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Cascade Reactions Catalyzed by Bionanostructures

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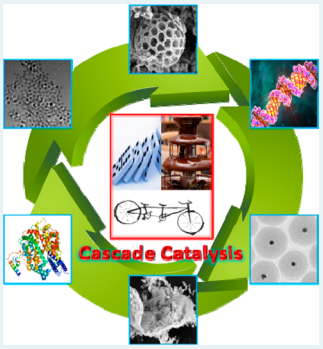
ABSTRACT: Cascade reactions are an emerging technology in organic chemistry, introducing elegance and efficiency to synthetic strategies. This Review provides an overview of the novel and recent achievements in cascade processes catalyzed by bionanostructures. The examples here selected demonstrate the advances related to the application of heterogeneous nanocatalysts—nanostructures and biomolecules combined by different manner—in efficient cascade processes. Metallic nanoparticles supported on biomolecules, multienzymatic systems or bionanohybrid structures with multicatalytic activities (containing both organometallic and biocatalytic activity) were selectively and efficiently used alone or in cooperative fashion. This Review highlights examples of efficient and interesting catalytic cascade processes in organic chemistry, ultrasensitive biosensing, or energy storage and conversion, underscoring their tremendous future potential in chemical synthesis.



KEYWORDS: *nanoparticles, nanostructures, biohybrids, cascade catalysis, tandem reaction, domino reaction*

INTRODUCTION

Cascade reactions, typically defined as a consecutive series of chemical reactions proceeding in a concurrent fashion, have attracted scientists' attention in recent years. One of the main areas in which this strategy plays a pivotal role is in nature with the biosynthesis of natural products.[1−3] Generally, this typology of reaction can be classified in domino, one-pot, or tandem reactions, and the intrinsic advantages correlated to these types of consecutive reactions are clear: atom economy; step-saving and, therefore, high yield; and efficiency of the chemical process.[4−9] From a practical point of view, homogeneous organometallic complexes, organocatalytic molecules, and enzymes have represented and still are successful catalysts for these types of reactions by combining them in different manners.[1,4,6,10−16] However, to efficiently catalyze a cascade reaction, the preparation of solid heterogeneous catalysts with precise control over the location of different functionalities would be generally preferable, but it is still a great challenge.[17−19]

During the past decade,[20−24] nanostructured materials (specially the active nanoparticles (NPs)) and biomaterials (as remarkable heterogeneous catalysts for different organic reactions) has undergone explosive growth, thanks to the development of more efficient synthetic methodologies.[25−27] Under a catalytic point of view, nanostructures present many advantages, especially their large surface-to-volume ratio compared to bulk materials. Consequently, as catalysts, NPs can be directly used as such or supported as different nanostructures (nanorods, nanotubes, etc.)[28−31] on a wide set of surfaces, such as inorganic materials (silica, carbon, metal oxides, etc.) or biomolecules (RNA, DNA, polysaccharides, peptides, or proteins).[32−38]

In particular, this last strategy possesses the *extra* capability to generate a greener and sustainable process because these biomolecules can be used as such or as an additive tool to mediate the formation and geometry of nanoparticles in the presence of a reducing agent (typically ascorbic acid or sodium borohydride).[39−41] For example, proteins have been involved in the synthesis of metal nanoparticles[35−38,42] and hybrid nanostructures[34] in aqueous media and at room temperature.

Bionanostructures, in which an enzyme is specifically encapsulated in a nanocluster or immobilized on biofunctionalized nanoparticles,[43−45] are another category of catalysts with excellent features in cascade reactions. In particular, heterogeneous nanohybrid enzyme−metal nanoparticle composites are especially of interest in organic synthesis because of their double or multiple catalytic activities fused in the same entity and simple reutilization strategy (a quite relevant feature from an industrial point of view).[35]

Hence, we focus this Review on the most recent advances achieved in this new area of nanocatalysis regarding the use of such bionanostructures in cascade catalysis. Among the various examples encountered in the literature, we selected the most representatives ones describing their synthesis protocols and main application areas, always with the final scope to generally underline to the reader the tremendous intrinsic potential enclosed in this novel but quickly growing strategy.

1. CASCADE REACTION IN ORGANIC SYNTHESIS

1.1. Metallic NP-Catalyzed Cascade Processes. Most of the chemical applications of metallic NPs in catalysis are based on their use as simple NPs or supported in inorganic material.[28−31] However, in recent years, a new approach based on the application of biological entities (RNA, DNA, polysaccharides, peptides, or proteins) as materials for NPs' immobilization, creating a new type of bionanocomposites, has been developed.[32−38] The possibility to immobilize the NPs by

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these strategies generates heterogeneous nanocatalysts with better performance, for example, in chemical synthesis of complex molecules.[46]

Carbohydrate-based materials, such as polysaccharides, are attracting growing interest as substitutes for classical inorganic and organic supports. They are inexpensive, nontoxic, environmentally friendly, and readily available in nature.[47]

For example, gum acacia (GA), a highly branched neutral or slightly acidic arabinogalactan polysaccharide, naturally obtained from the stems and branches of the *Acacia senegal* tree, has been used as a support to depose palladium NPs.[47] This strategy permits access to heterogeneous Pd catalysts, where the small NPs are generated in situ by the polymer, which acts in reducing and stabilizing the metal (Figure 1A).

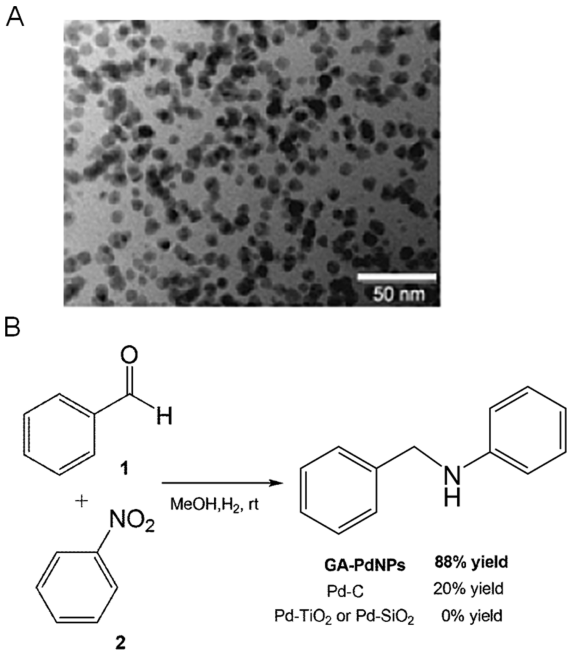
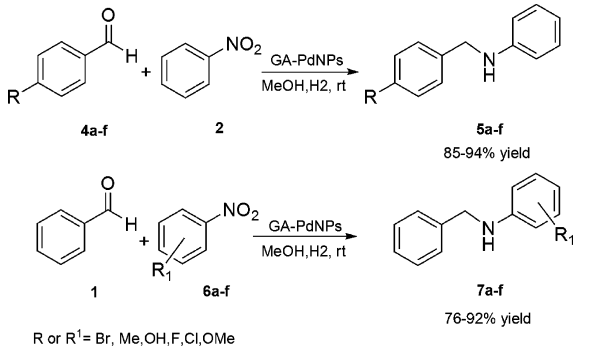


Figure 1. (a) TEM image of GA-Pd nanoparticles (reproduced with permission from ref 47). (b) Synthesis of amine **3** by reductive amination catalyzed by different Pd NP catalysts.

The GA-Pd NPs were used as excellent catalysts in the direct one-pot reductive amination of aldehydes with nitroarenes in a domino fashion in the presence of hydrogen as the reductant.[47] This catalyst exhibited the highest yield of the reductive amination reaction between benzaldehyde (**1**) and nitrobenzene (**2**), in comparison with other typical Pd-supported catalysts, 88% of amine **3** in 6 h against 20% for Pd−C or even no conversion for Pd−TiO_2 or Pd−SiO_2 (Figure 1B). This result was successfully extended to a combination of substituted aldehydes (**4a−f**) and nitrobenzene or benzaldehyde and different substituted nitroarenes (**6a−f**) under the same conditions, obtaining good or excellent yields in all cases (76−96%) (Scheme 1).[47]

Following this methodology, Khazaei et al.[48] have recently prepared Pd NPs supported on pectin for a copper-free Sonogashira reaction, an interesting process that can be conveniently coupled for a domino reaction. Other natural biopolymers, particularly natural animal fibers (wool), have been used as efficient polymeric supports in several important palladium-catalyzed transformations.[49,50]

Scheme 1. Reductive Amination Reaction Catalyzed by GA-Pd NPs



Moreover, bionanostructures based on the creation of AuNPs aggregated into nanowires on DNA have been designed with excellent catalytic possibilities, such as in reduction processes.[51] The accommodation of the AuNPs on DNA strains generate a better nanocatalyst compared with others in which the Au nanocomponents are synthesized using polymeric amines,[52] ionic liquids,[53] or surfactant.[54]

Peptide-capping nanoparticles has represented another elegant strategy to generate catalytic bionanostructures.[55,56] The investigation of the interactions between metal nanoparticles and peptides is still in its infancy, but this area has attracted tremendous attention in the scientific community in the past few years.

