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Pd nanoparticles-Polyethylenemine-Lipase Bionanohybrids as heterogeneous catalysts for selective oxidation of aromatic alcohols

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Abstract

A new kind of bionanohybrids, synthesized by using 63 kDa-lipase from *Candida rugosa* (CRL) in the presence of polyethyleneimine (PEI) by *in situ* formation of Pd (0) nanoparticles (PdNPs) in aqueous media at room temperature were obtained. Addition of Triton X-100 as additive was also used. XRD confirmed the formation of crystalline Pd (0) as metal species. TEM analyses revealed PdNPs ranging from 6 to 8 nm, whereas using Triton X-100 the size range of the nanoparticles was 9 to 24 nm (mainly being 13 nm). PdNPs of biohybrid created only with Triton X-100 ranged from 8 to 21 nm. The different heterogeneous nanobiohybrids were applied as catalyst in the oxidation of benzyl alcohol under mild conditions. In all cases, they were selective to the synthesis of alcohol to benzaldehyde without traces of carboxylic acid. More than >99% conversion was obtained in THF at 50ºC under air as oxidant. The addition of hydrogen peroxide improved the conversion initially but the reaction was stopped at around 50-68% conversion after 24 h in the best of cases. These new nanocatalysts synthesized using PEI showed a very high recyclability, maintaining >95% activity after 5 cycles of use. Also successful results were obtained in the oxidation of other aromatic primary and secondary alcohols. The new PEI-nanobiohybrids conserved the enzymatic activity and showed excellent results in metallic-enzymatic domino reaction.

Keywords: nanohybrid, metal nanoparticles, selective oxidation, heterogeneous catalysis

Introduction

Selective oxidation of alcohols to more valuable aldehydes is one of the most significant transformations in chemical industry [1-2].

However, many of them are synthesized using expensive and toxic oxidants, base additives or high experimental temperatures (80ºC) [3-5]. Recently the application of more sustainable oxidants such as oxygen or hydrogen peroxide has been successfully applied [6-7]. However, for an economically and environmental-industrial application process, the required high oxygen pressure increases the system's complexity.

Therefore, the development of sustainable catalysts, which can perform this reaction at moderate temperature, free of additives or solvents, are extremely required.

In this term, the application of nanotechnology has been one of the last tools applied for obtaining selective oxidation processes [8-12].

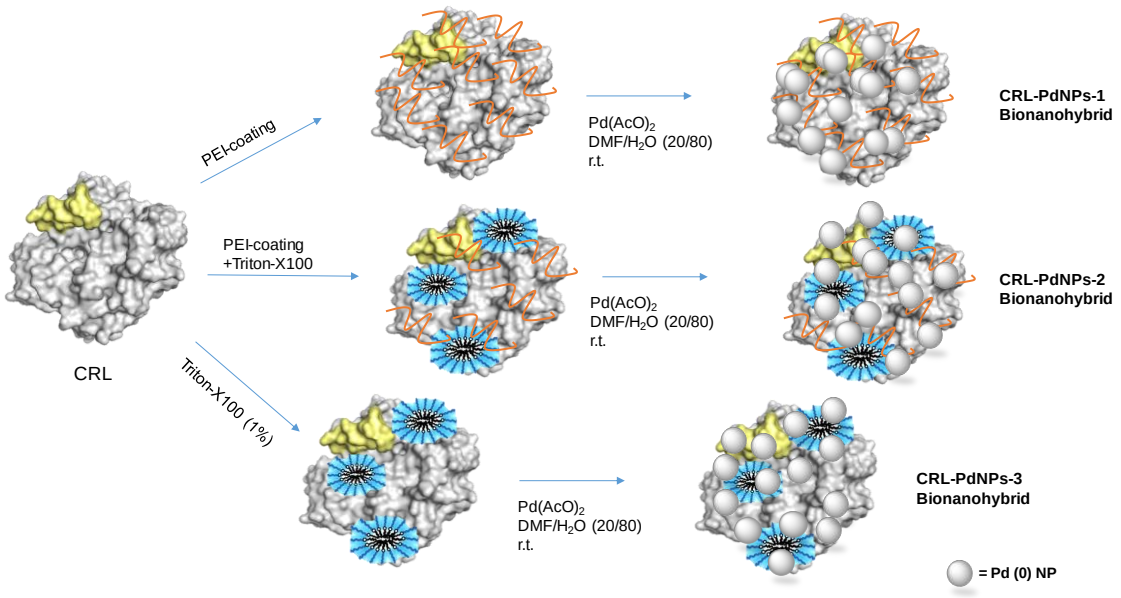
The application of metal nanoparticles as catalyst presents important advantages compared to the bulk material, such as the large surface-to-volume ratio [13].

