Multivalent Cooperative Catalysts

Leonard J. Prins, Fabrizio Mancin and Paolo Scrimin*

University of Padova, Department of Chemical Sciences and ITM-CNR, Padova Section, 35131 Padova, Italy

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 mostly (but not only) from our own laboratory we show what conditions are required for obtaining cooperativity in catalysis. Systems considered range from simple, discrete catalysts to dendrimers and monolayer-projected gold nanoparticles. Reactions taken into considerations for our analysis are the hydrolyses of carboxylate- and phosphate esters.

1. INTRODUCTION

It is a widely accepted paradigm that multivalent systems [1], i.e. systems topologically presenting a collection of identical (or similar) functional groups on their periphery, show enhanced binding ability against multivalent counterparts. This is the case of systems governing cell-cell, protein-protein, protein-cell interactions, for instance [2]. A large amount of data show how affinity constants between these systems may increase by several order of magnitude with respect to those of the monovalent ones. This is the result of cooperativity between the several units present in the recognition site.

The ability of multivalent systems to perform better in catalysis than their monovalent counterpart has been less studied, notwithstanding the fact that 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. Thus, catalysis takes advantage of a concerted action of a collection of functional groups as molecular recognition does. However, catalysis is much more complex to achieve than molecular recognition and, as a result, the design and synthesis of catalysts in which collection of functional groups work cooperatively still remains a major challenge for scientists. The aim of this review is to analyze catalytic, multivalent systems, mostly studied by our group during the last several years, to pinpoint properties required for such systems to become better catalysts than their monovalent counterparts.

One of the earliest multivalent systems studied was constituted by aggregates of lipids or surfactants forming vesicles or micelles. In studying micellar catalysis Fred Menger [3], reviewing the field more than fifteen years ago, wrote: “...groups of molecules, properly assembled, can obviously accomplish much more than an equal number of molecules functioning separately”. A careful dissection of the contribution to the catalytic activity of these systems led, however, the groups of Bunton [4], Romsted [5] and others [6] to reach the conclusion that these systems were not performing much better than the monomeric ones. In fact, although experimental data show very impressive rate accelerations, the analysis of their kinetic behavior indicated that the rate accelerations observed were mostly due to local concentration effects (including the ‘apparent’ reduction in reaction volume, but also the change of pH at the reaction loci). Thus cooperativity in these multivalent systems remains a controversial issue whose analysis is complicated by the fact that catalytic mechanisms with these systems proceed via pre-binding of the substrate (like in enzyme catalysis). The correct analysis of the cooperativity in catalysis thus requires that molecular recognition (binding) and catalysis be analyzed separately. As we will see the separation of the two processes is not always an easy task to perform. Further, by working under saturation conditions a multivalent catalyst may bind several substrate units. For proper comparison with monovalent systems this fact must be considered to avoid misinterpretation of the kinetic data.

For the ease of comprehension we will analyze multivalent systems starting with the simplest ones and then increase complexity ending with dendrimers, polymers, and functionalyzed metal nanoparticles.

2. ASSESSING COOPERATIVITY IN CATALYSIS WITH MULTIVALENT SYSTEMS

Quite often false claims of cooperativity in catalysis with multivalent systems are due to a non-correct interpretation of the kinetic data concerning the activity of these systems. We will consider a multivalent catalyst as a cooperative catalyst whenever: a) its activity is larger than the summation of the contribution of the individual components (this is often referred to as positive cooperativity); or b) the individual components play different roles not attainable with a monomeric catalyst resulting in rate acceleration. The following examples should clarify the concept.

Let’s first consider the kinetic behavior obtained by increasing the concentration of a multivalent catalyst and a monomeric one. In Fig. (1) the rate profile obtained with a micellar system, compared with a monomeric one bearing the same functional group, is reported [7]. 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. The micellar system is obviously much more active than the monomeric one in the

*Address correspondence to this author at the University of Padova, Department of Chemical Sciences and ITM-CNR, Padova Section, 35131 Padova, Italy; Tel: ---------; Fax: ---------; E-mail:
concentration interval explored. The enzyme-like reactivity profile indicates binding of the substrate to the catalyst but doesn’t 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 catalyst. This can be done by using a multivalent catalyst, in which active and non-active components are mixed together, and the mole fraction of the active component is increased. Under these conditions a non-cooperative catalyst will give a linear increase of the rate constant with concentration (Fig. 2, trace a) while a cooperative catalyst will deviate from linearity (Fig. 2, traces b and c).

