Review

Cooperative nanosystems[‡]

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Abstract: Multivalent systems are well known for their enhanced ability to bind multivalent counterparts. This contribution addresses the question whether they can also behave as cooperative catalysts. Analyzing examples from our own laboratory we show that self-assembled systems obtained by covering gold nanoclusters with thiol-terminated amino acids and peptides behave indeed as cooperative catalysts. By comparing their activity profiles with those of discrete, multivalent systems we show what are minimal conditions to elicit cooperativity in multivalent systems. Reactions taken into considerations for our analysis are the hydrolyses of carboxylate- and phosphate esters. Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.

INTRODUCTION

Most, if not all, of the biological catalysts exert their catalytic activity as the result of the cooperative action of several functional groups present in their catalytic site. Nevertheless the design and synthesis of synthetic catalysts in which functional groups work cooperatively still remains a major challenge for scientists pursuing the goal of preparing effective biomimetic catalysts [1]. Cooperativity in catalysis represents a step forward with respect to cooperativity in molecular recognition and one may consider, as a starting point in the design of such catalysts, that most of the principles that provide cooperative systems in molecular recognition should work also in catalysis.

In this regard, multivalent systems have recently attracted considerable interest in molecular recognition and a large amount of data show how affinity constants between multivalent systems may increase in several orders of magnitude with respect to those of the monovalent systems [2–5]. The question we asked ourselves was: are multivalent systems also cooperative catalysts? More than 15 years ago Fred Menger, reviewing the activity as catalysts of aggregation colloids (micelles and vesicles), wrote: '... groups of molecules, properly assembled, can obviously accomplish much more than an equal number of molecules functioning separately' [6]. Although experimental data indicate that indeed the catalytic activity of these systems can be much higher than that obtained with the corresponding monomeric systems, a thorough analysis of their kinetic behavior has, so far, clearly indicated that rate accelerations observed are mostly due to local concentration effects (this including, for instance, the 'apparent' reduction in reaction volume, but also the change of pH at the reaction loci) [7,8]. In no case clear evidence of a cooperative system has been reported.

Recently, we have started to study self-assembled monolayers of amino acids and di- and oligopeptides on Au-nanoparticles as novel nanosized biocatalysts [9]. Several oligopeptides bound to a cluster of gold atoms resemble a protein in size, structural properties and, potentially, functional properties, too. The spontaneous formation of these nanostructures renders them synthetically very easily accessible, allowing a high flexibility in the type and number of oligopeptides present on the surface. More importantly, the close packing of numerous components on the nanoparticles, their very limited freedom of movement, and the particular solvation [10] attained within the organic monolayer induce a strong cooperativity between several units giving rise to very potent catalytic systems. Here, we will illustrate the strong advantages of using Au-nanoparticles by comparing their catalytic behavior with analogous small molecular systems, in which similar amino acids or oligopeptides are ligated to small molecular scaffolds.

So far, we have mainly concentrated on the development of catalytic systems for the cleavage of carboxylic esters and phosphate esters. The first group, because of the ease of study and the facile assessment of cooperativity, renders these catalysts very suitable for proof-of-concept studies. On the other hand, phosphate esters

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BIOGRAPHY

Cristian Guarise obtained his degree in Pharmaceutical Biotechnology at the University of Padova in 2003, working on protease sensors based on gold nanoparticles. After a post-degree project, in 2005 he started as a Ph.D. student in Prof. Scrimin's group at the same University. The area of his research interest is the functionalization on solid phase of tripodal scaffolds for recognition and catalysis.

Flavio Manea obtained his degree in Chemistry at the University of Padova in 2003. He did his Ph.D. with Prof. Scrimin, working on the functionalization of gold nanoparticles and dendrimers and studying them as multivalent catalysts for the cleavage of phosphate esters. He is currently a post-doctoral associate at the University of Bristol, UK, with Prof. A. Davis.

Giovanni Zaupa obtained his degree in Chemistry at the University of Padova in 2005. After a post-degree project, in 2006 he started as a Ph.D. student in the group of Prof. Scrimin at the same University. His research interests are the synthesis and functionalization on solid phase of dendrimers and other multivalent structures, and the study of their catalytic activity.

Lucia Pasquato graduated in Chemistry at the University of Padova, in 1983, under the supervision of Prof. G. Modena. In 1985 she was appointed as researcher with the CNR (National Council of Research). In 2002 she moved to the University of Trieste to become Associate Professor. She has been a research associate at the University of Würzburg with Prof. W. Adam during 1985–1986. Her current research interests focus on metal nanoparticles for applications in catalysis and as templates to design functional 3D self-assembled monolayers for biochemical and material sciences applications.

BIOGRAPHY

Leonard J. Prins studied Chemical Technology at the University of Twente (Netherlands) and obtained his Ph.D. degree in 2001 at the same University working with Prof. D.N. Reinhoudt. He worked as a post-doctoral researcher with Prof. P. B. Dervan at the California Institute of Technology, Pasadena, and with Prof. G. M. Licini at the University of Padova, Italy. In January 2004, he took up a position as researcher at the same University associated to the group of Prof. P. Scrimin. His research interests are the application of dynamic combinatorial chemistry for catalyst discovery and the development of multivalent, enzyme-like catalysts.

Paolo Scrimin after obtaining his Doctor of Chemistry degree at the University of Padova with Prof. Modena in 1976, did post-doctoral work in the same group and got a Lecturer position at the University of Ferrara in 1979. In 1983 he returned to Padova where he is now full Professor. He has been the Head of the Department of Organic Chemistry from 1999 to 2003. Prof. Scrimin has been a visiting Fulbright Scholar at Rutgers University (1985–1986), at the University of California, Santa Barbara (1992–1993), and Luis Pasteur University in Strasbourg, France (2006). His scientific interests are in the chemistry of aggregates and of supramolecules with particular emphasis on catalysis of hydrolytic processes by transition metal complexes. The study of multivalent catalysts represents one of the most recent developments of the research of his group.

both classes of compounds we will give examples starting from molecularly well-defined systems and passing over to self-assembled monolayers of amino acids and peptides on Au-nanoparticles.