Knecht and co-workers have recently prepared a set of different peptide−Pd NP conjugates (Figure 2).[56a] They

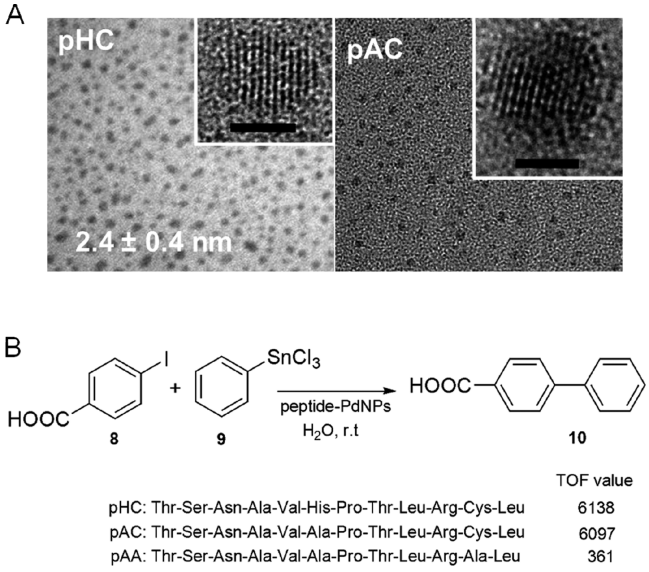


Figure 2. (A) TEM analysis of the Pd nanoparticles capped with pHC and pAC of the self-assembly of Au NP arrays into Au nanowires on DNA (reproduced with permission from ref 56). (B) Stille C−C coupling reaction catalyzed by different peptide−Pd NP conjugates.

demonstrate that using different peptide sequences with only small differences (one or two amino acids), the functionality of the Pd NPs was altered while their nanomorphology was conserved (NPs around 2.4 nm) (Figure 2). Parameters as binding affinity between the NPs and the peptide residues or flexibility seem to be critical for the final catalytic properties of the bionanostructures. In this way, these differences were

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extremely marked in the Stille C−C coupling reaction. Different peptide−Pd NP conjugates catalyzed the coupling of 4-iodobenzoic acid **8** with $PhSnCl_3$ **9** achieving biphenylcarboxylic acid **10** in water at room temperature. The best catalytic results in term of TOF (turnover frequency) values were obtained using pHC (Thr-Ser-Asn-Ala-Val-**His**-Pro-Thr-Leu-Arg-**Cys**-Leu) and pAC (Thr-Ser-Asn-Ala-Val-**Ala**-Pro-Thr-Leu-Arg-**Cys**-Leu) peptides, almost 20 times more active when using pAA (Thr-Ser-Asn-Ala-Val-**Ala**-Pro-Thr-Leu-Arg-**Ala**-Leu) peptide, showing that a Cys in a particular position is critical for the functionality of the nanoparticles (Figure 2B). These results could demonstrate the applicability of these new nanohybrids in a possible combination of this Stille reaction with a C−H transformation[56b] or, more recently, as another example of the same authors in a light-activated reductive dechlorination of polychlorinated biphenyls.[56c]

Another application in the combination of metal NPs and peptides has been reported by Zaramella et al.[57] They developed heterofunctionalized multivalent peptide−Au NP complexes, which accelerate the hydrolysis of *p*-nitrophenyl esters by at least 2 orders of magnitude. This reaction could be coupled with the reduction of the nitro group for the typical domino reaction producing aminoarenes.[35,58]

1.2. Biocatalytic Cascade Reactions. In nature, cellular biochemistry requires the orchestration of metabolic pathways in which cascades of many enzyme-catalyzed processes working simultaneously are critical. These cascade processes are possible thanks to evolved enzymes working in a complex and concerted media. In this way, part of the research in cascade catalysis has been dedicated to imitating nature by combining different enzymatic systems and exploiting the high specificity, stereo-, and regioselectivity of these biological catalysts in the production of complex chemical compounds.

However, compatibility problems and mutual inactivation are often encountered. Therefore, this drawback may be circumvented by performing cascades in sequential steps, by site-isolation of the individual catalysts through immobilization, heterogeneous, or biphasic reaction conditions, or creating encapsulated multienzymatic reactors.[59−69]

One of these examples has been developed by van Hest and co-workers,[65] who prepared a polymersome nanoreactor containing three different enzymes for cascade catalysis (Figure 3). The porous polymersome was created by block copolymers of isocyanopeptides and styrene and three different chemically activated enzymes. Glucose oxidase (GOx), lipase (CalB), and horseradish peroxidase (HRP) were located on the polymersome at different locations (Figure 3a).

This nanoreactor was successfully applied in a three-enzyme cascade reaction starting from 1-acetylated glucose converted to glucose by CalB, which was oxidized by GOx to gluconolactone in a second step. The hydrogen peroxide produced was used by HRP to oxidize 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to $ABTS^+$. This strategy could represent a very valuable example to be applied in cascade processes.

More recently, Palivan and co-workers have designed novel nanovesicles in which they encapsulated two different enzymes, superoxide dismutase (SOD) and lactoperoxidase (LPO) (Figure 4).[66] Similarly to nature, this nanoreactor was used to combat oxidative stress by reducing in situ superoxide anion radicals present in the environment into molecular oxygen and

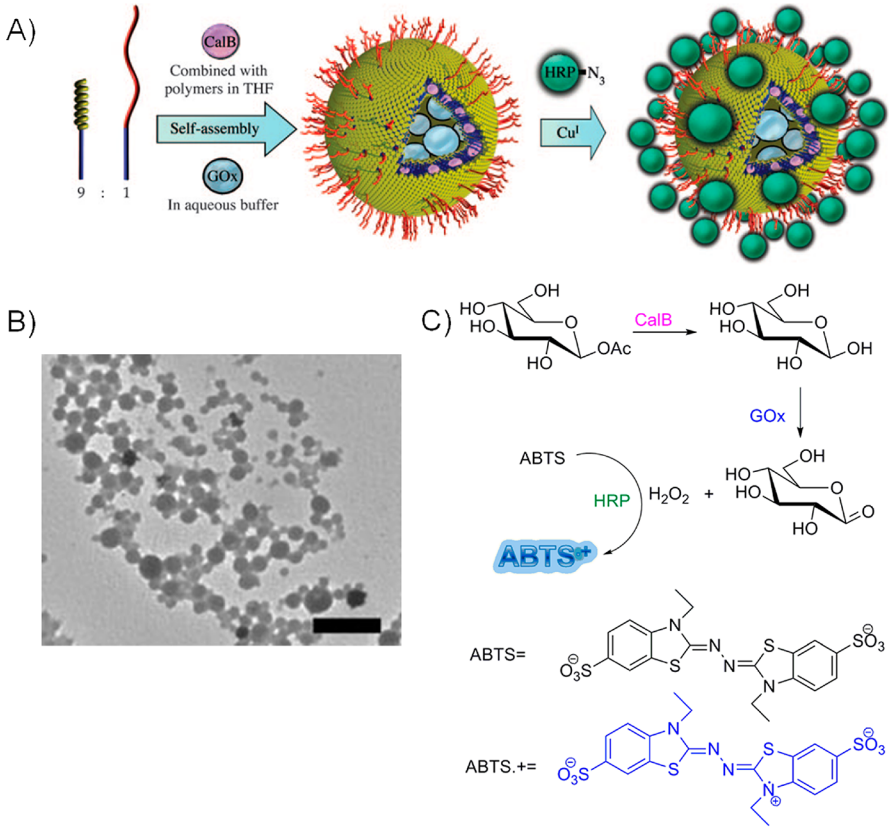

Figure 3. (A) Positional assembly of enzymes in a polymersome.[a] (B) TEM image of the biohybrid polymersomes. The scale bar represents 500 nm. GOx in the lumen, CalB in the membrane, HRP on the surface.[a] (C) Mutienzymatic cascade process. ([a]Reproduced with permission from ref 65.)

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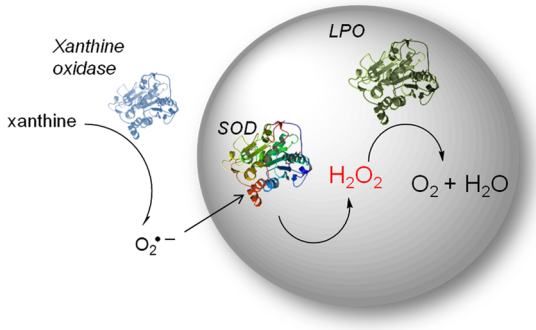


Figure 4. Schematic representation of enzyme cascade reactions inside polymeric nanocontainers to combat oxidative stress.

water via hydrogen peroxide inside the vesicle cavities. The process was performed by a tandem catalysis starting from the creation of the superoxide anion radicals as the source by the known combination of xanthine/xanthine oxidase. The cascade reaction starts with SOD activity (one part of the natural antioxidant defense mechanism) catalyzing the transformations of the superoxide radicals in hydrogen peroxide with a high turnover number. Subsequently, H_2O_2, as the only harmful product of the SOD reaction, is degraded in the second step of the cascade reaction by LPO, producing oxygen and water (Figure 4).

