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Viewpoint

Mimicking Enzymes: The Quest for Powerful Catalysts from Simple Molecules to Nanozymes

Yanchao Lyu and Paolo Scrimin*

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INTRODUCTION

Enzymes are highly evolved catalysts that perform their tasks with high efficiency.[1] Hence, they may constitute a very attractive reference in the development of a synthetic catalyst. The synthesis of molecules operating with the same principles used by natural enzymes, enzyme mimics,[2] is consequently a rapidly growing field of research.[3] The challenges an enzyme must overcome, such as substrate selectivity and the feedback control in response to events occurring in the cell where it operates, are typically absent in synthetic catalysts that do not necessarily require all of the sophisticated control mechanisms present in natural catalysts. For this reason, the design strategy of enzyme mimics is often minimalist, relying on those features that are deemed essential in enzymes for catalytic efficiency and neglecting all the rest.[3−5] What are these features and what are the properties of a catalyst to deserve to be called an enzyme mimic? In this viewpoint we try to answer these two questions, relying on the work we have done in the field in the last several years, particularly in the mimicry of hydrolytic enzymes. Enzyme mimics can be simple molecules as well as more complex nanostructures, and they do not necessarily catalyze reactions for which there is an enzymatic counterpart. Rather, it could be very important to develop catalysts for reactions not catalyzed by natural enzymes perhaps operating under conditions hostile to a protein. Nevertheless, we think that common features can be identified independently from the tasks these synthetic catalysts perform.[5] A recent editorial[6] in this journal has raised the question of whether many nanostructures, dubbed nanozymes,[7] really merit that name. We will indicate what, in our opinion, should be the guidelines in that regard as well.

We identify four common features present in enzymes that derive from the study of natural enzymes and enzyme models[5] and are at the basis of their activity. In preparing an enzyme model, a scientist typically rationally designs a catalyst to underpin specific features of a catalytic transformation performed by a particular enzyme. Ideally, an enzyme mimetic system should present all of them, a task rather difficult to achieve, particularly in simple structures.

(i) Enzymes bind, and thus stabilize selectively, the transition state of the reaction they catalyze. The intuition of Fischer, who introduced the lock and key principle,[8] proved unsuitable to explain enzyme catalysis. Pauling was the first[9] in 1948 who suggested enzymes' trick was to stabilize the transition state of a reaction rather than the ground state.[10] More recently Jencks has authoritatively supported that suggestion.[11,12] Transition-state binding equates to a lowered reaction barrier and thus to an increased turnover rate in comparison to the uncatalyzed reaction in solution.

(ii) The binding of the reactants in the catalytic site transforms an intermolecular reaction into a (pseudo) intramolecular one. As noted by Kirby,[12b] "The only simple reactions that can rival their enzyme-catalyzed counterparts in rate are intramolecular reactions such as cyclizations, and specifically intramolecular nucleophilic reactions." This aspect is so important that several enzymes are believed to use the trick of covalently binding the transition state.[13]

(iii) Functional groups are present in the catalytic site to transfer protons, stabilize charges, coordinate metal ions, and act as nucleophiles/electrophiles. They must be properly oriented and located to optimize their interaction with the substrate in its transformation into the transition state. This led to the introduction of concepts such as the "near attack conformation"[14] and the "spatiotemporal theory",[15] the "entatic state"[16] for metal complexes. Furthermore, and particularly important, they must operate in a concerted way, leading to cooperativity.[17]

(iv) The catalytic site of an enzyme differs from the bulk solution in terms of solvation properties[18] and local pH[19] in relation to the functional groups present (polar/apolar, anionic/cationic). This may affect, for instance, the nucleophilicity and the pK_a of the functional groups involved.

We advocate that the knowledge of the functional groups present in the putative catalytic site is an important requisite for a catalyst to deserve to be called an enzyme mimic. This applies to small molecules as well as to more complex nanostructures. Furthermore, such a knowledge allows one to formulate and experimentally support a mechanistic hypoth-

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esis. It is often assumed that the "binding" of the substrate to the catalyst leading to saturation kinetics in rate vs substrate concentration profiles is the only requisite of an enzyme mimic. We do not agree. Saturation kinetics can be observed in the presence of little specific adsorption of the substrate to the surface of a nanocatalyst devoid of any of the features that we have reported above. The knowledge of the catalytic site is also indispensable to assess the activity of such a catalyst. To derive the value of k_{cat} from the classical Michaelis–Menten kinetic analysis, one needs to know the concentration of the catalyst. This can be an issue in the case of nanosystems[20] where this information is missing, and several (10^2–10^3 or more) catalytic sites are typically present on the same nanostructure.