In particular, palladium nanoparticles are one of the most successfully applied in catalysis [14-16], specially on alcohol selective oxidation [17-21]. The synthesis of small size Pd nanoparticles have been performed by different methodologies. In some cases, these approaches involve very harsh conditions, high temperature and pressure, the use of highly flammable toxic organic solvents and the generation of hazardous by-products [22]. The application of biological methods has represented a green alternative. Biomolecules can be specifically selected to recognize a chosen surface through a biomimetic molecular evolution process, and have potential in the generalization of the nanomaterial synthesis process. For example, RNA

sequences or polypeptides were discovered to mediate the formation of nanoparticles by using a reducing agent (typically ascorbic acid or sodium borohydride) [23-24].

Biosynthesis using microorganisms or proteins has also been developed [25-29] in the latter case generating monodispersed nanoparticles, avoiding aggregation phenomenon [27-29]. Among all the biomacromolecules, enzymes are natural nanodimentional protein catalysts (up to 10^{17}-fold rate accelerations) that can catalyze a wide set of reactions with exquisite control of regio and stereochemistry.

Herein, we describe the synthesis of small-size palladium (0) nanoparticles induced by the lipase from *Candida rugosa* (CRL) –an enzyme widely used in selective biotransformations [30]- generating the so-called CRL-PdNPs bionanohybrids (Scheme 1). This lipase presents a 63kDa size with a relative low stability. In this way, different strategies in order to conserve the enzyme stability by coating with a polymer, or maintaining the open conformation of the enzyme by the presence of Triton-X100 [31-32] was used for the first time for preparing the bionanohybrids. These new heterogeneous catalysts were successfully applied for the selective oxidation of benzyl alcohol and other aromatic alcohols.



Scheme 1. Illustration of the synthesis of the different CRL-PdNPs bionanohybrids

Experimental section

General. *C. rugosa* lipase, polyethylenimine Mw 800 (PEI), Triton X-100, palladium acetate, benzyl alcohol, benzaldehyde, p-methoxybenzyl alcohol, p-methoxybenzaldehyde, p-nitrobenzyl-alcohol, p-nitrophenyl propionate, p-nitrophenol, p-aminophenol, THF, DMSO, toluene, acetophenone, (R/S), (R) and (S)-phenyl ethanol were from Sigma-Aldrich. Inductively coupled plasma atomic emission spectrometry (ICP-OES) was performed on a Perkin Elmer OPTIMA 2100 DV equipment. A non-monochromatic magnesium X-ray source with a power of 200 W and voltage of 12 kV was used. The X-Ray diffraction (XRD) pattern was obtained using a Texture Analysis Diffractometer D8 Advance (Bruker) with Cu Kα radiation. The transmission electron microscopy (TEM), high resolution TEM microscopy (HRTEM) were performed on a JEOL 2100F microscope equipped with an EDX detector INCA x-sight (Oxford Instruments). The scanning electron microscopy (SEM) imaging was performed on a TM-1000 (Hitachi) microscope. The spectrophotometric analyses were run on a V-730 spectrophotometer (JASCO, Japan). HPLC spectrum P100 (Thermo Separation products) was used. Analyses were run at 25°C using an L-7300 column oven and a UV6000LP detector. Column chromatography was carried out on silica gel (Silica gel 60, from Merck, Germany). TLC analysis was performed on Merck silica gel 60 F_{254}. The CRL structure analysis was performed using the PyMOL (DeLano Scientific) software.

General Synthesis of CRL-PdNPs Bionanohybrids.