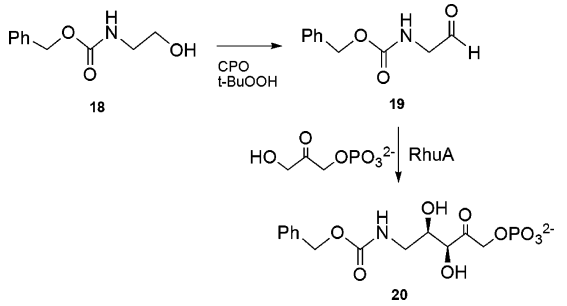
The creation of potential artificial enzymatic cascade reactions has been recently demonstrated by Ward and coworkers.[67] The idea is based on the combination of natural enzymes, or improved evolved variants, with fully compatible artificial designed metalloenzymes (introducing an organometallic compound in a host protein) for concurrent tandem catalysis. An artificial transferhydrogenase (ATHase) was created by incorporation of a d_6-piano stool Ir complex on streptavidin by biotin supramolecular binding (Figure 5).

The generality of the method was tested combining the ATHase with several cofactor-dependent enzymes, resulting in orthogonal redox cascades.[67]

Excellent conversion and enantioselectivities (ee > 99%) were achieved (i) by a three-enzyme cascade reaction combining ATHase with evolved monoamino oxidase (MAO-N) and catalase in a double stereoselective amine deracemization of **11**, **12** or stereoinversion of natural nicotine **13** (Figure 5a,b); (ii) by a four-enzyme tandem catalysis combining ATHase with L-amino acid oxidase (LAAO), D-amino acid oxidase (DAAO), and catalase in the formation of L-pipecolic acid **15** from L-lysine **14** (Figure 5c); and (iii) two-enzyme tandem using 2-hydroxybiphenyl monooxygenase (HbpA) in hydroxylation of 2-hydroxybiphenyl **16** coupled to an ATHase-catalyzed NADH regeneration process (Figure 5d).

Nevertheless, the application of immobilization techniques to create isolated supported enzymes or multienzymatic coimmobilized catalysts has been an advantage over soluble enzymes in enzymatic cascade processes.[68,69] For example, the combination of two different immobilized preparations of chloroperoxidase (CPO) and rhamnulose-1-phosphate aldolase (RhuA) permitted the successful coupling of enzymatic oxidation and aldol addition reactions for the synthesis of a Cbz-aminopolyol **20** from a Cbz-amino alcohol **18** in a multienzymatic one-pot system (Scheme 2).[68] These immobilized systems permitted the synthesis of the product up to 18-fold faster if compared with the use of soluble enzymes.

Scheme 2. Synthesis of Cbz-Aminopolyol 20 from a Cbz-Amino Alcohol 18 in a Multienzymatic One-Pot System



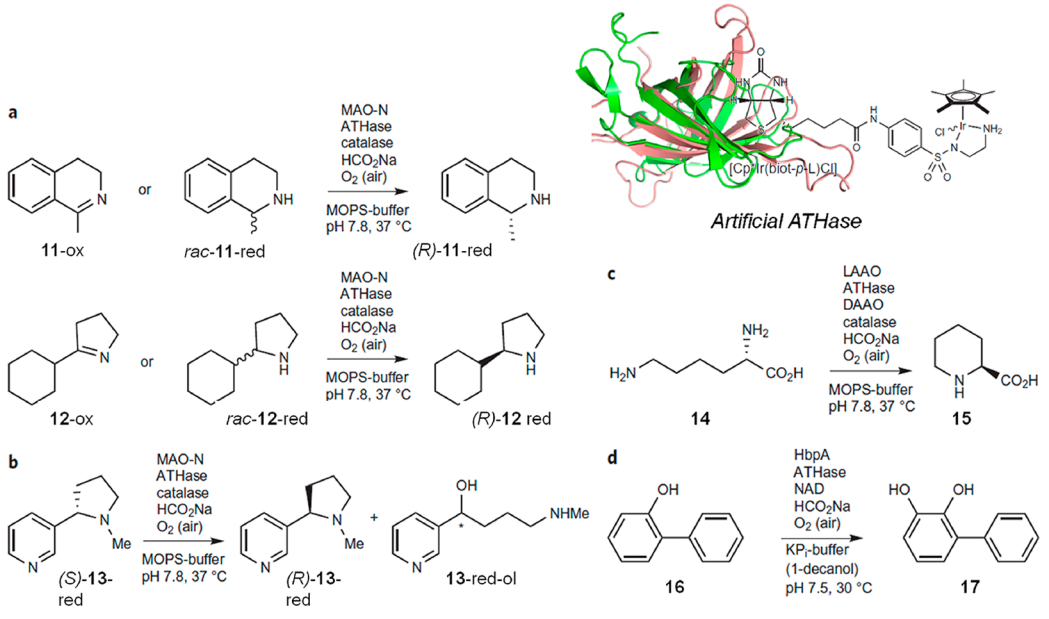


Figure 5. Stereoselective cascade reactions catalyzed by evolved, natural and artificial metalloenzymes. Adapted from ref 67.

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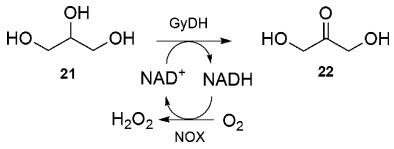
The coimmobilization of several enzymes has been beneficial to perform bioreduction processes.[69] One of the main advantage of this strategy is the cofactor regeneration. For example, this immobilization approach was successfully used in the transformation of glycerol (**21**) in dihydroxyacetone **22** by glycerol dehydrogenase (GlyDH) combined in a cascade with a NADH oxidase (NOX) to regenerate the cofactor necessary for the first enzyme (Scheme 3).

Scheme 3. Multienzymatic One-Pot Synthesis of Dihydroxyacetone 22 Catalyzed by Coimmobilized Biocatalysts



1.3. Metal-Enzyme Cascade Synthesis. The main application of the cascade reactions is complex chemical processes with the aim to produce high value-added products. Generally, to achieve this scope, the most used strategy combines organometallic compounds and enzymes as separate catalysts added on the same reaction medium.[70] In this way, the product of the first catalytic reaction became the substrate for the second reaction, the general development being regulated with a step-by-step mechanism (domino cascade reactions).

Following this strategy, for example, Kim and co-workers reported the "one-pot" synthesis of enantiomerically pure amides by a dynamic kinetic resolution (DKR) of racemic amine catalyzed by Pd nanoparticles entrapped in aluminum hydroxide combined with a lipase (commercially available Novozym435).[71]

The cascade process combined a lipase kinetic resolution of chiral amines by an amidation process and the racemization of the unreacted amine enantiomer by the Pd nanocatalyst. Using benzylamine as the model reaction and ethylmethoxyacetate as acyl donor, the enantiomerically pure amide was obtained in 98% isolated yield with an ee of >99% (Scheme 4).[71]

To expand the scope of the methodology, both catalysts were successfully applied in the DKR of different racemic benzylamines, alkyl-amines, and amino acids, obtaining the corresponding amides in good or excellent yields (85−99%) and high enantiomeric excesses (97−99%).[71] Furthermore, the stability of the Pd nanocatalyst−lipase hybrid system was examined through recycling experiments. After 10 cycles, the hybrid catalysts still showed good activity (89% yields), with only a slight decrease in enantiomeric excess (ee = 92%).

Another interesting application of the combination of these two different catalysts has been shown by Toste and co-workers, in which supramolecular metallic host−guest complexes were used collaboratively with enzymes in different catalytic tandem reactions (Scheme 5a).[72] Supramolecular Au (I) and Ru (II) complexes were prepared by their encapsulation in a tetrahedral Ga_4L_6 (L = *N*,*N*′-bis(2,3-dihydroxybenzoyl)-1,5-diaminonaphthalene) structure (Scheme 5a), providing a well-defined cavity for reaction, good stability at neutral pH in water, and good tolerance by the enzymes.[73]

This system was applied successfully, for example, when substrate **23** was subjected to tandem enzymatic kinetic resolution using Amano lipase and Au complex-mediated cyclization to afford substituted tetrahydrofuran **25** in 33% conversion and 96% ee (Scheme 5b).[72] A second example consisted of the transformation of 1-propenol (**26**) into propanol (**28**) by tandem catalysis via Ru-mediated olefin isomerization to produce propanal **27** and alcohol dehydrogenase (ADH) to reduce the aldehyde to alcohol. A second enzyme, formate dehydrogenase (FDH) using formic acid as the substrate was coupled to the system for ADH cofactor, NADP(H), regeneration (Scheme 5c).