To make our point, we will first present examples of relevant, groundbreaking enzyme mimics that have been developed in the last 50 years or so and that we believe constitute the reference in the field. Afterward, by focusing on a specific reaction, the cleavage of the phosphate bond, we will show how an enzyme mimetic catalyst can be developed and further evolved from very simple molecules to more complex nanosystems.

GROUNDBREAKING EXAMPLES OF ENZYME MIMICS

By using the principles reported above, several catalysts acting as enzyme mimics have been developed. The term catalyst in some cases may be inappropriately used, as no turnover is observed. In these cases, they are intended as mimics of specific steps of a transformation process such as, for instance, the transacylation of an ester substrate. These catalysts are based on natural molecules, such as cyclodextrins[21] and catalytic antibodies[22] (i.e., proteins), or synthetic complex systems, such as functional macrocycles,[23] imprinted polymers,[24] and dendrimers.[25] Complex nanosystems obtained by self-assembling protocols have been also studied, such as micellar and vesicular aggregates[26] or nanoparticles.[27,28] These latter catalysts are frequently multivalent, as they feature several identical copies of the same catalytic site. Selected examples and kinetic results obtained with these catalysts are reported in Figure 1 and Table 1 to give an idea of their typical performance and the source of catalysis as enzyme mimics with reference to points i–iv reported in the Introduction. Interested readers are referred to the references provided. The parameters used for assessing their activity are explained in the footnotes to Table 1.

On analyzing Table 1 one might wonder what the standard performance of a natural enzyme is. Their typical apparent affinity constant for the transition state (K_{tr}) exceeds 10^{15} M.[29] With the exception of the partial transacylase mimic reported by Cram[30] (entry 2 in Table 1), all of the enzyme mimetic catalysts reported in Table 1 show $K_{tr} < 10^{10}$. Cram's catalyst takes advantage of a very strong binding of the substrate related to the use of an organic solvent.[31] The message emerging from the analysis of Table 1 is that a single or just a few of features i–iv present in an enzyme mimetic catalyst are not sufficient to provide the catalytic efficiency present in a real enzyme. In real enzymes, all of them operate in a concerted way, taking advantage of the structural complexity of a protein. Achieving this goal in a single synthetic construct requires more complex systems and a more sophisticated design of the catalytic site. We do not think it is by chance that the best-performing catalyst operating in an aqueous solution among those reported in Table 1 is that based on monolayer

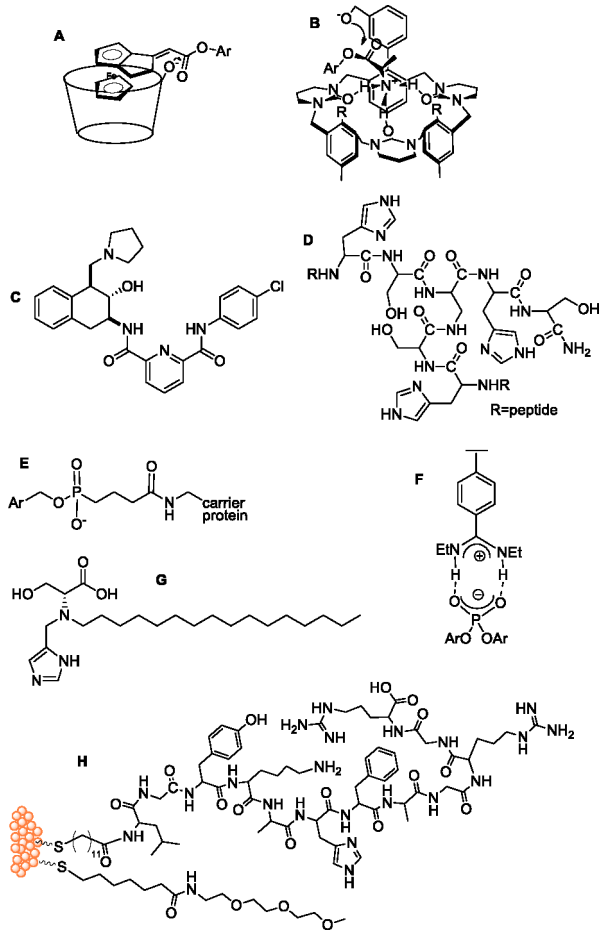

Figure 1. Structure of enzyme mimetic catalysts whose reactivity is reported in Table 1: (A) β-cyclodextrin with ferrocenyl derivative; (B) macrocyclic host with a complexed protonated alanine *p*-nitrophenyl ester substrate; (C) rigid receptor for methanolysis; (D) dendrimers; (E) hapten used for the development of catalytic antibodies for ester hydrolysis; (F) catalytic site of an imprinted polymer with transition state analogue for carbamate hydrolysis; (G) functional peptide for ester hydrolysis; (H) peptide-functionalized gold nanoparticle for ester hydrolysis.