ASSESSING COOPERATIVITY IN CATALYSIS WITH MULTIVALENT SYSTEMS

Quite often false claims of cooperativity in catalysis with multivalent systems are due to an incorrect interpretation of the kinetic data concerning the activity of these systems. A multivalent catalyst is a cooperative catalyst whenever: (i) its activity is larger than the summation of the contribution of the individual components, or (ii) the individual components play different roles not attainable with a monomeric catalyst resulting in a rate acceleration. The following examples should clarify the concept.

are attractive targets for catalysis because of their biological relevance (DNA, RNA), giving access to artificial catalysts as novel biomedicine or diagnostic tools. For

Let us first consider the kinetic behavior obtained by increasing the concentration of a multivalent catalyst and a monomeric one. In Figure 1 is reported the rate profile obtained with a micellar system compared with a monomeric one bearing the same functional group [11]. The reactivity of the monomeric catalyst increases linearly with concentration while that of the micellar one (but for the inflection at low concentration due to the formation of the aggregates) follows a Michaelis–Menten-type profile. Obviously, the micellar system is much more active than the monomeric one in the concentration interval explored. The enzyme-like profile indicates binding of the substrate to the catalyst but does not provide any indication whether the catalyst is a cooperative catalyst or not. The rate acceleration could be simply due to a higher local concentration of catalytic units rather than to their enhanced activity (in other words, catalysts and substrates are confined to a smaller volume which results in an increased rate). To assess cooperativity we need to determine the rate acceleration by changing the concentration of catalytically active units within the multivalent catalysts. This can be done by using a multivalent catalyst in which active and nonactive components are mixed together and the mole fraction of the active component is increased. Under these conditions a non cooperative catalyst (profile a) will give a linear increase of the rate constant with concentration (Figure 2) while a cooperative catalyst (profile b and c) will deviate from linearity (Figure 2).

Alternatively, one should demonstrate that different species within the multivalent catalyst play different roles in a concerted manner. A very simple example is that provided by a hydrolysis catalyst in which the same functional unit may act as a base or nucleophile in its
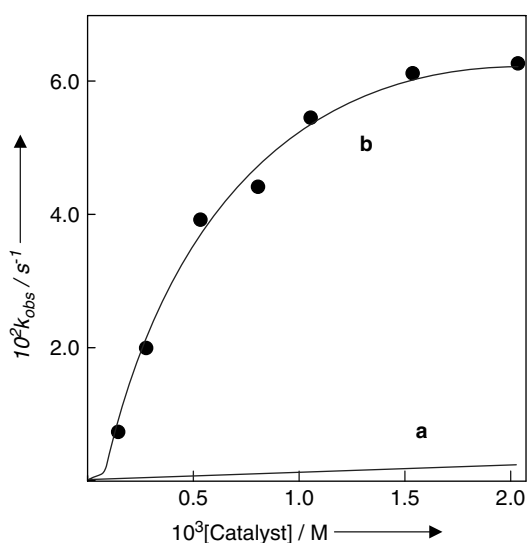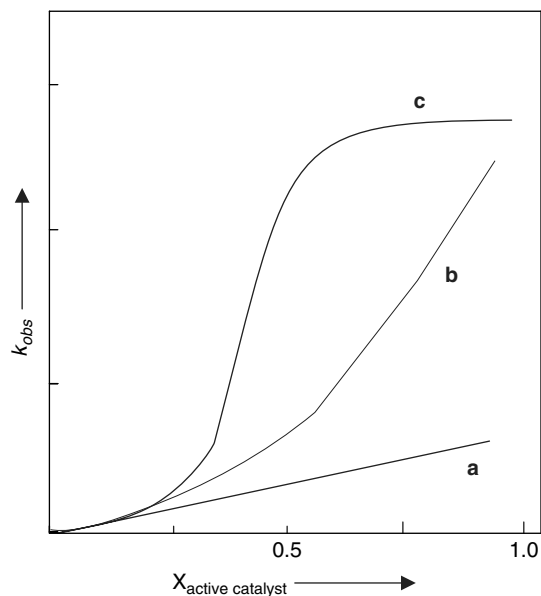
Figure 2 Kinetic profiles observed with multivalent catalysts upon changing the mole fraction of active component. Profile a is observed with a noncooperative catalyst while profiles b and c are typical of cooperative catalysts. The difference between profile b and c is that, with the latter, maximum efficiency was obtained at a mole fraction of active component lower than one.

nonprotonated form and as an acid in its protonated form. Figure 3 shows an example of such a catalyst (trace b). In this figure, the rate constant is plotted as a function of pH. Trace a is the one typically observed with monomeric catalysts for which the deprotonated species is catalytically more active than the protonated one. Accordingly, the curve is just the kinetic version of a titration. Curve b goes through a maximum at a pH corresponding to the pK_a of the kinetically relevant species. But maximum activity is observed when both protonated and unprotonated species are present and they must work cooperatively.

DEVELOPMENT OF ARTIFICIAL ESTERASES BASED ON THE COOPERATIVE ACTION BETWEEN IMIDAZOLES

It is widely known that multiple imidazole units can cooperatively cleave carboxylic esters around pH 7, providing both nucleophilic and general acid/base contribution (see profile b of Figure 3). We were curious to know the minimal conditions for inducing such cooperativity. Thus, mixtures of catalysts having up to three imidazole units connected to a 1,3,5-triethylbenzene scaffold were prepared simply by adding histamine and *N,N*-dimethylethylendiamine in varying amounts to the scaffold (Figure 4) (Zaupa G, Prins, LJ, Scrimin P, unpublished results.).