1 g of *Candida rugosa* lipase (CRL, 0.04 mg of protein per mg of powder) was dissolved in 40 mL of distilled water under gentle agitation at 4ºC, obtaining a 1 mg/mL protein solution. This solution was centrifuged at 8000 rpm for 5 minutes and the supernatant was recovered, obtaining a 1 mg/mL aqueous solution of CRL. 40 mL of distilled water containing either 100

mg of polyethyleneimine branched (Mw 800), 10 mL of a 10% w/v solution of Triton X-100 or both of them were also added to the 40 mL of CRL aqueous solution. 20 mL of palladium acetate ($Pd(OAc)_2$) solution in DMF, at 5 mg/mL, were then added and the solution was kept at room temperature under gentle magnetic stirring for 24-48 hours, increasing the turbidity of the solution through time. 20% v/v of the organic co-solvent were needed to ensure the complete dissolution of the Pd salt. Then, it was centrifuged at 10000 rpm for 10 minutes and the supernatant was discarded. The precipitate was washed twice with an aqueous solution of DMF (20% v/v), twice with water and then dried with acetone and diethyl ether, centrifuging each time at 8000 rpm for 10 minutes.

Three different types of bionanohybrids were synthesised using variation of the general method with addition of polyethylenimine (PEI), Triton X-100 or combination of both in the enzyme solution. The bionanohybrid prepared using PEI was named CRL-PdNPs-1, the one using PEI + Triton X-100 (10% w/v) with standard procedure adjusting to same final volume was named CRL-PdNPs-2. The one using Triton X-100 (10% w/v) was named CRL-PdNPs-3. Also the same protocol was used to synthesize the blanks, combining Pd solution with additive solution without protein or protein solution plus additive solution without metal solution. The content of Pd in each case was measured by ICP-OES. Different bionanohybrids were fully characterized by XRD, SEM, TEM, HRTEM.

General procedure for oxidation of benzyl alcohol. Benzyl alcohol (1 mM) was dissolved in different solvents in a round-bottom flask containing a magnetic stirrer with a hard plastic cap. 5 mg of nanohybrid was then added to the solution and the mixture was placed in an oven at 50^{o}C on a magnetic stirrer. In some case, H_2O_2 (33% v/v) was also used in the procedure at 10 μL 20 μL and 50 μL. The solution was tested for the presence of

benzaldehyde by TLC and HPLC analysis. TLC conditions were hexane: ethyl acetate (1:1) solution. The TLC plate was placed under UV lamp at 254 nm to determine spot placement. R_f at 0.58 and 0.37 for benzyl alcohol and benzaldehyde respectively. HPLC conditions were 30:70 acetonitrile: milliQ water at 1 mL/min, λ=254 nm, benzylalcohol was obtained at R_t=5 min , benzaldehyde at R_t=10.7 min. Standard pure products of both substrates were used.

Recycling of nanobiohybrids. Nanobiohybrids were recycled 5 times in the selective oxidation of benzyl alcohol at 50ºC. After each cycle (stopping the reaction around 50% conversion), the reaction solution was centrifuged, and the nanobiohybrid was washed two times with THF and left to dry for 15 minutes in a fume hood.

Enzymatic hydrolysis of 4-nitrophenyl propionate (pNPP). In order to determine the enzymatic activity of the bionanohybrids, the increment in absorbance at 348 nm (∈= 5.150 $M^{-1}cm^{-1}$) produced by the release of *p*-nitrophenol (pNP) in the hydrolysis of 1.2 mM *p*NPP in 25 mM sodium phosphate buffer at pH 7 and 25°C was spectrophotometrically measured. To initialize the reaction, 1 mg of solid was added to 2.5 mL of substrate solution. One unit of activity (U) was defined as the production of 1 μmol of p-nitrophenol per minute per mg of hybrid under the conditions of the assay.

Oxidation of p-substituted aromatic alcohol and enantiomers of phenylethanol. Different substrates were dissolved in 2 ml of THF at 1 mM concentration and 12 mg of CRL-PdNPs-1 bionanohybrid was used, the reaction was incubated at 50ºC. Then samples were taken and analysed by TLC and HPLC using standard aldehyde or ketone as control

Catalytic reduction of 4-nitrophenol (pNP) to 4-aminophenol (pAP). Solid $NaBH_4$ (0.0008 mol; 0.0302 g) was added to 10 mL of aqueous solution of pNP (5 mM). In these conditions, upon the addiction of $NaBH_4$, the initial absorbance band of the solution of pNP

undergoes to an immediate shift from 317 to 400 nm due to the formation of 4-nitrophenolate ions. Immediately after that, 1 mg of the CRL bionanohybrid was added under gentle mechanical stirring at 25 ºC. The reaction progress was monitored by taking out an aliquot of the solution (0.05 mL) at different times, diluting it with distilled water (2 mL) and measuring the absorption spectrum between 500 and 300 nm in a PMMA cuvette.