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 non-protonated form and as an acid when protonated. Fig. (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 that typically observed with a 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 pKₐ of the kinetically relevant species. But maximum activity is observed when both protonated and deprotonated species are present and they must work cooperatively.

3. SIMPLE, MINIMAL, MULTIVALENT CATALYSTS

The simplest example of a multivalent catalyst is that provided by a system presenting at least two catalytic units. Although within the scope of this review such a catalyst may appear trivial, it is quite useful to start our overview from very simple systems because even such catalysts may provide important hints in understanding multivalency in catalysis.

The catalysts we will consider have all in common the ability to catalyze the cleavage of a carboxylate or phosphate ester. Reactions mechanisms for these systems are reasonably well understood and hence, their analysis is facilitated.
While carboxylate ester constitute a simple proving ground for such catalysts, phosphates are quite more challenging, being rather stable compounds. Their hydrolysis at physiological pH and room temperature proceeds with a half life of $10^{10}$ years (dimethyl phosphate) [8]. Excellent catalysts both natural (enzymes) and artificial for phosphate esters hydrolysis, typically require at least two metal ions for activity [9,10]. These two metal ions work cooperatively playing distinct roles in the cleavage process.

### 3.1. Peptide-Based Catalysts

The group of Baltzer, exploiting the conformational preference of designed 42 amino acid sequences assuming a helix-loop-helix conformation (Fig. 4), has systematically modified specific residues in key positions of the oligopeptide in order to catalyze the hydrolysis and transesterification reactions of $p$-nitrophenyl esters [11,12].

Imidazole-functionalized peptides obtained by introducing several histidines in the sequence were able to provide substrate recognition and accelerations exceeding three orders of magnitude compared to N-methylimidazole. Sequences like that depicted in Fig. (4) hydrolyze 2,4-dinitrophenyl acetate with a second order rate constant of $0.18 \text{ M}^{-1} \text{s}^{-1}$ at pH 3.1 compared with $9.9 \times 10^{-5} \text{ M}^{-1} \text{s}^{-1}$ for N-methylimidazole. In this case it has been demonstrated that the reaction mechanism takes advantage of the cooperativity of two adjacent histidines, one acting as the nucleophile and the other one as a general base as shown in Fig. (5). This mechanism corresponds to that reported in Fig. (3) above.

Our contribution to these simple catalysts has been the synthesis of the artificial amino acid ATANP [13] and its incorporation in oligopeptides [14]. ATANP is characterized by the presence of a lateral 1,4,7-triazacyclononane unit, which forms 1:1 complexes with metal ions such as Cu(II) and Zn(II) with strong affinities. As said above these metals play an essential role in accelerating the hydrolysis of DNA and RNA. Thus, in collaboration with Baltzer’s group a 42-mer peptide analogous to the one reported in Fig. (4) was synthesized, with the difference that these new sequences incorporated up to 4 copies of ATANP (Fig. 6). Also these new peptides form helix-loop-helix motifs and bind Zn(II) ions with the triazacyclononane subunits present in the lateral arms of ATANP.

It was observed that metal complexation causes a decrease in the helical content of the peptide. However, even upon a partial unfolding of the structure, an acceleration of the cleavage of HPNPP (2-hydroxypropyl-$p$-nitrophenyl phosphate, an RNA model substrate) was observed with sig-
significant rate acceleration with respect to the mononuclear catalyst. In this case too cooperativity is rather evident and is elicited by the convergence of several metal centers where the binding of the substrate occurs, i.e. to one of the two faces of the double-helical hairpin.

A very appealing method for the construction of very short, but highly organized peptides, is the use of C^\text{\textsuperscript{4\_}}}-tetrasubstituted amino acids. Research by the group of Toniolo [15,16] has demonstrated that most of the C^\text{\textsuperscript{4\_}}}-tetrasubstituted amino acids, of which \text{\textit{\textalpha}-aminoisobutyric acid (Aib) is the simplest example, are very strong promoters of the 3\textsubscript{10}/\textalpha-helical conformations. The main factors that determine the type of helix formed are the main-chain length, fraction of Aib, and amino acid sequence. Although in low polarity solvents the formation of a stable 3\textsubscript{10}-helix is quantitative, in polar solvents, like water, folding generally results mostly in \textalpha-helices [17]. The heptapeptide of Fig. (7), containing five Aib residues, prevalently adopts a 3\textsubscript{10}-helical conformation also in aqueous solution [15].