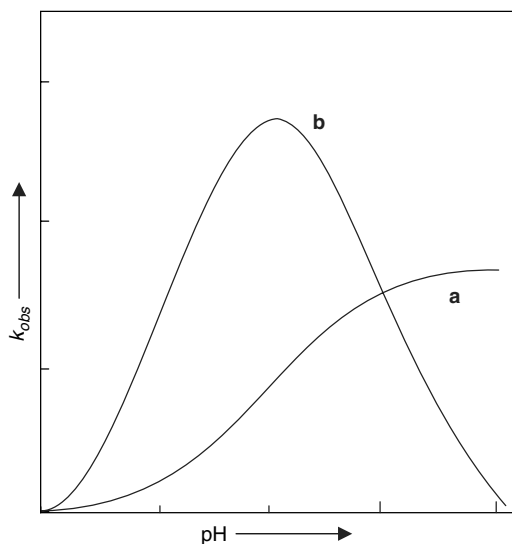The ratio between the four resulting catalysts was determined by the ratio of the reagents added. Direct

Figure 1 Kinetic behavior of monomeric (a) and multivalent (b) catalysts plotted as a function of the total catalyst concentration.

Figure 3 A kinetic profile of a cooperative kinetic process involving an acid and its conjugate base, b; line a report the profile observed for a noncooperative process involving the conjugate base of a protic catalyst.

screening for catalytic activity and subsequent deconvolution then gave direct access to the individual contribution of each catalyst [12]. The mixtures were tested for activity in the cleavage of *p*-nitrophenylacetate (PNPA) and the resulting pseudo-first order rate constants plotted against the relative amount of imidazole subunits present in the mixture. The straight lines obtained at both pH = 7 and 8 (similar to curve a in Figure 2) clearly indicate that, in this particular system, cooperativity between imidazoles is completely absent. This tendency was observed for other substrates also.

On the contrary, the incorporation of imidazole subunits in a self-assembled monolayer (SAM) covering an Au-nanoparticle gave results supporting cooperativity [13]. The system (Figure 5) consisted of a mixed monolayer of dodecanethiol and the *N*-methylimidazole-terminated thiol (**1**) obtained via a place-exchange reaction. Proton NMR studies revealed a 1:1 ratio of the thiols in the monolayer. The catalytic activity of the Au-nanoparticles was tested on the substrate 2,4-dinitrophenylacetate (DNPA) in methanol–water (6:4) solutions in the pH-range 4.5–7.2. The resulting dependence of the second-order rate constants is shown in Figure 6 together with those obtained for the monomeric catalyst. Packing of the imidazole units on the Au-nanoparticle surface induces a modest 30-fold rate acceleration with respect to the monomer. However, the bell-shaped dependence of k_2 with a maximum in the proximity of the pK_a (similar to curve b in Figure 3), supports cooperativity between two methylimidazoles in the DNPA cleavage by the nanocluster (general acid/base or nucleophilic catalysis). The kinetic behavior is very similar to that reported by Baltzer *et al.* who studied four helix bundle-forming peptides bearing multiple imidazole subunits [14]. The simple conclusion we obtained from these studies is that in order to elicit cooperativity between imidazoles it is necessary to reduce their mobility by confining them in an environment like that provided by the monolayer covering the gold nanocluster.

DEVELOPMENT OF ARTIFICIAL ESTERASES BASED ON CARBOXYLATE–IMIDAZOLE COOPERATIVITY

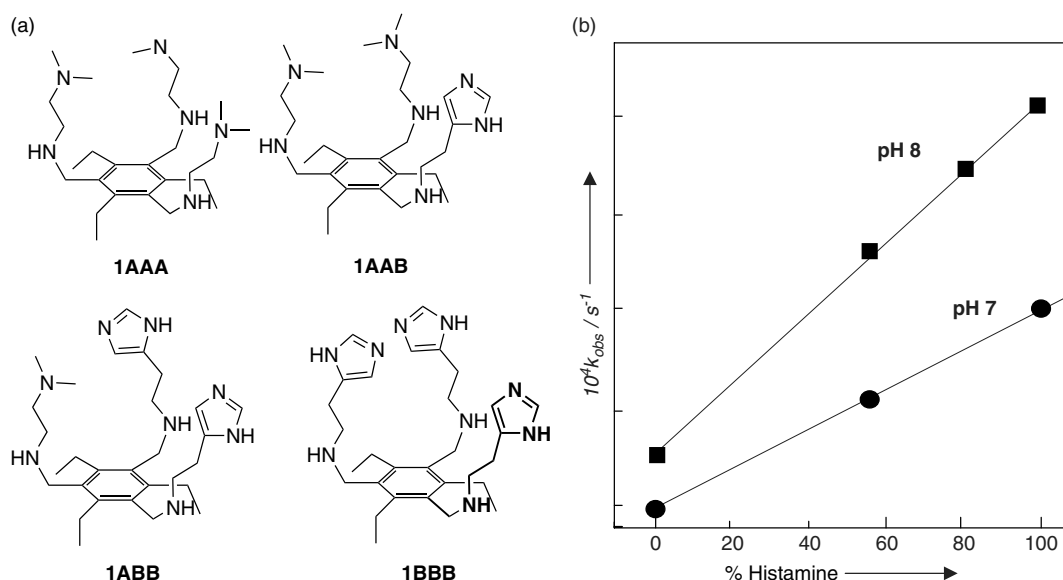As a bridge towards larger peptides, we designed catalytic systems aimed at inducing cooperativity