Domino transformation of 4-nitrophenyl propionate (pNPP) to 4-aminophenol (pAP). 60 µL of a solution of pNPP in pure acetonitrile was added to 2.40 mL of 25 mM sodium phosphate buffer (1.2 mM pNPP) under magnetic stirring at 25°C and let to homogenize. After that, the CRL-PdNPs-1 or CRL-PdNPs-2 biohybrid (1 mg) were added. The reaction was kept on gentle stirring until complete hydrolysis of pNPP to p-nitrophenol (pNP) (about 50 min). Subsequently, to initialize the Pd-catalyzed reduction of pNP to pAP, solid $NaBH_4$ (5 mg) was directly added to the reaction mixture under gentle magnetic stirring at 25 ºC. The reaction progress was monitored by taking out an aliquot of the solution (0.02 mL), diluting it with water (2 mL) and measuring the absorption spectrum between 600 and 200 nm in a quartz cuvette. The complete conversion of pNP to pAP was achieved immediately.

Results and discussion

Preparation of novel CRL-PdNPs bionanohybrids

Lipase from *Candida rugosa* (CRL) commercial powder was dissolved in distilled water to 1 mg/mL solution and solid polyethyleneimine (PEI) Mw800, Triton X-100 (up to 1% w/v) or both were added. Then, palladium acetate dissolved in an aqueous solution of DMF (80:20 water:DMF) was added and the mixture was stirred at room temperature. After 30 min in all cases, the clear initial solution turned into a cloudy suspension. This is indicative of the formation of the heterogeneous biohybrid. The content of Pd in the solution and solid were analyzed by ICP-OES, showing a decrease of Pd in the supernatant of 30% for the preparation using PEI (CRL-PdNPs-1) and 20% when Triton X-100 was used as additive (CRL-PdNPs-2, CRL-PdNPs-3). The ICP analysis of the solid confirmed that CRL-PdNPs-1 contained 2% Pd (w/w) whereas CRL-PdNPs-2, CRL-PdNPs-3 contained 6.5% Pd (w/w).

The solid was not formed in any case for the enzyme without Pd or for the Pd salt without protein. In the three cases SEM analysis revealed the formation of an aggregate with a mesoporous amorphous superstructure (Figure 1) which is composed by palladium atoms dispersed into an organic matrix (enzyme).

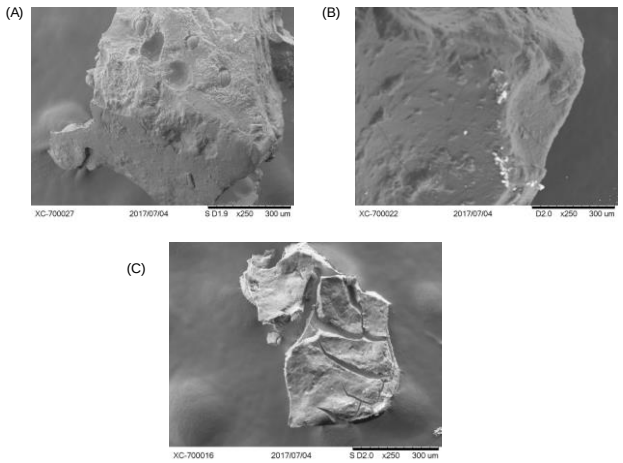


Figure 1. SEM images of the different bionanohybrids. A) CRL-PdNPs-1; b) CRL-PdNPs-2; c) CRL-PdNPs-3.

XRD analyses were performed in order to elucidate the Pd oxidation state. XRD pattern showed the presence of a face-centered-cubic structure assigned to metal palladium in all cases (Figure 2).