passivated gold nanoparticles[27a] (AuNPs, entry 8). In these nanosystems a collection of functional groups is typically present, and they may cooperate in the confined space of the monolayer, passivating the gold cluster. Furthermore, control of the solvation and local pH is possible, analogously to what happens in the catalytic site of an enzyme. In the nanoparticle reported in Figure 1H the carboxylate became a nucleophile at low pH regimes, the local pH was different from that of the bulk solution, and the turnover of the catalyst was controlled by the lipophilicity of the substrate,[32] all properties found in natural enzymes. For this reason, we have coined the term nanozymes when we first studied such systems in 2004.[33] It is difficult to install all these features in a simple, unimolecular, synthetic catalyst.

Each system reported in Table 1 is mostly based on a specific feature pertaining to enzyme catalysis. They are mechanistically rather heterogeneous and not much useful to guide a reader in the process of developing improved enzyme mimics. For this reason, in the following sections, we will focus on a very specific reaction (the cleavage of the phosphate bond) to highlight the rational process behind the design of

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Table 1. Examples of Enzyme Mimetic Catalysts, Their Performance, the Reactions They Catalyze, and the Main Source of Their Catalytic Efficiency

entry	catalyst/substrate (panel in Figure 1)[a]	catalyzed reaction	main source of catalysis[b]	$k_{rel} = k_{cat}/k_{uncat}$[c]	k_{cat}/K_M[d] or k_2[e] (s^{-1} M^{-1})	$K_{tr} = (k_{cat}/K_M)/k_{uncat}$[f] ($M^{-1}$)	ref
1	cyclodextrin/carboxylate ester (A)	transacylation	iii	5.9×10^6	16	1.0×10^9	34
2	macrocycle/carboxylate ester (B)	transacylation	ii, iii	1.7×10^3	2×10^9	2×10^{12}	30
3	rigid molecular receptor/carboxylate ester (C)	transacylation	iii	very high[g]	3.2×10^{-4}[h]	nd	35
4	dendrimer/carboxylate ester (D)	hydrolysis	iii, iv	3.9×10^4	3.2×10^2	1.3×10^9	36
5	catalytic antibody/carboxylate ester (E)	hydrolysis	i	1.3×10^5	214	4.6×10^8	37
6	imprinted polymer/diphenylcarbamate (F)	hydrolysis	i	3.8×10^3	0.24	1.2×10^6	38
7	micelles/carboxylate ester (G)	hydrolysis	ii–iv	1.6×10^4	1.3×10^3	1.1×10^7	39
8	gold nanoparticles/carboxylate ester (H)	hydrolysis	ii–iv	1.2×10^5	6.2×10^5	4.8×10^9	32

[a]Refer to Figure 1 for the structure of catalysts and substrates. [b]The indications i–iv refer to the four common features present in enzymes that derive from the study of natural enzymes and enzyme models and are at the basis of their activity discussed in the Introduction. [c]The rate acceleration exerted by the catalyst that gives an immediate evaluation of how much faster the catalyzed reaction proceeds in comparison to the uncatalyzed catalyst (i.e., the pseudo-first-order rate constant in the absence of catalyst). [d]k_{cat} is the first-order rate constant that evaluates the rate of transformation of the substrate at saturation of the catalyst, and K_M is the reciprocal of the affinity constant of the substrate for the catalyst (assuming a binding equilibrium faster than the rate of product formation). The ratio has the dimensions of a second-order rate constant and is a measure of how efficiently the catalyst converts the substrate into products under subsaturation conditions, also taking into consideration the efficiency of its binding; [e]Second-order rate constant in the absence of binding. [f]Expressed in units of concentration and, quoting its definition, "represents the lower limit of the enzyme's affinity for the altered substrate in the transition state".[29] [g]The background reaction is immeasurably slow under the experimental conditions; [h]Estimated from the turnover frequency; the affinity of the catalyst for the substrate is low (0.79 M^{-1}).

improved enzyme mimics, relying on a precise knowledge of the source of their catalytic performance. We will show that, through the incremental addition of key contributions and by an increase in the complexity of the system, the efficiency of the catalyst is significantly increased.