Figure 4 (a) Catalysts containing up to three imidazole moieties. (b) Observed rate constants for the hydrolysis of phenyl acetate at pH = 7 and 8 as a function of the relative amount of histamine present in the catalyst mixture.
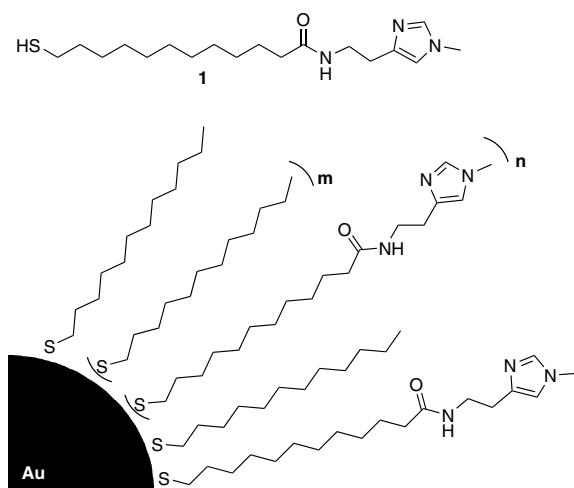
Figure 5 Synthesis of methylimidazole-functionalized gold nanoparticles.
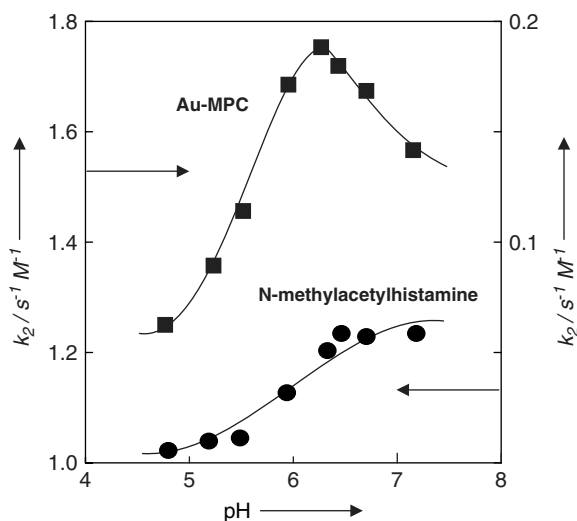

Figure 6 Dependence on pH of the second order rate constant for the cleavage of DNPA with methylimidazole-functionalized nanoparticles (bell shaped line) and monomeric *N*-methylacetylimidazole.

between carboxylates and imidazoles, by preparing simple dipeptides containing an histidine and a carboxylate either from a terminal amino acid or from aspartic acid. In the catalytic site of many esterases these functional groups operate in a concerted fashion as general acid and general base (or nucleophile) in the catalytic process.

On the molecular level, we prepared catalyst (**2**) (Figure 7) incorporating three AspHis-dipeptides on the 1,3,5-triethylbenzene scaffold (Guarise C, Prins LJ, Scrimin P, unpublished results). For the synthesis, we used a new synthetic methodology that allows us to functionalize any molecular scaffold with peptides on solid support [15]. As part of a combinatorial search for small peptide-catalysts we studied the catalytic activity of (**2**) in the hydrolysis of *p*-nitrophenylbutyrate (PNPB)

at pH 6.5 ([cat] : 0.1 mM; [PNPB] : 20 μM). We observed a significant rate enhancement of 53-fold compared to the uncatalyzed background reaction, and a rate increase of 20-fold with respect to the catalytic activity of the monomeric unit. This could be an evidence for cooperativity in this small molecular system although the kinetic analysis carried out so far has not provided sufficient information on this regard. On the basis of these initial positive results, we are extending our search including structural variations both in the peptide sequence and the scaffold.

Positive proof of cooperativity was found by self-assembling the same functionalities on Au-nanoparticles [16]. The complementary role of a car-boxylate and an imidazolium ion was demonstrated by studying the hydrolysis at low pH. Thiol (**3**) (Figure 8) contains a HisPhe–OH terminal sequence and was used to passivate an Au-nanoparticle in conjunction with a tris-ethyleneglycol methyl ether (TEG)-containing thiol, which makes the system water-soluble. As a reference catalyst, Ac-HisPhe–OH was used, which contains the same functional groups but is unable to aggregate.

Figure 8 reports the activity against pH of these functional nanoparticles and the monomeric catalyst in the hydrolysis of 2,4-dinitrophenylbutanoate (DNPB). At all pH values the Au-nanoparticle catalyst outcompetes the monomeric catalyst, but, extremely interesting, the two curves show strikingly different profiles. The monomeric catalyst behaves as a system in which a catalytically relevant nucleophile is generated with pK_a 6.6, which is consistent with the basicity of the imidazole. This is the logarithmic version of curve a of Figure 3. On the contrary, the nanoparticle shows a more complex profile: a first nucleophilic species is generated with pK_a 4.2, then the curve flattens up to pH 7 where a second nucleophile is generated with pK_a 8.1. These pK_a values can be assigned to the carboxylic acid and the imidazolium, respectively. The reason for the higher pK_a value of the imidazolium in the nanoparticle is due to the anionic nature of the nanoparticle, which disfavors the deprotonation of the imidazolium cation. What is particularly remarkable is the high activity, at acidic pH, of the nanoparticle-based catalyst showing over
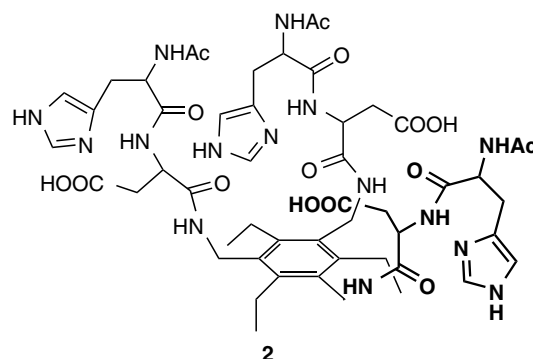