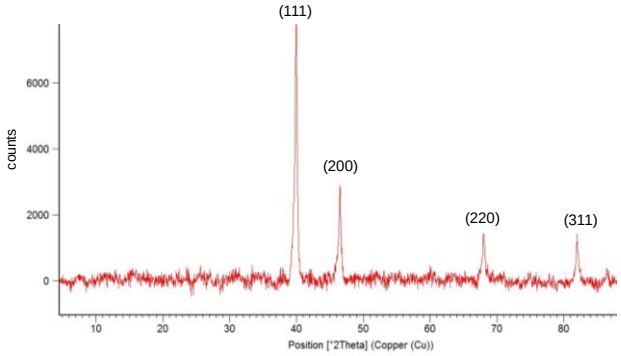


Figure 2. XRD pattern of Pd(0) in bionanohybrids.

The morphology and the distribution of such metallic nanoparticles embedded in the enzymatic net were investigated by TEM and HRTEM microscopy (Figure 3). TEM microscopy of the respective lyophilized forms of the three newly synthesized biohybrids (CRL-PdNPs-1, CRL-PdNPs-2, CRL-PdNPs-3) were performed. TEM analysis revealed the formation of Pd nanoparticles without using any reducing agent during the synthesis. It showed that the morphology and distribution of the formed PdNPs were different depending on the additive used in the synthesis (Figure 3, Table 1). The synthesis using PEI as additive (CRL-PdNPs-1) generated a biohybrid containing the smallest PdNPs of them all, showing an average diameter size of 6-8 nm (Figure 3a). The addition of Triton X-100, a non-ionic detergent, which maintains the lipase in an open conformation [33] together with PEI (CRL-PdNPs-2) (Figure 3b) or alone (CRL-PdNPs-3) (Figure 3c) caused the generation of bigger-sized Pd nanoparticles (Table 1). In the case of the CRL-PdNPs-2 biohybrid, the main percentage of nanoparticles showed an average diameter of 13 nm, although larger nanoparticles were found (17 and 24 nm) (Table 1).

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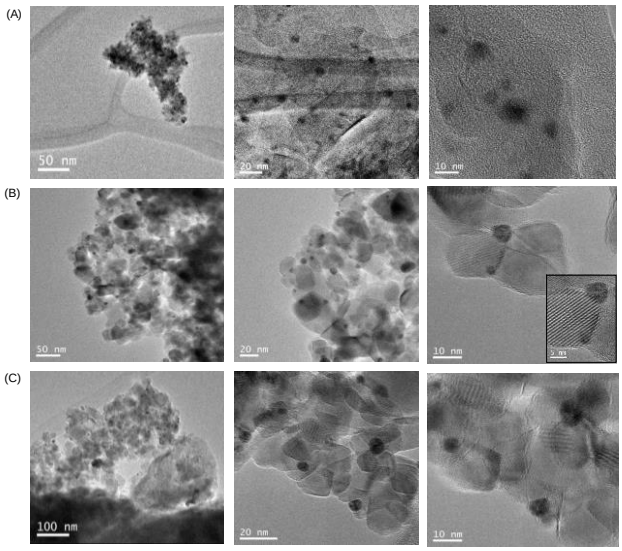

Figure 3. TEM and HRTEM images of the different bionanohybrids.
A) CRL-PdNPs-1: b) CRL-PdNPs-2; c) CRL-PdNPs-3.

In the case of the CRL-PdNPs-3 biohybrid, distribution of size was observed from 8 nm to much higher average diameter size (21 nm) (Table 1).

Table 1. Nanoparticle size of palladium (0) of the different bionanohybrids

Biohybrid[a]	PdNPs size, nm [distribution size, %]				
CRL-PdNPs-1	6 [40]	8 [60]			
CRL-PdNPs-2	9 [19]	13 [56]	17 [13]	24 [12]	
CRL-PdNPs-3	8 [30]	16 [30]	18 [5]	19 [10]	21 [25]

Bioinformatics analysis of the residual distribution on the tridimensional structure of CRL, revealed a high amount of Ser and Thr residues around the hydrophobic structures of the protein (Figure 4). These two amino acids have been described to having a key role in the nanoparticle formation mechanism [27,34]. This distribution ensures a high number of peptides sequences with the capacity to transform the Pd^{2+} bounded to the protein to Pd (0) [27,34].