Recently, Prastaro and co-workers combined the use of a protein−Pd NP nanobiohybrids with an enzyme to catalyze a cascade of Suzuki−Miyaura cross-coupling reaction with an asymmetric reduction.[74]

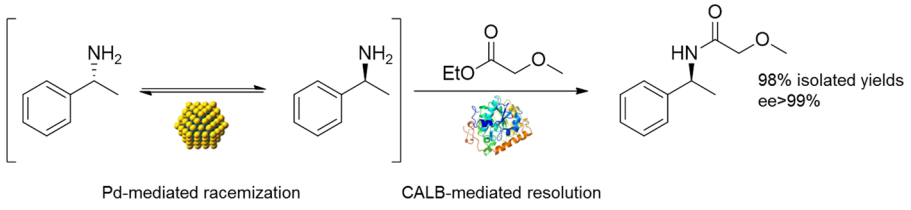
Pd NPs were generated in situ on a biological matrix, a DNA binding protein from starved cells of the thermophilic bacterium *Thermosynechoccus elongatus* (Te-Dps), in the presence of a strong reducing agent ($NaBH_4$) (Figure 6). This Pd NPs/Te-DPs bionanohybrid was successfully used to catalyze the formation of biaryl products from different aryl halides and arylboronic acids.[77] In the case of aryl iodide **29** and aryl boronic acid **30**, the corresponding biaryl **31** was synthesized in 87% yield (Figure 6b).

In this way, this C−C bond formation was followed by an asymmetric reduction of the ketone in **31** by an alcohol dehydrogenase [(*R*)-LBADH] to produce the enantiomerically pure biaryl alcohol **32** in 91% yield and ee >99% in a two-step one-pot domino reaction (Figure 6).[74]

Moving a step over the basic concept of two separated catalyst acting in a "one-pot" tandem fashion, very recently, several research groups have described the fusion of both independent catalysts in a unique hybrid multiactive nanostructure.[35,75−77]

For example, San and co-workers described an elegant methodology to synthesize protein-shelled nanoparticles.[78] Using aminopeptidase from *Streptococcus pneumoniae* (PepA, a tetrahedral protein with a well-defined cavity and four wide channels that ensure the substrate-product exchange with the outside environment) as biotemplate, in the presence of strong reducing agent $NaBH_4$, platinum NPs were synthesized inside the protein cavity starting from a K_2PtCl_4 solution. Both catalytic activities (enzymatic and metallic) were characterized with good results by means of a cascade transformation composed by the first hydrolysis of glutamic acid-*p*-nitroanilide (peptidase activity assay), followed by the reduction of

Scheme 4. Dynamic Kinetic Resolution of Racemic Aryl Amines



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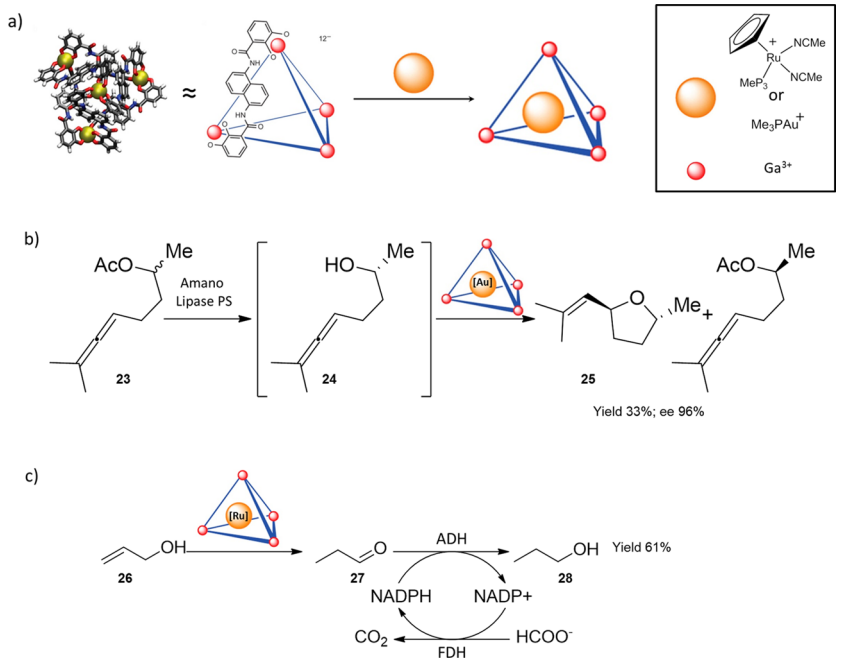
Scheme 5. (a) A Schematic View of the Supramolecular Assembly of Ga_4L_6 Host With Metallic Complexes Guests; (b) Tandem Enzymatic Kinetic Resolution and Cyclization with [Au] Supramolecular Host–Guest Complex; (c) Ru(II)-Mediated Olefin Isomerization of Allyl Alcohol To Give Propanal Followed by Reduction to Propanol via ADH[a]



[a]Adapted from ref 75.

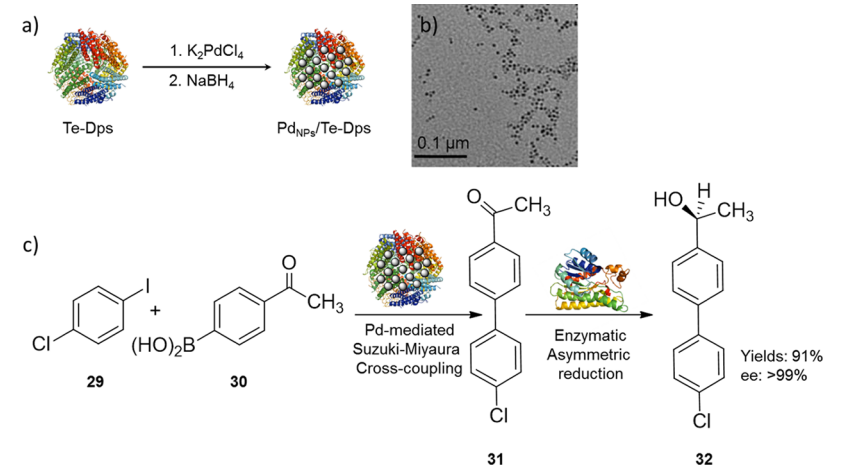


Figure 6. (a) DNA-binding protein (TeDps)-assisted synthesis of Pd nanoparticles. (b)TEM micrograph of generated Te-Dps Pd NPs. (c) One-pot chemoenzymatic synthesis of chiral biaryl alcohols. Adapted from ref 74.

previously released *p*-nitrophenol to *p*-aminophenol (hydrogenation activity assay).[78]

One recently reported alternative consists of the preparation of functionalized metallic NPs for specific adsorption of enzymes.[43,75,77] For example, Ganai et al. described the synthesis of nanoparticles with a core–shell architecture composed by a gold nanoparticle covered by mesoporous silica ($mSiO_2$, MSN). Finally, a glucosidase was grafted onto the outside silica surface (Figure 7).[75] The dual-catalytic performance of this bionanostructure was evaluated in a concurrent cascade transformation (Figure 7c). 4-Nitrophenyl-*β*-glucopyranoside **33** was hydrolyzed by the glucosidase, and in the subsequent step, the 4-nitrophenol **34** released from the first reaction was reduced to the corresponding amine **35** by the gold nanoparticle in the presence of $NaBH_4$. Unfortunately, the recyclability of this hybrid catalyst was found to be poor, with a rapid activity decrease in successive cycles. Nevertheless, this result demonstrates that this system could be very interesting for obtaining several compatible catalytic activities in the same catalyst.

The same approach based on the direct creation of metallic-biologic hybrid nanostructures has been very recently followed by Engström et al. describing the coimmobilization of the lipase from *Candida antarctica* together with palladium NPs (generated by previous chemical reduction) inside the compartments of siliceous mesocellular foams.[79] The catalytic activities of this artificial bionanostructure were characterized by the dynamic kinetic resolution (DKR) of