The sequence contains also two ATANPs (see above) for metal ions complexation. The dinuclear Zn(II) complex turned out to be a good catalyst for the cleavage of both the RNA model substrate HPNPP [18] and plasmid DNA [19]. In the latter case, cooperative action between the metal centers was demonstrated by comparison of the activity of the mononuclear versus the dinuclear complex. Tentatively, a mechanism was proposed which requires the formation of a supramolecular DNA-peptide complex as shown in Fig. (7).

The k\text{\textsubscript{a}} for the cleavage process (~1\times10^5 s\textsuperscript{-1}) allows the estimation of a rate acceleration of about ten million fold over the uncatalyzed cleavage process. In this case the pH vs. rate profile goes through a maximum suggesting complementary roles for the two metal ions involved in catalysis. This result also supports cooperativity.

### 3.2. Tripodal Catalysts

Tripodal platforms also constitute simple examples of multivalent catalysts. Analogously to the multiple-imidazole-functionalized peptide of Baltzer reported above mixtures of catalysts having up to 3 imidazole units connected to a 1,3,5-triethylbenzene scaffold were prepared simply by connecting histamine and N,N-dimethyllethylendiamine in varying amounts to the scaffold (Fig. 8).

The ratio between the four resulting catalysts was determined by the ratio of the reagents added [20]. Direct screening for catalytic activity and subsequent deconvolution then gave direct access to the individual contribution of each catalyst. 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 clearly indicate that, in this particular system, cooperativity between imidazoles is completely absent. The simple functionalization of the platform with the three arms is, accordingly, not enough to elicit cooperativity between them.

A further elaboration of the system was the use of 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.

---

**Fig. (7).** The octapeptide functionalized with five Aib and two copies of the triazacyclonane-bearing amino acid ATANP is active in the cleavage of plasmid DNA. On the right is reported the bell-shaped rate profile as a function of pH.
These were the functional groups of the catalyst shown in Fig. (9), incorporating three AspHis-dipeptides on the 1,3,5-triethylbenzene scaffold [21]. For the synthesis we used a new synthetic methodology which allows us to functionalize any molecular scaffold with peptides on solid support. As part of a combinatorial search for small peptide-catalysts we studied the catalytic activity in the hydrolysis of p-nitrophenylbutyrate (PNPB) at pH 6.5 ([cat]; 0.1 mM; [PNPB]; 20 μM). We observed a significant 53-fold rate enhancement compared to the uncatalyzed background reaction, and a 20-fold rate increase with respect to the catalytic activity of the monomeric unit. This could be evidence for cooperativity in this small molecular system although the kinetic analysis carried out so far has not provided clear-cut evidence on this regard.

However cooperativity between metal centers was observed in a tripodal system obtained by connecting several ligand amino acids (analogous to ATANP) to a tris-aminoethylamine (TREN) platform and inducing proximity between the metal complexes by using an allosteric metal center (Fig. 10) [22,23]. At variance with the previous system close proximity between the arms was ensured by a fourth metal ion. Its indispensable role was confirmed by acylation of the amines of the platform thus blocking their ability to bind this metal ion: the resulting catalyst was not behaving in a cooperative fashion. Allosteric control of conformation worked also nicely for the obtainment of a three helix bundle comprising three peptide sequences each containing an ATANP unit [24]. Cooperative catalysis was clearly apparent with the RNA model substrate HPNPP but not in the case of an oligonucleotide catalyst. One may speculate that in this latter case several phosphate groups bind to the three metal complexes thus spoiling the system of any possible cooperativity in catalysis.

This observation brings about an important point: a multivalent catalyst must be able to converge its catalytic units on the functional group to be transformed. Thus this section message is that structurally well-defined structures (as conformationally stable peptides) behave cooperatively in catalysis. More loose systems like the tripodal ones on the contrary do not, unless they are put to order, like, for instance, by an allosteric regulator.