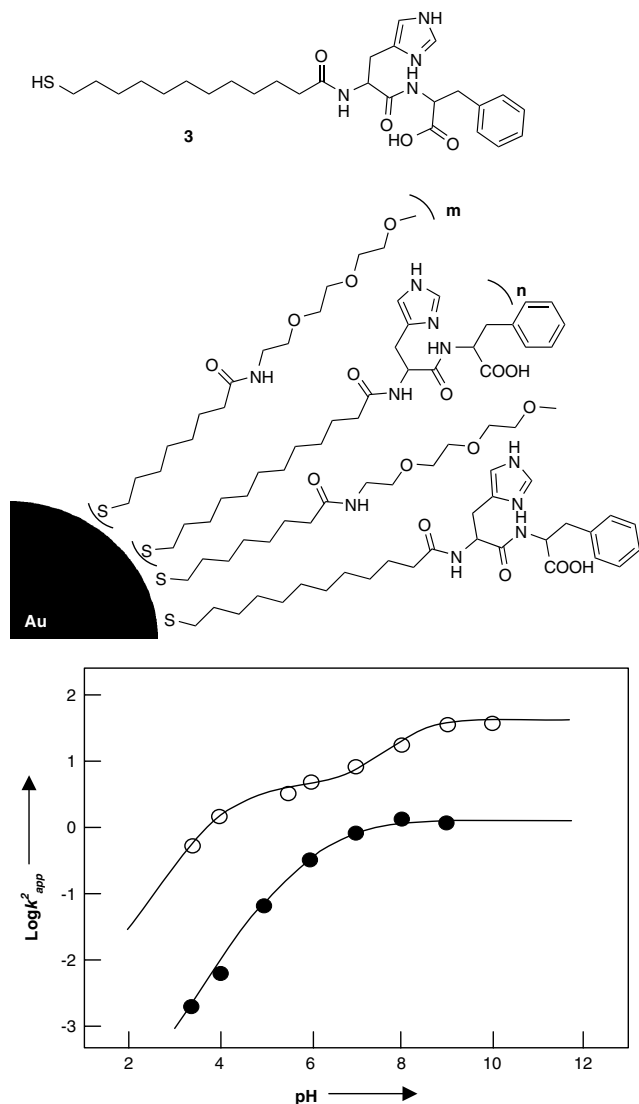**Figure 7** Structure of tripodal catalyst **2**.

Figure 8 Dipeptide-functionalized nanoparticle and dependence of the rate of cleavage of DNPB on pH for the nanoparticle-based catalysts (empty circles) and the acetylated monomeric catalyst (filled circles).

300-fold rate acceleration with respect to the acetylated dipeptide. Mechanistically, this has been interpreted by involving a carboxylate anion in the cleavage that acts as a general base deprotonating a water molecule and a protonated imidazole acting as a general acid. The absence of this mechanism in the monomeric system clearly indicates that this behavior results from the confinement of the dipeptide on the monolayer covering the nanoparticle. Any influence of the TEG units appears to be highly unlikely.

Moving on toward real 'nanozymes', i.e. nanoparticle-based models of enzymes, a dodecapeptide was grafted on Au-nanoparticles in a collaborative study with the group of Baltzer (see Figure 9) [17]. A combination of a His, two Arg, and a Lys residue was expected to enable nucleophilic, general-acid, and/or general base catalysis, but also stabilization of the

negatively charged transition state that arises along the pathway of ester hydrolysis. Au-nanoparticles were functionalized in a similar way as before and the catalytic activity of the resulting nanosystem in the hydrolysis of DNPB was studied as a function of pH. The results are reported in Figure 10 together with those related to the activity of the monomeric S-acetylated peptide. At low pH values, the nanoparticle-based catalyst behaves very similarly to the previous dipeptide-based system, although the dodecapeptide-nanoparticle has an additional 10-fold gain in activity. This amounts to a 3000-fold rate acceleration over that exerted by the simple dipeptide. The larger catalytic efficiency is ascribed to a stronger acidity of the protonated imidazole group, which in the dodecapeptide has a pK_a value of 0.9 units lower than in the dipeptide. This is due to the fact that now the nanoparticle has no longer a net negative charge as this is compensated by the presence of the cationic guanidinium groups.

At higher pH values, the activity of the peptide-nanoparticle increases significantly with respect to the dipeptide-NP reaching an additional 40-fold rate acceleration. This can be ascribed to the presence of an additional nucleophile (the phenoxide of tyrosine) with an apparent pK_a of 9.9.

We may conclude this section by pointing out that peptide-functionalized nanoparticles provide an easy access to cooperative catalysts introducing a new catalytic pathway in hydrolytic reactions otherwise not accessible with monomeric systems. Our preliminary results with discrete systems constituted by single molecules also indicate that cooperativity might also be present with these, very simple multifunctional catalysts. At variance with the previous imidazole-functionalized catalysts sharing the same platform which were unable to work cooperatively, in this case the dipeptides may provide a decrease of the conformational freedom, for instance, via H-bonding, resulting in a significant rate acceleration.