THE CHALLENGE TO CLEAVE THE PHOSPHATE BOND

Among the slowest hydrolytic reactions known are phosphate diester cleavages. Measurements at elevated temperatures indicate that a diphosphate anion undergoes hydrolysis in water (P–O bond cleavage) with a $t_{1/2}$ value of 3×10^7 years at 25 °C. Kinetic constants reported for staphylococcal nuclease indicate that this enzyme enhances the rate of phosphorus–oxygen cleavage by a factor of at least 10^{17}-fold and that the formal association constant with the transition state (K_{tr}) is higher than 10^{22} M^{-1}.[40] This is the challenge that phosphate-cleaving enzymes pose to a scientist willing to prepare a synthetic catalyst able to rival them. Phosphoryl transfer reactions have been the subject of intense studies, and a fairly good picture is available concerning the mechanism of these reactions (not yet completely understood, though).[41,42] Most of these enzymes use metal ions as cofactors.[43,44] Metal ions contribute to the catalytic process in several ways. Among them are (i) the activation of the phosphoryl group toward nucleophilic attack by acting as a Lewis acid and reducing the total charge at the phosphate more at the transition state than at the ground state and facilitating the encounter of two negatively charged species, (ii) the assistance in leaving group departure (by decreasing its pK_a) through coordination of the alcoholic oxygen, (iii) the transformation of the nucleophilic attack from a intermolecular process into a (pseudo)-intramolecular one, and (iv) an increase in the fraction of the anionic nucleophile present (by decreasing its pK_a) if this is the rate-determining step of the reaction. Scientists have estimated that the above contributions, when they operate in a concerted fashion, may lead to rate accelerations of up to 10^{16}-fold (k_{rel}), in line with those found with nucleases.[41] Notably, in most nucleases more than one metal ion is present in the catalytic site, typically two.[43] Cationic side arms of amino acids may act as metal ion surrogates.[45]

EVOLUTION OF SIMPLE SYNTHETIC METALLOCATALYSTS FOR PHOSPHATE TRANSFER REACTION ACCELERATION

Metal ions in aqueous solutions at physiological pH are unstable and tend to form insoluble metal hydroxides. Furthermore, they must be properly placed in the catalytic site where the transformation of the substrate occurs. For this reason, in metalloenzymes functional groups create "coordination cages" using functional groups of lateral arms of amino acids such as imidazoles, carboxylates, and alcohols. The secondary and tertiary structures of the protein allow the correct placement of these coordinating groups. It is also believed that unusual, strained metal complexes can be formed, leading to an "entatic state".[16] In simple models, with the weight being a tiny fraction of that of a protein, preformed ligands are used for simplicity. We will show a few examples in which the performance of a metallocatalyst for the cleavage of a phosphate diester model of RNA (2-hydroxypropyl *p*-nitrophenyl phosphate, HPNP) has been progressively increased by taking advantage of simple concepts. They are the number of metal ions present in the catalyst, their precise orientation to elicit cooperativity, the introduction of hydrogen bond donors, and the control of the solvating medium properties. An important role of these metal ions is to decrease the pK_a of the nucleophilic species involved. This affects both the concentration of deprotonated nucleophile and its nucleophilicity. Second-order rate constants of metal-bound nucleophiles are typically lower when they are bound to a metal ion. This difference in reactivity is offset by the concentration at the physiological pH at which these reactions are carried out. This aspect will not be specifically considered, as the comparison between the different catalysts will be done under pH conditions at which these species are fully deprotonated.