4. DENDRIMERIC CATALYSTS

In the final part of this review we will discuss multivalent structures which cooperative phenomena have been frequently observed: dendrimers, polymers, and nanoparticles. We will focus the attention on how cooperativity is expressed by these systems and what its origin is. Dendrimers are monodispersed, hyperbranched polymers which expose a high surface density of functional groups that depends on the generation. The first catalytic peptide dendrimers were reported by Reymond and co-workers for the hydrolytic cleavage of esters [25,26,27]. These dendrimers were prepared by disulfide dimerization of second-generation dendritic peptides containing all possible combinations of the catalytic triad of the serine proteases (aspartic acid, histidine, and serine) resulting in 21 dimeric dendrimers (Fig. 11). Screening with a fluorogenic substrate showed that peptide dendrimers with triad amino acids having eight histidine groups at the surface were catalytically active and displayed enzyme-like Michaelis-Menten kinetics with substrate binding (KM ~ 0.1 mM) and rate acceleration (kcat/kuncat ~ 10^3). From a systematic alanine-scan it was concluded that catalysis most likely originated from cooperative action of two imidazoles, whereas the serine residues did not appear to play a significant role [26].

Therefore, in a subsequent study a series of dendrimers of different generation were prepared in which histidine-units were repeated in each generation (Fig. 12) [28]. The
catalytic activity was studied using a pyrene trisulfonate ester as a substrate. A systematic study of the dendritic effect in peptide dendrimer catalysis revealed that the catalytic rate constant $k_{\text{cat}}$ and substrate binding constant $1/K_M$ both increased with increasing generation number. The dendrimers showed rate accelerations up to $k_{\text{cat}}/k_{\text{uncat}} = 20000$ and $K_M$ values around 0.1 mM. The reactivity of histidine side chains within the dendrimer is increased up to 4500-fold when compared to 4-methylimidazole. A bell-shaped pH-rate profile with a maximum around pH 5.5 in the dendrimer-catalyzed reactions suggests that catalytic activity results from cooperative action of two histidines (adding both a nucleophilic and a general base component to catalysis, analogous to what reported in Fig. 3).

As a first entry into dendrimer synthesis, our group has functionalized 3rd generation commercial DAB (poly(propylene imine)) dendrimers [29] with a triazacyclononane (TACN)-bearing acetate via amide bond formation (see Fig. 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 [30]. 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, since both the size and the number of func-

---

Fig. (9). Structure of tripodal catalyst bearing AspHis dipeptides.

Fig. (10). Tripodal metallocatalysts subject to allosteric control by a remote metal ion. On the right, the kinetic profile as a function of the equivalents of Zn(II) added. The filled symbols refer to the tripodal catalyst while the open ones refer to the mixture of its components.

Fig. (11). Dendrimeric-peptide catalyzed ester hydrolysis.
Multivalent Cooperative Catalysts

Fig. (12). A strong positive effect in peptide dendrimer catalyzed ester hydrolysis. Higher generation dendrimers show a stronger substrate binding and stronger catalysis, resulting in a large enhancement of the specificity constant $k_{cat}/K_M$ (graph at lower right). Figure taken from ref. [27].

Fig. (13). Triazacyclononane-functionalized dendrimers bearing increased amounts of ligand units. On the right, the kinetic profile obtained as a function of the mole fraction of metallocatalyst present.

Additional groups present on the periphery are increased. This makes it 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 dendrimers with different catalyst (TACN)-loadings on the surface but at a constant catalyst (TACN) concentration we obtained the upward curved profile reported in Fig. (13), right. The fitting of this curve gave us the critical information that two metal ions are involved in the catalytic process. Likely this is also the catalytically active complex in the dendrimers. 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 that that takes advantage of the cooperative action of two metal centers.
In subsequent work, we studied the origin of this positive dendritic effect in more detail [31]. A series of peptide dendrons and dendrimers of increasing generation functionalized at the periphery with TACN were prepared. Kinetic studies showed that these metallo-dendrimers very efficiently catalyze the cleavage of the RNA model compound HPPNP, with dendrimer D32, containing 32 endgroups, exhibiting a rate acceleration of around 80 000 ($k_{\text{cat}}/k_{\text{uncat}}$) operating at a concentration of 600 nM. Although these are impressive numbers, a careful analysis revealed their true value. A theoretical model was developed to explain the positive dendritic effect displayed by multivalent catalysts in general. In this model the classical Michaelis-Menten model (describing interactions between an enzyme and a single substrate) was extended to cover a multivalent catalyst involving multiple simultaneous binding and catalytic events. Simulation of the catalytic behavior of a series of multivalent models and analysis of the saturation profile and the Michaelis-Menten parameters $k_{\text{cat}}$ and $K_M$ revealed that the positive dendritic effect is an intrinsic property of multivalent catalysts. The positive dendritic effect can be explained without the need to recall reasons such as changes in the catalytic site, increased substrate binding constant, or changes in the microenvironment. Rather it appears that the efficient catalytic behavior of multivalent catalysts is mainly determined by two factors: the number of catalytic sites occupied by substrate molecules under saturation conditions, and the efficiency of the multivalent system to generate catalytic sites in which multiple catalytic units act cooperatively on the substrate. In other words, clustering of multiple catalytic units in close proximity enhances the apparent concentration of dinuclear catalytic sites, which makes substrate binding appear stronger (Fig. 14). Obviously, under saturation conditions, this effect is lost and in fact, often the catalytic constant per catalytic unit is identical for dendrimers of different generation. Nonetheless, catalytic sites that cannot be saturated (for example because of poor accessibility) negatively affect the global catalytic constant of the system. Analysis of this type will be of increasing importance for the design and evaluation of new multivalent systems.