DEVELOPMENT OF ARTIFICIAL PHOSPHATASES BASED ON METAL IONS COOPERATIVITY

The hydrolytic cleavage of the phosphate bond under physiological conditions is incredibly slow. So slow that, in the case of phosphate diesters, the determination of reliable rate constants has been elusive, with numbers that have been updated to a slower figure almost yearly. The latest values indicate a half-life of 10^{10} years for the cleavage of dimethyl phosphate [18,19]. Not far from this sluggish reactivity should be the time required for hydrolytically cleaving the P–O bond of DNA. RNA is more labile because the nucleophilic attack on phosphorus is performed intra-molecularly by the –O(H) in the 2' position of the ribose. Thus the half-life is, in this case, only 10^4 years. These
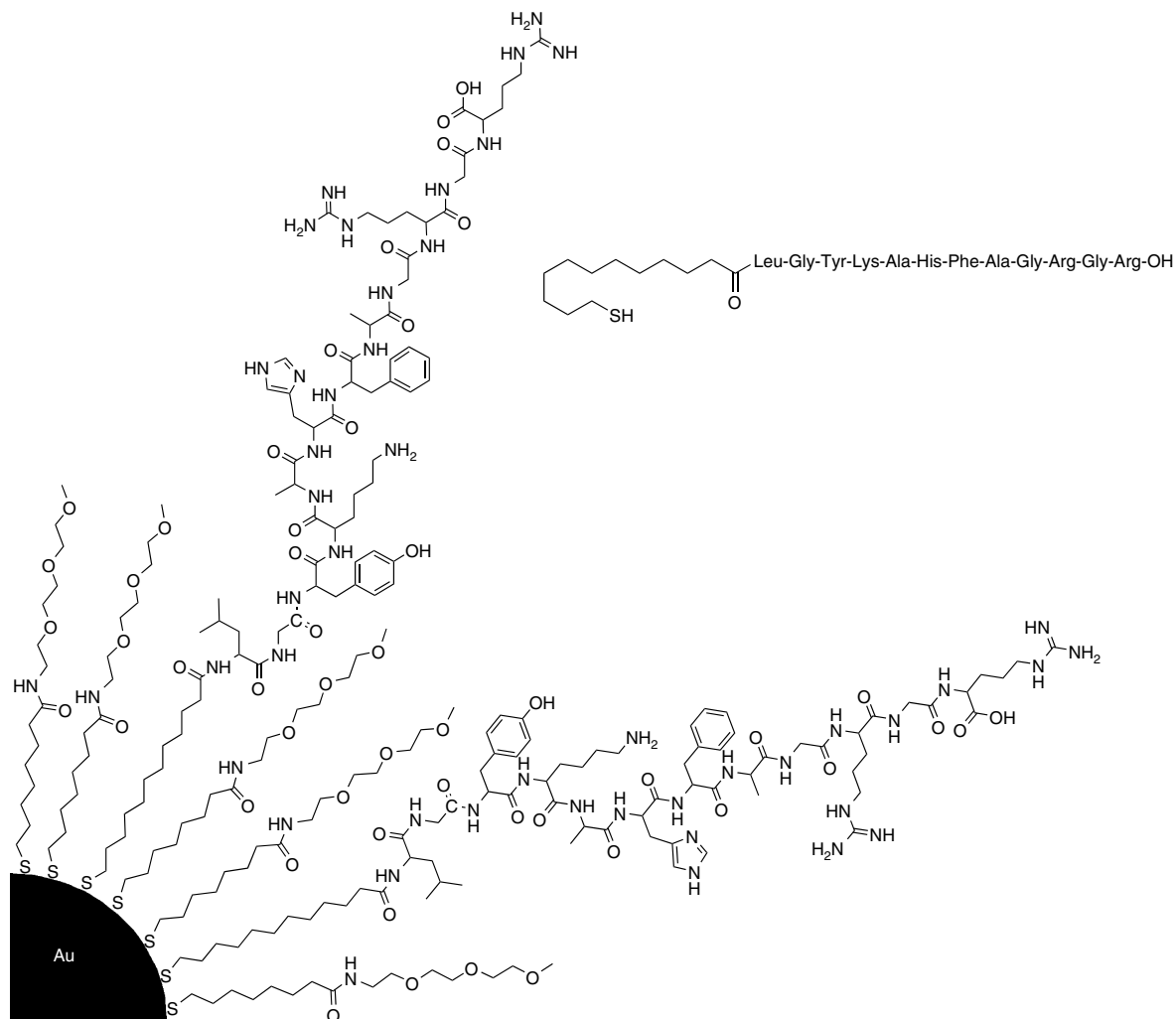
Figure 9 Dodecapeptide-functionalized nanoparticle and thiolated dodecapeptide used for the grafting on the gold cluster.
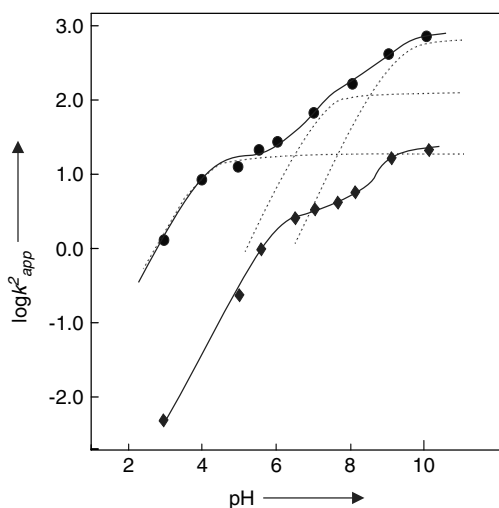

Figure 10 Dependence of the second order rate constant on pH for the cleavage of DNPB for dodecapeptide-functionalized nanoparticles (filled circles) and the acetylated monomeric peptide (see Figure 9 for its structure).

processes can be accelerated by several orders of magnitude by metal ions and a considerable number of laboratories has embarked in the challenging goal of preparing powerful, metal ion-based catalysts with, in some cases, interesting results [20]. Chin has estimated that, simply by adding-up independent contributions to the catalysis by two cooperating metal ions, a catalyst could bring up to about 18 orders of magnitude in rate acceleration [21]. Such a catalyst will, however, remain the Holy Grail for scientists for many years to come. The critical issue is, again, cooperativity in this case between the metal centers. We have studied a number of systems based on amino acids and small peptides taking advantage of different structural features in order to induce cooperativity between several metal ions. As it will be clear from the following discussion, multivalent systems are the most efficient ones.