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A comparison of catalysts **1** and **2** (Figure 2, red arrow a) shows that the dinuclear catalyst is 34- or 200-fold better than

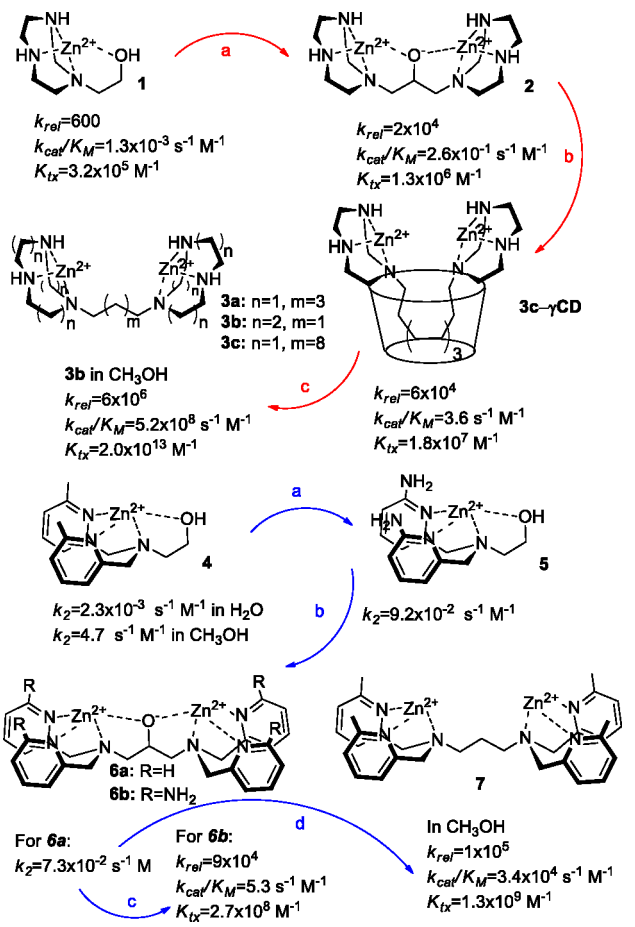

Figure 2. Evolution of the efficiency of simple synthetic metallocatalysts for the cleavage of the RNA model substrate HPNP. The red and blue arrows are meant to guide the eye in following the progressive improvement of the catalysts. In red: (a) from one to two metals; (b) the need for structure organization; (c) solvent effect. In blue: (a) the role of proton donors; (b) from one to two metal ions; (c) merging (a) and (b); (d) solvent effect. References: **1** and **2**, ref 47; **3a**, ref 48; **3b**, ref 49; **3c**, ref 46; **4**, ref 50; **5** and **6**, ref 51; 7, ref 52.

the mononuclear one when k_{cat} or k_2 (k_{cat}/K_M) is considered, respectively. The apparent better performance of **2** obtained when the k_2 ratio is compared indicates that there is a significant binding contribution in its favor. A similar increase in efficiency is observed for dinuclear catalyst **6b** in comparison to mononuclear **5** (576-fold, blue arrow b in Figure 2). The two metal ions must be, however, in close proximity: removal of the bridging alkoxide (compare **2** with **3a**), allowing rotational freedom to the connecting arm, decreases the acceleration to a mere 2-fold in terms of second-order rate constants. It is interesting to note, however, that while the alkoxide is useful for bringing the systems to order, it is detrimental in terms of intrinsic catalytic properties of the metal ions. In fact, we have demonstrated[46] that catalyst **3c**, which is as poor as **3a**, becomes very effective in the presence of a γ-cyclodextrin that, by binding the hydrocarbon tether, acts as an allosteric effector bringing the two metal complexes in close proximity (Figure 2). Its effect on reactivity is 3-fold (k_{cat}) or 14-fold (k_2) higher than that of **2** (red arrow b).

The evidence that hydrogen bond donors may further improve the efficiency of a dinuclear catalyst is provided by a comparison of catalysts **6a** and **6b**: the rate acceleration (k_2) is 726, almost 3 orders of magnitude (Figure 2, blue arrow c). We have mentioned before that these H bonds may act as metal ion surrogates. This is clearly shown by a comparison of the mononuclear catalyst **5** with the dinuclear catalyst **6a** devoid of them: the reactivities are practically the same (blue arrow b).

However, the most astonishing effect on reactivity is caused by a change in the solvent from water to methanol (or other light alcohols). The k_2 value for the mononuclear catalyst **4** is 46000-fold larger in methanol than in water (allowing for the different second-order rate constants of the reference reaction in the two solvents). The two dinuclear catalysts **3b** and **7**, devoid of the structural hydroxide and hence behaving as a mononuclear catalyst in water, bring about rate accelerations of 2×10^{11} and 1.3×10^7 (k_2) and 6×10^6 and 1×10^5 (k_{rel}), respectively, when they are used in methanol (Figure 2, red arrow c and blue arrow d). These rates are approaching enzyme-like rate accelerations. The message is clear: desolvation of the reacting species and, most important, an increase in the binding strength of the substrate to the metal ion(s) and, possibly, ionization effects cause an impressive beneficial effect on the efficiency. One may speculate that the polarity of methanol is more similar to that present in the catalytic site of an enzyme than water is. The solvating properties of the medium in the catalytic site exert an important, often neglected, contribution to catalysis in enzymes as well.[53]