5. POLYMERIC CATALYSTS

An approach pursued in the design of multivalent catalyst is that of functionalizing a polymer. Polymers, at variance with dendrimers, are not so well defined in terms of molecular weight and conformation. The difficulty in conformation control reflects itself in the uncertainty in knowing the location in the space of the functional groups, their proximity and, ultimately, their ability to cooperate one with the other. Nevertheless functional polymers have been widely used as cooperative catalysts and as enzyme mimetic systems behaving sufficiently well to warrant them the name synzymes [32]. After the excitation generated at the beginning research on this field somehow faded to be revitalized in the late ‘90s by Suh and his group [33]. The target they have selected is the hydrolysis of the peptide and phosphate bonds that very well fits within the scope of this review. The first systems reported was designed as a mimic of a protease and was obtained by constructing an artificial active site comprising three convergent salicylate residues on the backbone of a highly branched, polyethyleneimine polymer [34]. To overcome the problem of the correct folding of the polymer and, hence, of the appropriate location of functional groups, they assembled three molecules of 4-bromoacetyl-salicylate around an Fe(III) ion and then crosslinked with PEI. Once an amino group of PEI was linked to one of the three salicylates.
through a covalent bond, other amino groups of PEI were located in the right positions to attack the remaining two salicylates, thus completing the cross-linkage. After removal of Fe(III) ion by treatment with acid, a water-soluble polymer with catalytic sites comprising three proximal salicylates was obtained. The polymer was incubated with 

poly(chloromethylstyrene-co-divinylbenzene) (PCD) and poly(aminomethylstyrene-co-divinylbenzene) (PAD), which are cross-linked polystyrenes with styryl residues containing chloromethyl and aminomethyl groups [35]. Using the same catalytic units they succeeded in the cleavage of albumin with optimum activity at pH ca. 3. The concept was also extended to imidazole-functionalized PCD polymers by attaching imidazole to PCD through the carbon (C-2) atom [36]. The imidazole-containing PCD hydrolyzed albumin with a half-life of 1200 sec at pH 7 and 25 °C. The optimum activity was manifested at pH ca. 8. When the content of the imidazole was reduced by 4.4 times, the proteolytic activity was reduced by 24 times. Hence there was not a linear dependence of reactivity on imidazole functionalization of the polymer implicating collaboration of two or more imidazoles in the catalytic process. The mechanism proposed (see Fig. 15) is very similar to the one reported in Fig. (3) and involves cooperation between two imidazoles: one as a nucleophile and the other as a general acid.

A clever approach for the design of a catalytic site on a polymer was reported, among others, by the group of Wulff with the use ‘molecularly imprinted polymers’ [37,38]. For this, a crosslinked polymer is formed around a molecule that acts as a template. The monomer mixture contains functional monomers that can interact with the template through cova-