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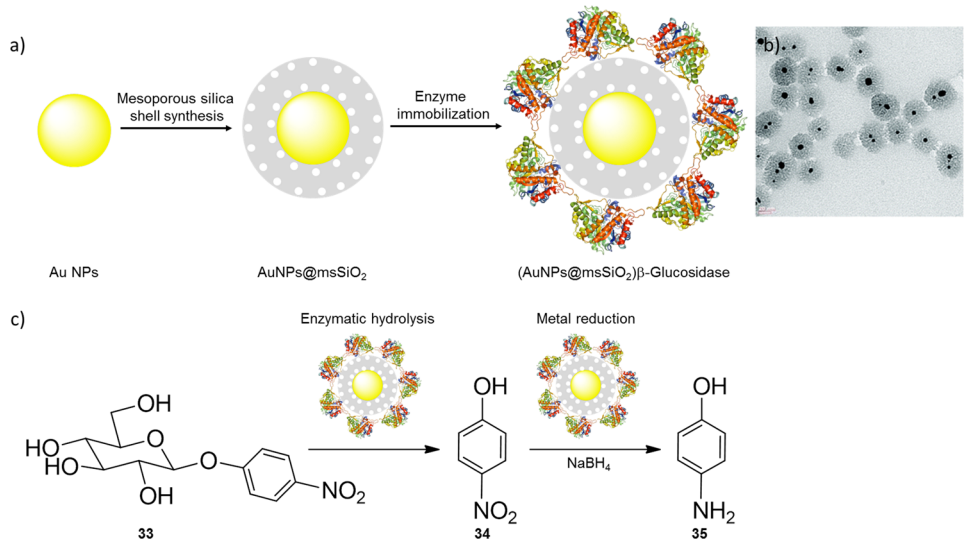
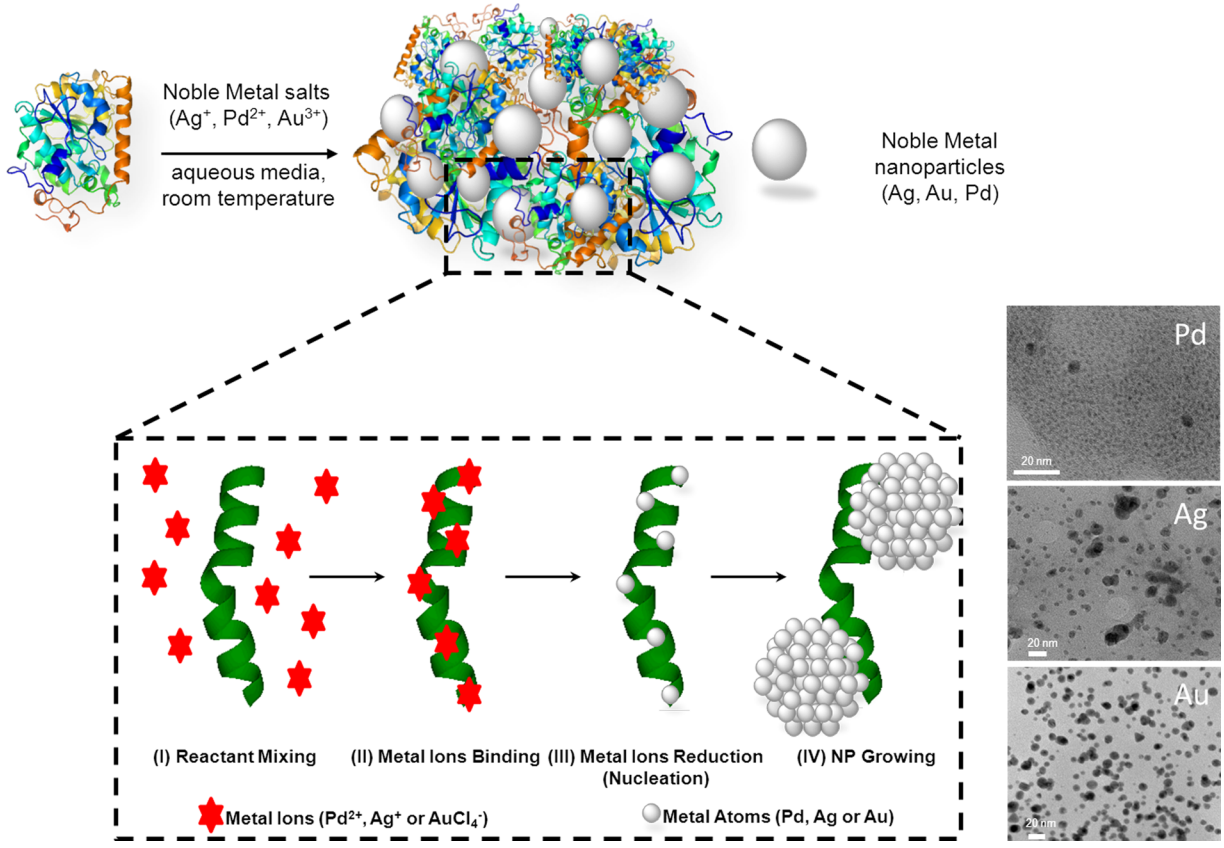


Figure 7. (a) Synthetic procedure for gold nanocrystal/mesoporous silica core−shell nanoparticles with immobilized *β*-glucosidase. (b) TEM micrograph of generated hybrid catalysts. (c) Model domino reaction catalized by the hybrid catalyst to synthesize 4-amino phenol **35**. Adapted from ref 75.

Scheme 6. Schematic Synthesis of the Noble Metal NPs/Enzyme Nanobiohybrids and Their Mechanism of Formation[a]



[a](inset) TEM micrographs of generated nanobiohybrids.

rac-phenylethylamine with excellent results (96% yield, 99% ee). Unfortunately, the recycle studies showed that results from the third cycle were moderate.[79]

Following these advanced research lines, a very interesting strategy affording multivalent hybrid nanocatalysts combining metallic and enzymatic catalytic activities has been recently described.[35] Starting from a homogeneous aqueous solution composed of a noble metal salt (Ag^+, Pd^{2+}, or Au^{3+}) in the presence of a free enzyme (lipase, CALB), a novel type of heterogeneous hybrid nanocatalyst composed by metal NPs embedded in an enzymatic net was generated in situ under very mild reaction conditions (Scheme 6).[35] The use of an enzyme in the methodology permitted generation in situ of small metal NPs (e.g., around 2 nm core size for Pd NPs) without the

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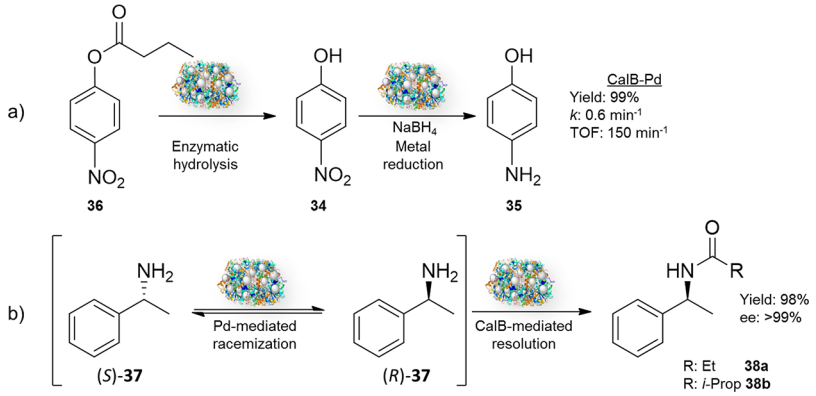
Scheme 7. (a) Model Domino Reaction Catalized by the Nanobiohybrid Catalysts To Synthesize 4-Amino Phenol 35, (b) Dynamic Kinetic Resolution of Racemic Aryl Amines



necessity of any external reducing agent, exploiting the reductive ability of the biomacromolecule (biomineralization), which moreover remains catalytically active at the end of the synthesis.

The catalytic potential of these heterogeneous nanobiohybrids was successfully applied in different reactions: hydrolysis; reduction; C−C bond formation; and, especially interesting, for successful one-pot cascade reactions selectively exploiting the catalytic activity of the metal, the enzyme, or both at the same time (domino and tandem reactions).[35] The model "one-pot" synthesis of aryl amines starting from 4-nitrophenyl esters was chosen as a representative example of a domino reaction to evaluate the catalytic capacity of the novel metallic NP−enzyme biohybrid (Scheme 7a). The lipase (CalB)-mediated hydrolysis of butyryl ester **36** to produce 4-nitrophenol **35** was reduced by the metalNPs to aminophenol **35** in the presence of $NaBH_4$. The results were very good or excellent with all the nanobiohybrids tested achieving suitable values of reaction rate constant (k) and TOF of 0.6 min^{-1} and almost 150 min^{-1}, respectively, using the lipase−Pd NP biohybrid.[35]

To further expand the scope of this novel strategy, the biohybrid Pd-nanocatalyst was evaluated in the dynamic kinetic resolution (DKR) of *rac*-phenylethylamine in organic medium, a tandem catalytic process (both enzymatic and Pd catalysis acting at the same time) (Scheme 7b). After a fine optimization of many reaction parameters, the quantitative formation of enantiopure (*R*)-benzylamide **38** with ee > 99% was achieved. These good results have been maintained, even after 3 reaction cycles (96% yields >99% ee), demonstrating the high operational stability and recyclability of this hybrid bionanostructure.[35] This interesting strategy result is noteworthy from different points of view (i.e., ease of method, mild reaction conditions, or variability of enzymes and metal) but mainly because, in this way, it is possible to generate a unique multivalent hybrid catalyst that is useful in a wide set of organic reactions and whose activity can be finely programmed, depending on the process requirement (use of each catalytic activity for a separate reaction or its complete multivalent activity in a domino cascade or tandem fashion).