INCREASING IN COMPLEXITY: NANOZYMES

The takeaway messages from the studies of simple systems are that a dinuclear catalyst appears to be better than a mononuclear one but that preorganization is required. As we will show below, this second metal ion can be replaced by other cationic groups playing a similar role. Ancillary H-bonding groups favor the catalytic process, and importantly, the polarity of the solvent is crucial. It is practically impossible to install all of these features in a low-molecular-weight catalyst.

The message drawn from an analysis of Table 1 was that systems based on monolayer-passivated metal nanoparticles may provide a simple and easily accessible way to address this problem. It is thus worth considering these systems in comparison with the above unimolecular enzyme models. Is this also true in this context? Are these systems still better catalysts than those reported in Figure 2? We have also included in our analysis micellar catalysts due to their similarity to gold nanoparticles. Obviously, we have examined the cleavage of the very same substrate, HPNP. The data reported in Figure 3 reveal that aggregates of amphiphilic molecules (**8**, Figure 3) featuring the very same Zn(II) complex of catalysts **1**–**3** provide higher rate accelerations (more than 1 order of magnitude in k_{rel}) in comparison to those catalysts. The anchoring of the complex on the surface of a small cluster of gold atoms (ca. 2 nm in diameter) results in a further 1 order of magnitude increase in rate acceleration (k_2 = 51.7 and k_{rel} = 1.5×10^5 for **10a**). The presence, in the tether connecting the metal complex to the nanocluster surface, of an amide bond is known to impart stiffness to the monolayer because of the formation of CO- - -HN bonds.[54] This feature results in a further increase of 1 order of magnitude in reactivity (see **9c** and **10b**, Figure 3). Indeed, **9c** is the best-known synthetic

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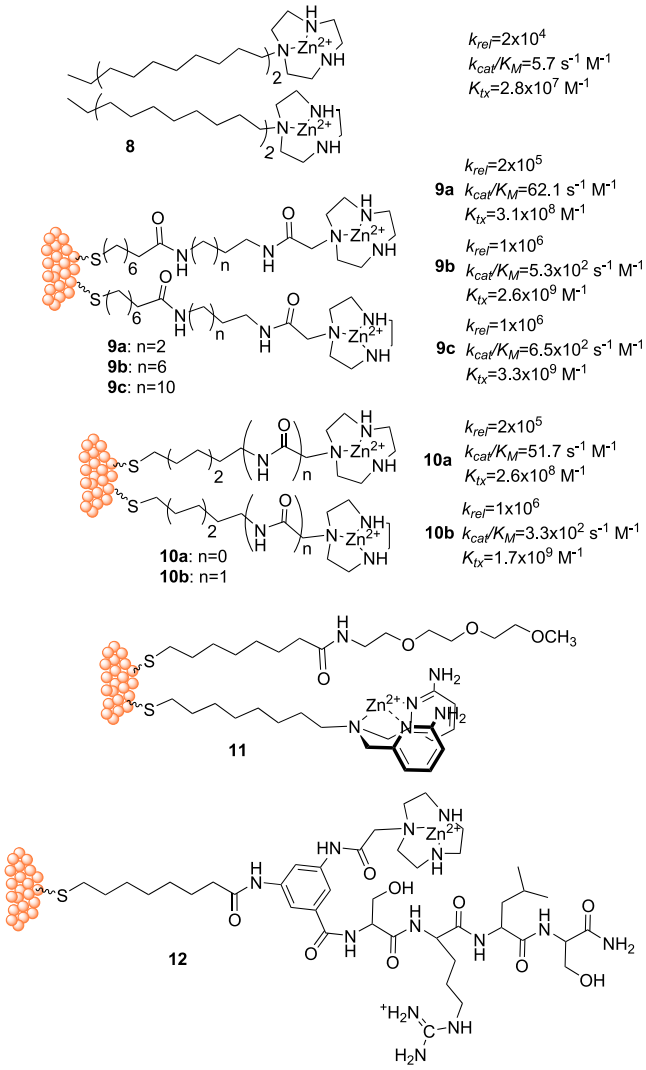

Figure 3. Multivalent nanocatalysts for the cleavage of HPNP (from **8** to **10**) and plasmid DNA (**11** and **12**), taking advantage of cooperativity, medium control, and (for **11** and **12**) very efficient binding to the substrate leading to their being dubbed nanozymes. References: **8**, ref 55; **9**, ref 56; **10**, ref 28; **11**, ref 61; **12**, ref 59.

catalyst for the cleavage of HPNP in water reported so far. A comparison among AuNPs **9a−c**, where the hydrocarbon portion of the tether was progressively increased in length (thus increasing the hydrophobicity of the monolayer), reveals that a more hydrophobic medium is advantageous (Figure 3).

