nanoparticle monolayer without the need of the bridging oxygen present in **5**. In methanol such structural element has a detrimental effect on the catalyst reactivity. In fact, complex **7** is 37000 times more reactive than the equivalent one comprising an alkoxy bridge.¹⁷ The bridge may decrease the net charge of the catalyst (decreasing both Lewis acidity and transition state

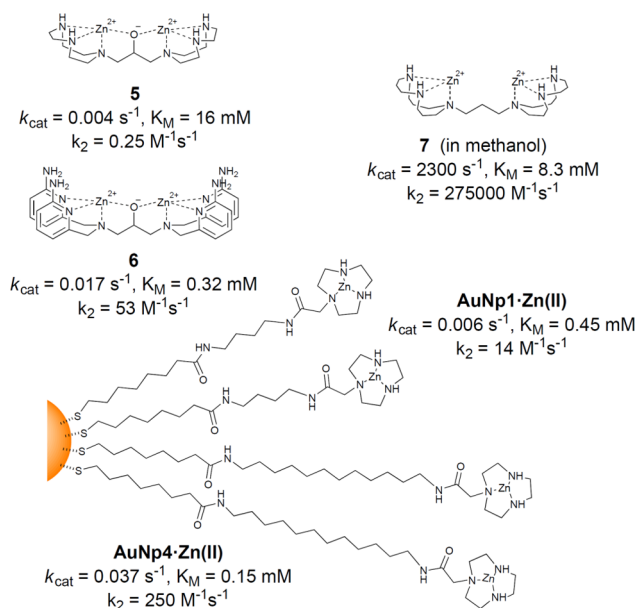


Figure 3. Structure and reactivity parameters (at 25 °C) for different HPNP cleaving bimetallic systems reported in literature or studied in this work. But for catalyst **7**, all the rest were studied in water.

stabilization) or reduce the flexibility of the complex preventing its reorganization during the reaction.¹⁸ In water, the presence of such a bridging alkoxy group is necessary to keep the two metal ions in close proximity, as demonstrated by the fact that **7** becomes a rather poor catalyst in these conditions. It is, however, still unclear whether this gain in organization of the catalysts pays a reactivity cost also in this solvent.¹⁷ The fact that the k_{cat} value we here measured for **AuNp1** is similar to that of **5** indicates that the detrimental effect of the alkoxy bridge may be smaller in water than in methanol.

Eventually, when the reactivity of **AuNp4** is examined, the advantage of nanozymes clearly emerges, as the decrease of medium polarity is awarded by a 10-fold increase of k_{cat} . Remarkably, the reactivity parameters of these nanoparticles are, although to not such a large extent, better also when compared with Williams bimetallic complex **6**, the current benchmark for HPNP cleavage in water, where the reactivity arises from highly efficient cooperation between metal ions and H-bond donors.¹⁹ Admittedly, when the comparison is made with Brown bimetallic complex **7** in methanol,^{7f} as already pointed out, the reactivity of **AuNp4** is at least 3 orders of magnitude lower. Interestingly, when Michaelis–Menten parameters for **5** and **7** are compared, it appears quite evident that moving the reaction from water to methanol affects more reactivity (k_{cat}) than binding (K_M).²⁰ The same occurs in the nanoparticles series **AuNp1–4**, supporting the hypothesis that the activity increase observed is related to a decrease of local medium polarity at the reaction site.²¹ The huge difference observed between k_{cat} values of **AuNp4** (in water) and catalyst **7** (in methanol) indicates that such a polarity decrease is quite small, as it may be expected since the reaction is still occurring at the monolayer/water interface, a still highly hydrated region.²² One may speculate that an higher activity could be obtained by moving the reaction site more deeply in the nanoparticle coating monolayer.²³

In conclusion, we have demonstrated that nanoparticles can be used not only to easily assemble active units capable to cooperate in a reaction taking advantage of their multivalency,

but also to modify the local medium in order to tune the reactivity. In this way, nanoparticles offer the possibility to create artificial "binding sites", something long sought in micellar catalysis²⁴ and rarely found because of the very fast monomer exchange occurring in such aggregates. An analogous medium effect has been also observed with synzymes, metal-based HPNP-cleaving polymers, but with far lower efficiency due probably to the lack of cooperativity between metal ions shown by these systems.²⁵ The fact that the rate accelerations here observed in water, although very high, are lower than those reported by Brown while working in nonaqueous solvents⁷ indicates that there is still room for improvement of nanozymes reactivity.

■ ASSOCIATED CONTENT

■ Supporting Information

Synthesis, characterization of 1–4 and AuNp1–4, and additional kinetic experiments. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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(22) We have unsuccessfully tried to carry out the reaction with AuNp2 in methanol for comparison. Regrettably, upon addition of the substrate, substantial precipitation occurs, rendering the experiment unfeasible.

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