Another very interesting approach to develop catalytic entities expecting the use of the potential of both biocatalysis and transition metal catalysis has been reported by Foulkes et al.[60] This strategy describes the use of engineered *Escherichia coli* bacteria to simultaneously intracellularly express monoamine oxidase (mao-N-D5 insert) and bind Pd nanoparticles on the outer membrane, finally creating a unique biometallic hybrid catalyst. The overall activity of this interesting bionanostructure has been successfully evaluated in the amine deracemization of racemic 1-methyltetrahydroisoquinoline (**39**) to (*R*)-**39** via the intermediate 1-methyl-3,4-dihydroisoquinoline **40**, with an enantiomeric excess of up to 96% (Figure 8).[60] Furthermore,

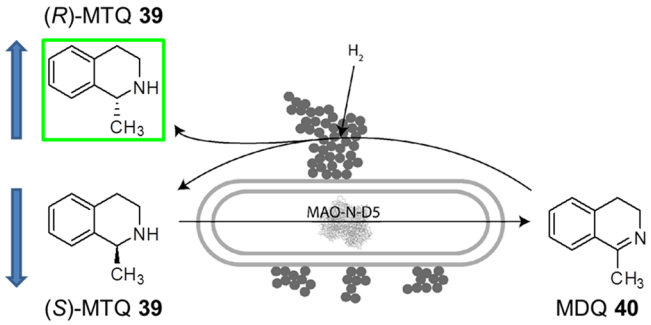


Figure 8. The cyclic deracemization of **39** via the imine **40** using the hybrid catalyst composed by the engineering inserted mao-N-D5 gene and Pd NPs bound on the outer membrane. Adapted from ref 60.

these good results were maintained for five subsequent reaction cycles, demonstrating in this way the high stability of this hybrid bionanostructure.

2. OTHER APPLICATION FIELDS OF CASCADE REACTION

2.1. Biosensor Design. In addition to its valuable application in organic and fine chemistry, the usefulness of cascade reactions (based on mixed chemo- and enzymatic catalyst as well as completely based on enzymatic catalytic cascade) has also been demonstrated in many other research fields, such as biomedical applications (diagnosis and drug delivery) or biosensing.[80−83] For example, among the wide set of applied reactions, the classic enzymatic cascade catalyzed by glucose oxidase (GOx) and horseradish peroxidase (HRP) has been thoroughly studied and widely used in glucose detection (e.g., glucometer).[84] However, it has been demonstrated that signal amplification by enzymatic reactions is not sufficiently high to achieve ultrasensitive detection of biomolecules, which is essential for the early and rapid diagnosis of diseases.[84] To overcome this limitation, enzymatic reactions have been efficiently combined with an additional cascade amplification process (i.e., redox cycling).[85] For example, Han and co-workers recently described a novel signal amplification strategy for

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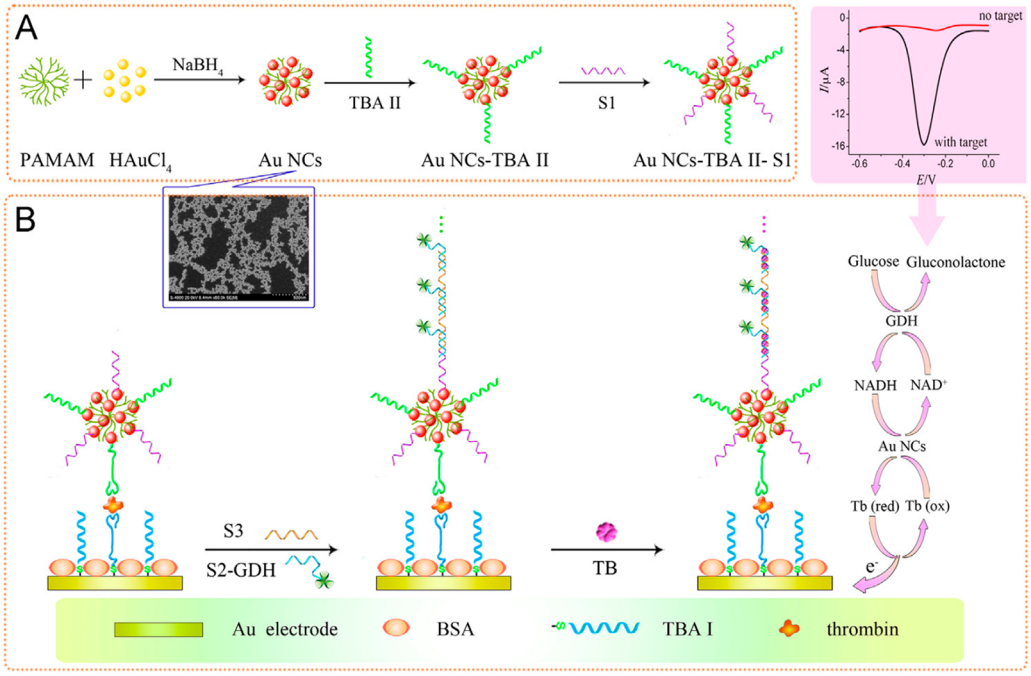

Figure 9. (A) Preparation procedure of AuNCs−TBA II−S1 bioconjugate; (B) schematic illustration of the electrochemical aptasensor for detection of thrombin based on cascade catalysis of AuNCs and GDH. BSA, bovine serum albumin; TBA, thrombin binding aptamer; PAMAM, polyamidoamine dendrimer; S1, initiator strand; TB, toluidine B; GDH, glucose dehydrogenase. Reproduced with permission from ref 86.

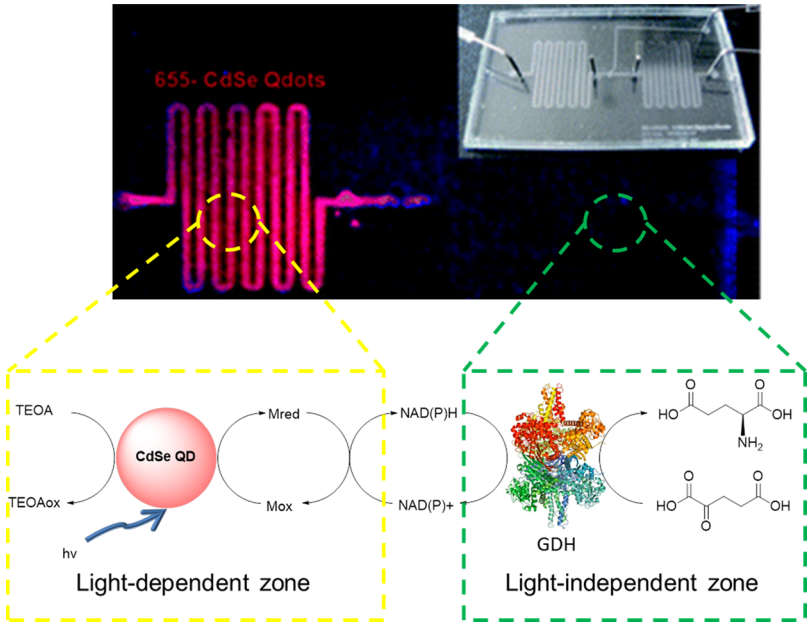

Figure 10. Microfluidic artificial photosynthesis system. The microfluidic platform incorporates CdSe quantum dots (QDs) and glutamate dehydrogenase (GDH) within the separate microchannel zones. The light-driven nicotinamide cofactor (NADH) regeneration takes place in the light-dependent reaction zone, which is then coupled with the light-independent enzymatic synthesis of L-glutamate in the downstream of the microchannel. Adapted from ref 91.

ultrasensitive detection of thrombin by cascade catalysis of gold nanoclusters (AuNCs) and glucose dehydrogenase (GDH).[86] With this strategy, the AuNCs were constructed not only as nanocarriers for anchoring the large amounts of secondary thrombin aptamers but also as nanocatalysts, promoting the efficient oxidation of NADH efficiently (Figure 9). Moreover, a large amount of GDH was loaded through the immobilization technology of DNA hybridization, and a large amount of toluidine blue (Tb, as redox probe) was intercalated into the DNA grooves via electrostatic interaction. In this way, the electrochemical signal was greatly enhanced by means of cascade catalysis: first, GDH catalyzed the oxidation of glucose to gluconolactone with the concomitant generation of NADH in the presence of NAD^+. Then, AuNCs catalyze the oxidation of NADH to NAD^+, reducing the Tb and generating a free electron (Figure 9). Under the optimal conditions, the proposed aptasensor exhibited a high sensitivity (low femtomolar detection) and good specificity for thrombin detection.[86]

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2.2. Energy Storage and Conversion. Undoubtedly, photosynthesis represents one of the most powerful and known examples of natural energy storage and conversion of solar energy into renewable resources.[87] During this process in green plants, light-dependent and light-independent reactions take place simultaneously in the organelle of micrometer-sized chloroplasts that contain light-harvesting thylakoid membranes.[88] Thus, many efforts have been made to clarify the mechanism of photosynthesis and to further mimic such natural process.[89,90]

With that in mind, for example, Lee and co-workers reported the development of a microfluidic artificial photosynthetic cascade system for in situ regeneration of reducing power (i.e., NADH cofactor) and redox enzymatic synthesis of fine chemicals under visible light.[91]

Microchannels were synthesized by using the imprint lithography technology, and subsequently, a light-harvesting photosensitizer (CdSe quantum Dots) and a redox enzyme (Glutamate dehydrogenase, GDH) were in situ immobilized in different compartments of such microchannels (Figure 10). Hence, this interesting example of cascade reactions results constituted by a light-dependent zone where CdSe QDs regenerate NADH recurring to triethanolamine (TEOA) and pentamethyl cyclopentadienyl rhodium bipyridine (M) as a sacrificial electron donor and electron mediator, respectively. The NADH cofactor is subsequently consumed by GDH in the light-independent compartment for the redox enzymatic synthesis of L-glutamate starting from α-ketoglutarate (Figure 10). Therefore, this system results in a successful, sustainable, recyclable, and integrated light-harvesting mimetic of the natural photosynthetic machinery.[91]

3. CONCLUSIONS

Catalytic cascade reactions have become one of the most active research areas in organic synthesis. In particular, the application of bionanostructures—nanostructures, and biomolecules combined in different manners—as novel nanocatalysts in these types of reactions has been reviewed in this work. Undoubtedly, this is a very recent research area whose general interest is undergoing impressive growth.