We have recently shown, using atomistic simulations integrated with NMR experiments, that this allows a better packing of the monolayer with the formation of an increased number of dinuclear, active catalytic sites.[57] However, even in these cases the rate accelerations observed are far lower than those obtained in methanol. It is important to point out that in all these systems the catalytic site is dinuclear and the cooperativity between the metal ions is induced by their aggregation (**8**) or self-assembly on the surface of the gold cluster (AuNPs **9** and **10**). This means that space confinement is a useful trick to induce cooperativity.

The experimental evidence supports the excellent properties of these nanosystems as enzyme mimics. It is important to point out that the relevant kinetic parameters for these systems were obtained by using as the concentration of the catalyst that of the metal complexes (and not that of the nanoparticles), adjusting for the nuclearity of the catalytic site when we could prove the mechanism required two metal ions for catalysis.

These systems are multivalent. It is thus not surprising that their catalytic activity is best performed in the presence of a multivalent substrate such as DNA. The AuNPs of Figure 3 are all cationic, polyvalent species, and they interact very strongly with polyanionic DNA. It has been estimated that the strength of binding of **9a** with anionic compounds increases by 1 order of magnitude per single charge increment (from millimolar with one charge to less than micromolar with four charges in terms of dissociation constants).[58] We have found, using molecular mechanics simulations, that there are at least 11 phosphates of a single DNA bound to these nanoparticles, very likely bringing the dissociation constant well below nanomolar concentration.[59] That number is very similar to that estimated for *Bam*HI restriction nuclease for supercoiled DNA (10^{-11} M).[60] AuNPs **11** showed the unique ability to contemporaneously cleave both strands of DNA,[61] suggesting that the strong binding of the polymer to the nanoparticle surface resulted in the contemporaneous cleavage of phosphate esters present in different strands. This property is found frequently in enzymes but is almost impossible to achieve with synthetic catalysts.

In terms of activity, AuNPs **12** are remarkable nuclease mimics and are "only" 7×10^4-fold less active than the natural enzyme.[59] An analysis of the catalytic mechanism operating with AuNP **12** reveals that a single metal ion is involved, with a key role being played by the guanidinium as a second metal ion surrogate. Notably, nanoparticles devoid of such guanidinium groups, such as **10b**, are totally inactive. The reason is that the plasmid binds to the nanoparticle surface with a zipper-like mechanism involving one phosphate and one Zn(II) ion. This precludes the cooperation between two metal centers. Nanoparticles **12** not only approach an enzyme-like activity but also show a mechanism similar to that of type IA and II topoisomerases, for which it has been proposed that only a metal ion is present in the catalytic site flanked by a guanidinium of arginine (Figure 4).[52b] In the enzyme the nucleophile is the phenol of a tyrosine, while in the nanoparticle it is the alcohol of a serine. Nanoparticles **12** represent the limit of sophistication and activity achieved so far in enzyme mimicry for DNA cleavage. The name nanozymes is, in this case, well deserved.[62]

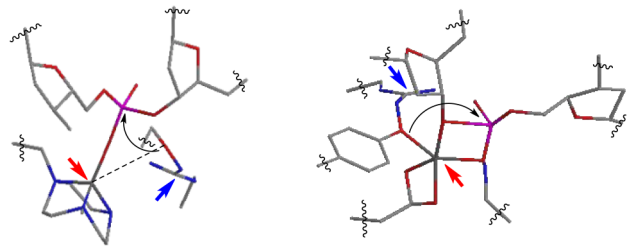

Figure 4. Comparison between the proposed catalytic mechanism for the cleavage of DNA by nanozyme **12** (left, ref 59) and type IA and II topoisomerases (right) as suggested by Berger (ref 45b). The enzyme is a dinuclear one, but only one metal ion was proposed as catalytically active. The red arrows denote the Zn(II) ions and the blue arrows the guanidinium groups, while the curly arrows indicate the attack of the nucleophile (serine or tyrosine) to the phosphorus atom of the phosphate.