lent or noncovalent interaction. After removal of the template, an imprint containing functional groups in a certain orientation remains in the highly cross-linked polymer. The shape of the imprint and the arrangement of the functional groups are complementary to the structure of the template. This corresponds to an implementation of the concepts reported above, as not only multiple functions are put in the catalytic site, but also diverse ones. The final goal is obviously to arrive to an array of functions acting in a concerted manner, i.e. cooperatively. If the template is a model of the transition state of the reaction the polymer is supposed to catalyze, it may stabilize it via complexation, thus increasing the rate of the process. The concept of transition state stabilization in (enzyme) catalysis, first introduced by Pauling [39], was very successfully applied to catalytic antibodies. The most recent advances in the field of imprinted polymers concern the formation of a polymer with carboxypeptidase A activity [40]. By using a phosphate diester as a mimic of the transition state of a carbonate ester hydrolysis Wulff and Liu have imprinted a polymer with amidinium and a metal ion binding site. By using diarylcarboxylic acids as substrates and Cu(II) as the metal ion, a rate acceleration over the uncatalyzed reaction of up to 217,000 was observed. Most important, the imprinting factor (or, in other words, the cooperativity factor) ranged from 50 to 80 to illustrate the great advantage of the use of an imprinted polymer over a nonimprinted one. The mechanism suggested, involving the cooperativity by the amidinium and the metal ion is illustrated in Fig. (16).

Switching to the cleavage of phosphate esters, recently Hollfelder et al. [41] reported the use of PEI derivatized with alkyl, benzyl and guanidinium groups to obtain catalysts with enzyme-like properties and rate accelerations ($k_{\text{cat}}/k_{\text{uncat}}$) of up to $10^4$ for the standard RNA-model substrate HPNPP in the absence of any added metal. The authors have shown that the efficacy of the catalysts is determined by the PEI derivatization pattern that exert a synergistic effect. This means that the catalysts act cooperatively because the combination of all these parts in the polymer increases catalysis by more than the sum of each modification taken independently. Highest rate was observed at pH 7.8 corresponding to
the combination of two effects: the decrease of the binding at higher pH due to the deprotonation of the amines and the decrease of the [OH-] at low pH. Catalysis is thus due to substrate binding by positively charged amine groups and the presence of hydroxide ions in active sites in a relatively hydrophobic environment provided by the presence of the long hydrocarbon chains.

The very same PEI polymer was functionalized again by Suh et al. by random attachment of lauryl groups and Ni(II) complexes of terpyridine to obtain a metallocatalysts for HPNPP cleavage [42]. In water, the lauryl and the terpyridyl residues are expected to form hydrophobic clusters on the backbone of PEI. An artificial active site comprising two or more metal ions can form in the cluster, whose structure depends on the contents of lauryl and terpyridyl residues. In this case too, the cooperativity between two (or more) metal ions was suggested as the possible mechanism for the accelerated rate of cleavage of the substrate (Fig. 17).

6. NANOPARTICLE-BASED CATALYSTS

Clusters of gold atoms covered by a monolayer of organic molecules constitute an interesting example of self-assembled multivalent systems [43]. Multivalency in these systems is ensured by the presence, on the monolayer covering the gold core, of several identical functions that, in principle, could cooperate in a catalytic process [44]. Typically, individual molecules are anchored on the gold surface via a thiol that ensures a strong (ca. 20 kcal/mole) interaction with the metal. Depending on the size of the nanoparticles these thiols are more (large and less curved clusters) or less (small and more curved clusters) tightly packed. Furthermore the use of mixtures of thiols with different functional groups may allow the modulation of their interaction and reactivity. Our first attempt to obtain a cooperative catalysts for the cleavage of a carboxylate ester was performed by incorporation of imidazole subunits in the self assembled monolayer (SAM) [45]. The system (Fig. 18) consisted of a mixed monolayer of dodecanethiol and the N-methylimidazole-terminated thiol. 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 Fig. (18) 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 Fig. 1), 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 and discussed in Section 3.1. The simple conclusion from these studies is that by confining the catalytic...
units in an environment like that provided by the monolayer covering the gold nanocluster we managed to elicit cooperation between them. This is probably due to their reduced mobility.

Slightly more complex systems were those constituted by dipeptides designed to induce cooperation between carboxylates and imidazoles by using histidine and a carboxylate either from a terminal amino acid or from aspartic acid. The concept is very similar to the one reported in Section 3.2.

Nanoparticles functionalized with these peptides gave positive proof of cooperation [46]. The complementary role of a carboxylate and an imidazolium ion was demonstrated by studying the hydrolysis at low pH. The thiol used for the passivation contains a HisPhe-OH terminal sequence and was used 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.