We first started our investigation by using peptide sequences incorporating two or more copies of the artificial amino acid, *S*-2-amino-3-[1-(Tacn)] propanoic acid (ATANP) [22], a derivative of serine in which the –OH has been replaced by triazacyclononane, a
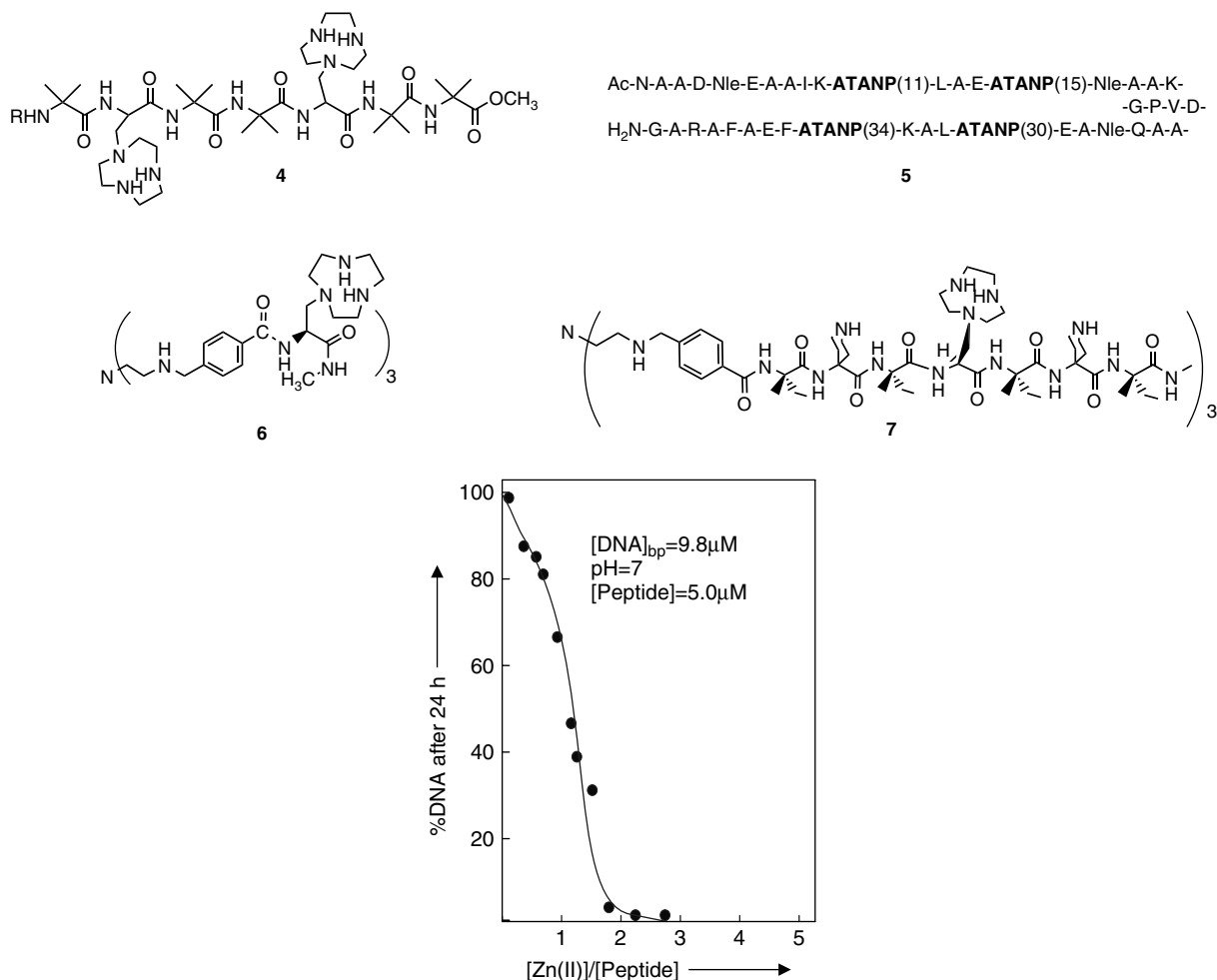
Ac-N-A-A-D-Nle-E-A-A-I-K-**ATANP**(11)-L-A-E-**ATANP**(15)-Nle-A-A-K-
                                                                        -G-P-V-D-
H₂N-G-A-R-A-F-A-E-F-**ATANP**(34)-K-A-L-**ATANP**(30)-E-A-Nle-Q-A-A-

5

Figure 11 Dependence of the amount of uncleaved plasmid DNA and the equivalents of Zn(II) added to a fixed concentration of peptide (**4**). Lesser the DNA present, the better the performance of the catalyst.

strong ligand for metal ions like Zn(II) and Cu(II). Because of the nature of the component amino acids, these sequences had the strong tendency to fold into helical conformations [23,24]. Upon folding, the ligand units are placed close to one another, thus, providing potentially cooperative metal ion-based catalytic sites. Peptide (**4**) folds into a 3_{10} helical conformation while peptide (**5**) folds into a hairpin-like α-helix. Both showed moderate efficiency in phosphate cleavage [25–27]. In the case of (**4**), the cooperativity between the two metal centers could be unequivocally demonstrated in the cleavage of plasmid DNA. Indeed (Figure 11), by keeping constant the concentration of the peptide and progressively saturating the two triazacyclononane units with Zn(II) ions the efficiency of the system increased in a nonlinear fashion with the dinuclear catalyst proving to be much more efficient than the mononuclear one.

We were also able to prove cooperativity between metal centers by connecting several ligand amino acids (analogous to ATANP) to a tris-aminoethylamine (TREN) platform (**6**) and inducing proximity between the metal

complexes by using an allosteric metal center [28]. This strategy worked nicely also for the obtainment of a three helix bundle comprising three peptide sequences each containing an ATANP unit (**7**) [29].

However, the most exciting results were obtained with multivalent systems: monolayer-protected gold nanoparticles and dendrimers. In the case of the nanoparticles, we prepared ATANP-functionalized thiol (**8**) and grafted it on the gold nanoparticle in a 1 : 1 mixture with dodecanethiol [30].

By studying the cleavage of the RNA-model substrate 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPPNP), by working at a fixed concentration of nanoparticle and progressively adding Zn(II) ions, we obtained the sigmoidal profile shown in Figure 12. Once again, this curve provides strong support in favor of a cooperative mechanism between several metal ions. However, it was not clear what 'magic' number of metal ions in the catalytic site was able to elicit maximum catalysis.