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CONCLUSIONS AND OUTLOOK

The above two paragraphs highlight the rational processes that, starting from the known mechanisms of phosphate-cleaving enzymes and information obtained from specific models, using structure−reactivity relationships led to the development and progressive improvement of synthetic enzyme mimics. Cooperativity in enzymes is important to enhance their catalytic efficiency. AuNPs constitute a valuable option to elicit cooperativity in a synthetic catalyst. Other nanoplatforms, but gold clusters are used for preparing nanocatalysts as resins,[63] polymers,[64] polymeric nanoparticles,[65] inorganic nanoparticles passivated with imprinted polymers,[66] carbon dots,[67] graphene oxide,[68] and metal−organic frameworks,[69] just to mention a few. In contrast to simple, unimolecular catalysts, these systems may allow one to take advantage of all four features, common to natural enzymes, that we have illustrated in the Introduction. This clearly results in a much better catalytic performance.

We can now answer our original question: is it correct to name these catalysts nanozymes? In the Introduction we have indicated two critical and minimal requirements for a system to deserve this name: the binding of the substrate before its transformation and the knowledge of the functional groups present in the putative catalytic site. Both are met by the nanosystems we have described here. Indeed, the kinetic parameters for the evaluation of their activity could be determined and the source of catalysis[70] assessed with confidence despite the multivalent nature of the catalysts.

These studies revealed that their catalytic efficiency derives from a relatively high affinity constant for the substrate.[70b] Furthermore, a linear energy relationship analysis indicated that a nanoparticle such as AuNP **10a** catalyzes the transphosphorylation reaction mostly via a nucleophilic mechanism with little stabilization of the pentacoordinated phosphorane and moderate assistance in leaving group departure.[70a] Molecular dynamics simulations revealed significant flexibility of the catalytic site, where functional groups may easily follow the substrate toward the transition state.[57,71] Richard, in commenting on the activity of catalytic antibodies, has pointed out that the catalysts are too stiff and that protein stiffness "must be combined with protein flexibility to obtain well-rounded and efficient catalysts".[72] His work provides the details of achieving transition state stabilization through the controlled reorganization of an enzyme structure when a substrate binds, a process, however, that is not necessarily true for all enzymes. We have already seen that the placement of the functional groups in the confined space constituted by the organic monolayer surrounding the metal cluster of gold atoms allows one to take advantage of the cooperativity between them without relying on other (often kinetically detrimental) structural features. We[32] and others[73] have observed that functional groups which are not active in simple, unimolecular catalysts may get involved in reactivity once they are embedded in the monolayer of the gold nanoparticles, opening new, more advantageous mechanistic pathways. This is the case of the carboxylate of nanoparticles H (Figure 1). Substrate selectivity[74] (including stereoselectivity)[75] is addressable with these systems.

An understanding of what happens at the catalytic site during the chemical transformation of the substrate allows one to design evolved generations of catalysts. Furthermore, the combinatorial approach[76] permitted by the self-assembled nature of the monolayer passivated AuNPs may enable one to combine rational design with the selection of relatively large pools of catalysts. We are confident that this will allow, in a relatively short time, to further narrow (or even close) the gap in kinetic performance between natural enzymes and these nanozymes.

Enzyme mimics (and artificial enzymes as well) may also be obtained using approaches different from those that we have described here. For instance, artificial metalloenzymes[77] result from the incorporation of an abiotic metal cofactor within a protein scaffold. Iterative cycles of mutagenesis and screening or selection are applied to modify existing protein properties to enhance their catalytic activities or develop completely new catalysts for non-natural chemical transformations.[78] The availability of sophisticated computational methods such as machine learning will possibly constitute one of the prominent approaches in the future for the engineering of new enzymes or enzyme mimics.[79−81]

AUTHOR INFORMATION

Corresponding Author

Paolo Scrimin − *University of Padova, Department of Chemical Sciences, 35131 Padova, Italy;* orcid.org/0000-0002-6741-3374; Email: paolo.scrimin@unipd.it

Author

Yanchao Lyu − *University of Padova, Department of Chemical Sciences, 35131 Padova, Italy;* Present Address: Yangzhou University, School of Chemistry and Chemical Engineering, Yangzhou, Jiangsu, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acscatal.1c01219

Notes

The authors declare no competing financial interest.

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