Fig. (19) 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 interestingly, 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 Fig. (1). 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 of the higher value of the $pK_a$ of the imidazolium in the nanoparticle is due to the anionic nature of the nanoparticle that disfavors the deprotonation of the imidazolium cation. What is particularly remarkable is the high activity, at acidic pH, of the nanoparticles-based catalyst showing over 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 towards 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 Fig. 20) [47]. 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 Fig. (20) (top, right) 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 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.

When addressing phosphate cleavage with these systems we prepared an ATANP-functionalized thiol and grafted it on the gold nanoparticle in a 1:1 mixture with dodecanethiol [48].

By studying the cleavage of the usual RNA-model substrate HPNPP, we obtained, by working at a fixed concentration of nanoparticle and progressively adding Zn(II) ions, the sigmoidal profile shown in Fig. (21). Once again this curve provides strong support in favor of a cooperative mechanism between several metal ions. Indeed this catalyst was also active with dinucleotides, UpU in particular.

A step further in the improvement of the catalytic unit was that to functionalize the monolayer with a more elaborate ligand in which the action of the metal ion could be assisted by that of ancillary groups (able to H-bond with the substrate, for instance). Accordingly, we prepared a bis-(2-amino-pyridinyl-6-methyl)amine (BAPA) functionalized thiol (Fig. 22) as the BAPA-Zn(II) complex is known to be able to elicit the cooperation between metal Lewis acid activation and hydrogen-bonding to achieve increased hydrolytic activity toward phosphate diesters [49,50]. The nanoparticles were prepared following a recent protocol [51] reported by us. The resulting Zn(II) nanoparticle proved to be one of the most effective catalysts reported so far for the cleavage of the DNA model phosphate, bis-p-nitrophenyl phosphate (BNPP) [52]. The second-order rate constant observed was more than 60,000 higher than that of the base-catalyzed reaction and more than 100-fold better than that of the mono-

![Fig. (21). Triazacyclononane-functionalized nanoparticles behaving cooperatively in the cleavage of HPNPP as evidenced by the sigmoidal reactivity profile shown on the right reporting the reactivity as a function of the equivalents of Zn(II) added.](image-url)
increase in rate with respect to the monomeric complex. Both condition represent a significant change occurs with the linearization of the double strand in close proximity to the previous one a further cut occurs in the other strand. However, if a second cut occurs in the other strand in close proximity to the previous one a further cut occurs with the linearization of the double strand (form III). Statistically this second cleavage occurs in the proper position only in 1% of the cases. Strikingly, in our case linear DNA started forming after only 10% of the original supercoiled DNA was cleaved and already with 16% of it cleaved it was the most abundant form present (Fig. 23). This clearly indicates that the multivalent nanoparticle was able to perform multiple cuts on the double strand, the obvious consequence of its multivalent nature. One may speculate that the nanoparticle by sticking to the polymer is able to hydrolyze at the same time several phosphate bonds belonging to both strands of the polymer thus linearizing it very efficiently, a behavior never observed with other catalysts. This is a very important result because for the first time a multivalent catalyst takes advantage of its multivalency on two counts. First, it cleaves efficiently a specific functional group by converging several cooperating catalytic units on it. Second, using the same mechanism it cleaves simultaneously several functional groups on a multivalent target, the polymeric DNA backbone.

CONCLUSIONS

We have tried to show in this overview, how to turn multivalent catalysts into cooperative ones. The clear message is that maximum cooperativity is the result of a decrease of the conformational freedom of the multivalent catalysts and of the enhanced proximity of the putative catalytic units converging in the catalytic site. With small, discrete multivalent catalysts, cooperativity is more difficult to obtain. Cooperativity not only provides very large rate accelerations, but also induces quite new reaction mechanisms, which are not accessible with monomeric, analogous systems. The best performing multivalent catalysts are dendrimers, imprinted polymers, and functional gold nanoparticles. The latter present the enormous advantage of being self-assembled systems, thus reducing to the minimum the synthetic efforts for their preparation.

REFERENCES

[4] Scrimin, P.; Tecilla, P.; Tonellato, U.; Bunton, C. A., Nucleophilic catalysis of hydrolyses of phosphate and carboxylate esters by met-
allomeric structures of the 1,4,7-triazacyclononane ligand,


(a) Guari, C.; Prins, L. J.; Scrimin, P. Fully symmetrical functionalization of multivalent scaffold molecules on solid support, Tetrahedron, 2006, 62, 11670-11674; (b) Guari, C.; Prins, L. J.; Scrimin, P. Unpublished results