This information was obtained by studying another class of multivalent systems: metallodendrimers. At variance with the nanoparticle-based multivalent
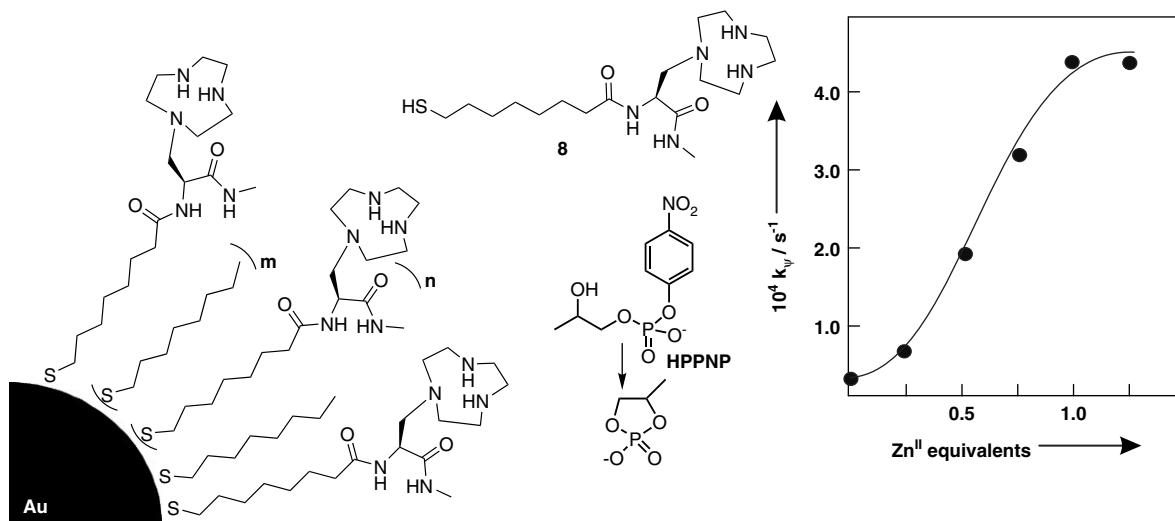
Figure 12 ATANP-functionalized nanoparticles and thiolated aminoacid (**8**) used for the passivation of the gold core. On the right is the rate profile observed in the cleavage of HPPNP as a function of Zn(II) equivalents.

catalysts, dendrimers are not self-assembled and are the result of quite demanding synthetic efforts and purifications. We have functionalized 3rd generation DAB (poly(propylene imine)) dendrimers [31,32] with a triazacyclononane-bearing acetate via amide bond formation (see Figure 13). In order to specifically address the issue of cooperativity between different metal ions present on the periphery of the dendrimer, we have prepared derivatives with different degrees of functionalization [33]. In this way the relative concentration of the complexes on the surface of each dendrimer could be changed, thus, allowing us to test how this affects the activity of the different catalysts. Usually, the activity of increasing generation of functional dendrimers is compared, but this is not a reliable way to assess cooperativity. In fact, by increasing dendrimer generation both the size and the number of functional groups present on the periphery are increased. It is thus difficult to correlate the activity with the concentration of the active functions at the reaction loci (i.e. the dendrimer interfacial region). By reporting the reaction rates determined with different generations of dendrimers at constant catalyst concentration (this amounts to changing the mole fraction of catalysts on the dendrimer surface) we obtained the upward curved profile reported in Figure 14. The fitting of this curve gave us the critical information we were looking for: the number of metal ions involved in the catalytic process. This number is two. Likely, this is also the catalytically active complex in the nanoparticles. The data clearly indicate that in spite of the fact that more than two metal ions are potentially accessible to the substrate the most efficient catalytic process is the one that takes advantage of the cooperative action of two metal centers.
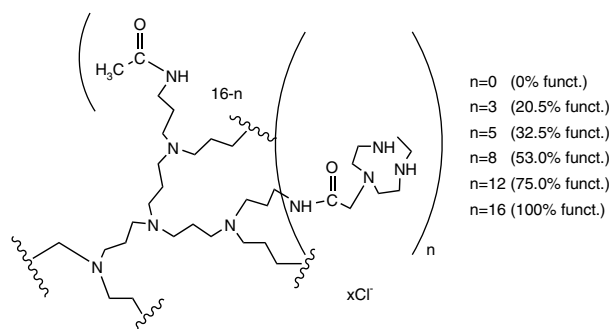

Figure 13 Triazacyclononane-functionalized dendrimers bearing increased amounts of ligand units.
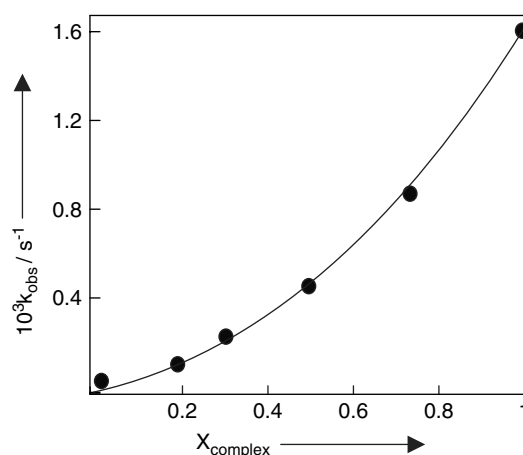

Figure 14 Dependence of the rate constant for the cleavage of HPPNP on the mole fraction of Zn(II) complex present on the periphery of the dendrimers.

CONCLUSIONS

We have tried to show in this overview, focused on our own work, how, by self-assembling amino acids and

peptides on gold nanoparticles, very peculiar multivalent nanocatalysts are obtained with unique properties. The most intriguing one is cooperativity. Cooperativity is not only at the basis of the very large rate accelerations measured, but also of quite new reaction mechanisms observed with these catalysts that are not accessible with the monomeric counterparts. The systems showing similar features are functional dendrimers. These latter share a number of properties with functional nanoparticles but for the very important difference that they are not self-assembled and, quite often, tedious syntheses and purifications are necessary for their obtainment. We have also shown that with small, discrete multivalent catalysts, cooperativity is more difficult to obtain. Likely, the advantages provided by the functional nanoparticles are twofold: first an higher packing of the functional groups with consequent decrease of the conformational freedom at the reaction loci, second the desolvation attained within the organic monolayer covering the gold nanoparticle which is responsible of enhanced nucleophilicity of the active functional groups. All these features indicate that it has been appropriate to call these nanosystems 'nanozymes' [30].

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