The recent explosion of nanotechnologies in catalysis together with the recent advances in protein chemistry (such as the creation of semisynthetic proteins, expanding the catalytic properties of enzymes toward nonnatural substrates)[35,70,92] have made possible this new approach in cascade reactions.

In this way, the fusion of these two worlds becomes a priority of many research groups. Because of this integration between nano- and biocatalytic structures, we discussed in this work several efficient and interesting cascade processes that have been developed and applied in many different and impacting areas, such as organic chemistry, ultrasensitive biosensing, or energy storage and conversion.

Moreover, in addition to their usefulness, with the representative examples here described, we have tried to demonstrate the tremendous potential underscoring this novel approach. In fact, each nanostructure here described (metallic as well as bio) has been selectively and efficiently used alone or in cooperative activity in cascade catalysis. Therefore, we envision that this method could provide a major advance in terms of the development of new and more efficient catalysts opening a new way to rationally exploit the advantages offered at nanoscale by the combination of organometallic chemistry and biocatalysis.

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Notes

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REFERENCES

(1) Ueberbacher, B. T.; Hall, M.; Faber, K. *Nat. Prod. Rep.* **2012**, *29*, 337−350.
(2) Vilotijevic, I.; Jamison, T. F. *Angew. Chem., Int. Ed.* **2009**, *48*, 5250−5281.
(3) Carlson, J. C.; Li, S.; Gunatilleke, S. S.; Anzai, Y.; Burr, D. A.; Podust, L. M.; Sherman, D. H. *Nat. Chem.* **2011**, *3*, 628−633.
(4) Grondal, C.; Jeanty, M.; Enders, D. *Nat. Chem.* **2010**, *2*, 167−178.
(5) Climent, M.; Corma, A.; Iborra, S.; Mifsud, M. *J. Catal.* **2007**, *247*, 223−230.
(6) Nicolaou, K. C.; Chen, J. S. *Chem. Soc. Rev.* **2009**, *38*, 2993−3009.
(7) Peters, R. J. R. W.; Louzao, I.; van Hest, J. C. M. *Chem. Sci.* **2012**, *3*, 335−342.
(8) Vriezema, D. M.; Aragones, M. C.; Elemans, J.; Cornelissen, J.; Rowan, A. E.; Nolte, R. J. M. *Chem. Rev.* **2005**, *105*, 1445−1489.
(9) Zhao, C.; Lercher, J. A. *Angew. Chem., Int. Ed.* **2012**, *51*, 5935−5940.
(10) Aleman, J.; Cabrera, S. *Chem. Soc. Rev.* **2013**, *42*, 774−793.
(11) Rong, H.; Cai, S.; Niu, Z.; Li, Y. *ACS Catal.* **2013**, *3*, 1560−1563.
(12) Shiroodi, R. K.; Gevorgyan, V. *Chem. Soc. Rev.* **2013**, *42*, 4991−5001.
(13) Zhang, Q.; Cai, S.; Li, L.; Chen, Y.; Rong, H.; Niu, Z.; Liu, J.; He, W.; Li, Y. *ACS Catal.* **2013**, *3*, 1681−1684.
(14) Padwa, A. *Chem. Soc. Rev.* **2009**, *38*, 3072−3081.
(15) Dang, T. T.; Zhu, Y.; Ngiam, J. S. Y.; Ghosh, S. C.; Chen, A.; Seayad, A. M. *ACS Catal.* **2013**, *3*, 1406−1410.
(16) Reezt, M. T. *J. Am. Chem. Soc.* **2013**, *135*, 12480−12496.
(17) Shylesh, S.; Wagener, A.; Seifert, A.; Ernst, S.; Thiel, W. R. *Angew. Chem., Int. Ed.* **2010**, *49*, 184−187.
(18) Katz, A.; Davis, M. E. *Nature* **2000**, *403*, 286−289.
(19) Phan, N. T. S.; Gill, C. S.; Nguyen, J. V.; Zhang, Z. J.; Jones, C. W. *Angew. Chem., Int. Ed.* **2006**, *45*, 2209−2212.
(20) Chang, L. L.; Erathodiyil, N.; Ying, J. Y. *Acc. Chem. Res.* **2013**, *46*, 1825−1837.
(21) Chepulskii, R. V.; Curtarolo, S. *ACS Nano* **2011**, *5*, 247−254.
(22) Häkkinen, H. *Nat. Chem.* **2012**, *4*, 443−455.
(23) Ranganath, K. V. S.; Kloesges, J.; Schfer, A. H.; Glorius, F. *Angew. Chem., Int. Ed.* **2010**, *49*, 7786−7789.
(24) Cantillo, D.; Baghbanzadeh, M.; Kappe, C. O. *Angew. Chem., Int. Ed.* **2012**, *51*, 10190−10193.
(25) Hebbalalu, D.; Lalley, J.; Nadagouda, M. N.; Varma, R. S. *ACS Sustainable Chem. Eng.* **2013**, *1*, 703−712.
(26) Holmberg, R. J.; Aharen, T.; Murugesu, M. *J. Phys. Chem. Lett.* **2012**, *3*, 3721−3733.
(27) Chaudhuri, R. G.; Paria, S. *Chem. Rev.* **2012**, *112*, 2373−2433.
(28) Shiraishi, Y.; Fujiwara, K.; Sugano, Y.; Ichikawa, S.; Hirai, T. *ACS Catal.* **2013**, *3*, 312−320.
(29) Li, Z.; Liu, J.; Huang, Z.; Yang, Y.; Xia, C.; Li, F. *ACS Catal.* **2013**, *3*, 839−845.

(30) Yuan, Y.; Yan, N.; Dyson, P. J. *ACS Catal.* **2012**, *2*, 1057−1106.
(31) Levi, N.; Neumann, R. *ACS Catal.* **2013**, *3*, 1915−1918.
(32) Song, C.; Wang, Y.; Rosi, N. L. *Angew. Chem., Int. Ed.* **2013**, *52*, 3993−3995.
(33) Dickerson, M. B.; Sandhage, K. H.; Naik, R. R. *Chem. Rev.* **2008**, *108*, 4935−4978.
(34) Ge, J.; Lei, J.; Zare, R. N. *Nat. Nanotechnol.* **2012**, *7*, 428−432.
(35) Filice, M.; Marciello, M.; Morales, M. P.; Palomo, J. M. *Chem. Commun.* **2013**, *49*, 6876−6878.
(36) Bu, X.; Zhou, Y.; He, M.; Chen, Z.; Zhang, T. *Dalton Trans.* **2013**, *42*, 15411−15420.
(37) Baksi, A.; Xavier, P. L.; Chaudhari, K.; Goswami, N.; Pal, S. K.; Pradeep, T. *Nanoscale* **2013**, *5*, 2009−2016.
(38) Samanta, D.; Sawoo, S.; Sarkar, A. *Chem. Commun.* **2006**, *32*, 3438−3440.
(39) Sanpui, P.; Pandey, S. B.; Sankar Ghosh, S.; Chattopadhyay, A. *J. Colloid Interface Sci.* **2008**, *326*, 129−137.
(40) Forbes, L. M.; Goodwin, A. P.; Cha, J. N. *Chem. Mater.* **2010**, *22*, 6524−6528.
(41) Chiu, C.-Y.; Li, Y.; Ruan, L.; Ye, X.; Murray, C. B.; Huang, Y. *Nat. Chem.* **2011**, *3*, 393−399.
(42) Willner, I.; Baron, R.; Willner, B. *Adv. Mater.* **2006**, *18*, 1109−1120.
(43) Marciello, M.; Bolivar, J. M.; Filice, M.; Mateo, C.; Guisan, J. M. *Biomacromolecules* **2013**, *14*, 602−607.
(44) Yusdy; Patel, S. R.; Yap, M. G. S.; Wang, D. I. C. *Biochem. Eng. J.* **2009**, *48*, 13−21.
(45) Zhang, B.; Liu, B.; Zhou, J.; Tang, J.; Tang, D. *ACS Appl. Mater. Interfaces* **2013**, *5*, 4479−4485.
(46) Cong, H.; Porco, J. A. *ACS Catal.* **2012**, *2*, 65−70.
(47) Sreedhar, B.; Reddy, P. S.; Devi, D. K. *J. Org. Chem.* **2009**, *74*, 8806−8809.
(48) Khazaei, A.; Rahmati, S.; Saednia, S. *Catal.Commun.* **2013**, *37*, 9−13.
(49) Wu, S.; Ma, H. C.; Jia, X. J.; Zhong, Y. M.; Lei, Z. Q. *Tetrahedron* **2011**, *67*, 250−261.
(50) Ma, H.-c.; Cao, W.; Bao, Z.-k.; Lei, Z.-Q. *Catal. Sci. Technol.* **2012**, *2*, 2291−2296.
(51) Kundu, S.; Jayachandran, M. *RSC Adv.* **2013**, *3*, 16486−16498.
(52) Lee, K. Y.; Hwang, J.; Lee, Y. W.; Kim, J.; Han, S. W. J. *Colloid Interface Sci.* **2007**, *316*, 476−481.
(53) Bai, T.; Gao, Y. A.; Liu, H. G.; Zheng, L. Q. *J. Phys. C* **2009**, *113*, 17730−17736.
(54) Huang, D.; Bai, X.; Zheng, L. *J. Phys. Chem. C* **2011**, *115*, 14641−14647.
(55) Stodulski, M.; Gulder, T. *Angew. Chem., Int. Ed.* **2012**, *51*, 11202−11204.
(56) (a) Coppage, R.; Slocik, J. M.; Ramezani-Dakhel, H.; Bedford, N. M.; Heinz, H.; Naik, R. R.; Knecht, M. R. *J. Am. Chem. Soc.* **2013**, *135*, 11048−11054. (b) Yue, W.; Lv, A.; Gao, J.; Jiang, W.; Hao, L.; Li, C.; Li, Y.; Polander, L. E.; Barlow, S.; Hu, W.; Di Motta, S.; Negri, F.; Marder, S. R.; Wang, Z. *J. Am. Chem. Soc.* **2012**, *134*, 5770−5773. (c) Zahran, E. M.; Bedford, N. M.; Nguyen, M. A.; Chang, Y.-J.; Guiton, B. S.; Naik, R. R.; Bachas, L. G.; Knecht, M. R. *J. Am. Chem. Soc.* **2014**, *136*, 32−35.
(57) Zaramella, D.; Scrimin, P.; Prins, L. J. *J. Am. Chem. Soc.* **2012**, *134*, 8396−8399.
(58) Jin, Z.; Xiao, M.; Bao, Z.; Wang, P.; Wang, J. *Angew. Chem., Int. Ed.* **2012**, *51*, 6406−6410.
(59) Mateo, C.; Palomo, J. M.; Fernandez-Lorente, G.; Guisan, J. M.; Fernandez-Lafuente, R. *Enzyme Microb. Technol.* **2007**, *40*, 1451−1463.
(60) Foulkes, J. M.; Malone, K. J.; Coker, V. S.; Turner, N. J.; Lloyd, J. R. *ACS Catal.* **2011**, *1*, 1589−1594.
(61) Yusop, R. M.; Unciti-Broceta, A.; Johansson, E. M. V.; Sanchez-Martin, R. M.; Bradley, M. *Nat. Chem.* **2011**, *3*, 239−243.
(62) Wasilke, J.-C.; Obrey, S. J.; Baker, R. T.; Bazan, G. C. *Chem. Rev.* **2005**, *105*, 1001−1020.
(63) Schoffelen, S.; Van Hest, J. C. M. *Curr. Opin. Struct. Biol.* **2013**, *23*, 613−621.
(64) Idan, O.; Hess, H. *Curr. Opin. Biotechnol.* **2013**, *24*, 606−611.
(65) van Dongen, S. F. M.; Nallani, M.; Cornelissen, J. J. L. M.; Nolte, R.J. M.; van Hest, J. C. M. *Chem.—Eur. J.* **2009**, *15*, 1107−1114.
(66) Tanner, P.; Onaca, O.; Balasubramanian, V.; Meier, W.; Palivan, C. G. *Chem.—Eur. J.* **2011**, *17*, 4552−4560.
(67) Kohler, V.; Wilson, Y. M.; Durrenberger, M.; Ghislieri, D.; Churakova, E.; Quinto1, T.; Knorr, L.; Haussinger, D.; Hollmann, F.; Turner, N. J.; Ward, T. R. *Nat. Chem.* **2013**, *5*, 93−99.
(68) Pesic, M.; Lopez, C.; Lopez-Santin, J.; Alvaro, G. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 7173−7183.
(69) Rocha-Martin, J.; de las Rivas, B.; Muñoz, R.; Guisan, J. M.; Lopez-Gallego, F. *ChemCatChem.* **2012**, *4*, 1279−1288.
(70) Hoyos, P.; Pace, V.; Alcantara, A. R. *Adv. Synth. Catal.* **2012**, *354*, 2585−2611.
(71) Kim, M.-J.; Kim, W.-H.; Han, K.; Choi, Y. K.; Park, J. *Org. Lett.* **2007**, *6*, 1157−1159.
(72) Wang, Z. J.; Clary, K. N.; Bergman, R. G.; Raymond, K. N.; Toste, F. D. *Nat. Chem.* **2013**, *5*, 100−103.
(73) Wang, Z. J.; Casey, C. J.; Bergman, R. G.; Raymond, K. N.; Toste, F. D. *J. Am. Chem. Soc.* **2011**, *133*, 7358−7360.
(74) Prastaro, A.; Ceci, P.; Chiancone, E.; Boffi, A.; Cirilli, R.; Colone, M.; Fabrizi, G.; Stringaro, A.; Cacchi, S. *Green Chem.* **2009**, *11*, 1929−1932.
(75) Ganai, K. R.; Shinde, P.; Dhar, B. B.; Gupta, S. S.; Prasad, B. L. V. *RSC Adv.* **2013**, *3*, 2186−2191.
(76) Margelefsky, E. L.; Zeidan, R. K.; Davis, M. E. *Chem. Soc. Rev.* **2008**, *37*, 1118−1126.
(77) Huang, Y.; Xu, S.; Lin, V. S. Y. *Angew. Chem., Int. Ed.* **2011**, *50*, 661−664.
(78) San, B. H.; Kim, S.; Moh, S. H.; Lee, H.; Jung, D. Y.; Kim, K. K. *Angew. Chem., Int. Ed.* **2011**, *50*, 11924−11929.
(79) Engström, K.; Johnston, E. V.; Verho, O.; Gustafson, K. P.; Shakeri, M.; Tai, C. W.; Bäckvall, J. E. *Angew. Chem. Int. Ed.* **2013**, *52*, 14006−14010.
(80) Song, S. P.; Qin, Y.; He, Y.; Huang, Q.; Fan, C. H.; Chen, H. Y. *Chem. Soc. Rev.* **2010**, *39*, 4234−4243.
(81) Pei, X.; Zhang, B.; Tang, J.; Liu, B.; Lai, W.; Tang, D. *Anal. Chim. Acta* **2013**, *758*, 1−18.
(82) Rotello, V. M.; Ghosh, P.; Han, G.; De, M.; Kim, C. K. *Adv. Drug. Delivery Rev.* **2008**, *60*, 1307−1315.
(83) Saha, K.; Agasti, S. S.; Kim, C.; Li, X.; Rotello, V. M. *Chem. Rev.* **2012**, *112*, 2739−2779.
(84) Yang, H. *Curr. Opin. Chem. Biol.* **2012**, *16*, 422−428.
(85) Enustun, B. V.; Turkevich, J. *J. Am. Chem. Soc.* **1963**, *85*, 3317−3328.
(86) Han, J.; Zhuo, Y.; Chai, Y.; Gui, G.; Zhao, M.; Zhu, Q.; Yuan, R. *Biosens. Bioelectron.* **2013**, *50*, 161−166.
(87) Gust, D.; Moore, T. A.; Moore, A. L. *Acc. Chem. Res.* **2009**, *42*, 1890−1898.
(88) Berg, J. M.; Tymoczko, J. L.; Stryer, L. *Biochemistry*, 6th ed.; W. H. Freeman: New York, 2007; pp 541−551.
(89) Lee, S. H.; Nam, D. H.; Kim, J. H.; Baeg, J.-O.; Park, C. B. *ChemBioChem* **2009**, *10*, 1621−1624.
(90) Ryu, J.; Lee, S. H.; Nam, D. H.; Park, C. B. *Adv. Mater.* **2011**, *23*, 1883−1888.
(91) Lee, J. S.; Lee, S. H.; Kim, J. H.; Park, C. B. *Lab Chip* **2011**, *11*, 2309−2311.
(92) Palomo, J. M. *Org. Biomol. Chem.* **2012**, *10*, 9309−9318.