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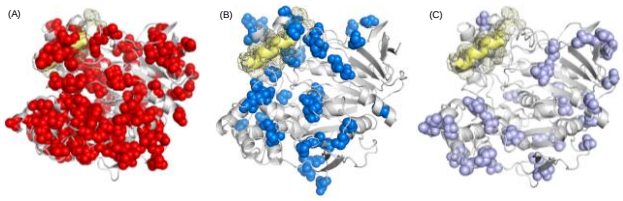

Figure 4. Three-dimensional structure of lipase from *Candida rugosa* (CRL). A) Hydrophobic residues (Phe, Ile, Leu, Val) marked in red. B) Serines marked in light blue. C) Threonines marked in purple. Lid oligopeptide is marked in yellow. The structure of CRL was obtained from the Protein Data Bank (pdb code: 1CRL) and the picture were created using Pymol v. 0.99**.**

Benzyl Alcohol oxidation using different CRL-PdNPs bionanohybrids

The new CRL-PdNPs Biohybrids were applied in the oxidation of benzyl alcohol at moderate conditions, under aerobic conditions in air at 50ºC (Table 2). First, the catalytic efficiency was tested performing the reaction in different solvents (Table 2). Toluene was the first one used with each nanohybrid tested in equal amounts to each other. After 24 h, the conversion was approximately 50% of benzaldehyde without any trace of benzoic acid in all cases, being slightly better for the CRL-PdNPs-1 biohybrid. However, the conversion remained unchanged after 72 hours (Table 2). DMSO was then used as solvent, as it could potentially aid in the oxidation process, but no product was formed in any case after 48 hours. Finally, THF was used for this reaction. In this solvent, excellent results were obtained and >99% of conversion was obtained using CRL-PdNPs-1 and CRL-PdNPs-2 which was completely selective towards benzaldehyde as the unique product (Table 2). Also a good result was obtained with the other biohybrid achieving 90% conversion at 120 h. Therefore, THF was the selected solvent.

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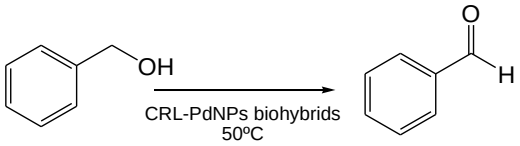
Table 2. Selective oxidation of benzyl alcohol to benzaldehyde catalyzed by CRL–PdNPs bionanohybrids.



Catalyst[a]	Solvent	Time [h]	Conversion[b] [%]	Selectivity[c] [%]
CRL-PdNPs-1	Toluene	72	56	>99
CRL-PdNPs-2	Toluene	72	51	>99
CRL-PdNPs-3	Toluene	72	54	>99
CRL-PdNPs-1	DMSO	48	0	
CRL-PdNPs-2	DMSO	48	0	
CRL-PdNPs-3	DMSO	48	0	
CRL-PdNPs-1	THF	120	>99	>99
CRL-PdNPs-2	THF	120	>99	>99
CRL-PdNPs-3	THF	120	90	>99

[a] Conditions used were: 1mM of benzylalcohol, 3 mL of solvent, 50ºC, 5 mg of catalyst.
[b] Conversion of product was determined by TLC and HPLC.
[c] Selectivity to benzaldehyde as unique product, determined by HPLC

Benzyl Alcohol oxidation using different concentrations of hydrogen peroxide.

The selective oxidation reaction catalyzed by these bionanohybrids was tested in the presence of hydrogen peroxide as an oxidant. Different amount of H_2O_2 were added to the reaction (from 0.1% to 0.5 % v/v) (Table 3). The reaction was much faster in the case of CRL-PdNPs-1 and CRL-PdNPs-2 in the presence of 0.5% (v/v) H_2O_2, obtaining even 66% conversion of benzaldehyde at 2h using CRL-PdNPs-2, although the reaction stopped with same results after 24 h. The best result was obtained with CRL-PdNPs-3, with a 68% conversion after 24 h although it did not change after 48 h (data not shown). Therefore, the addition of the oxidant did not improve the activity of the catalyst in the reaction.

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Table 3. Selective Oxidation of benzyl alcohol to benzaldehyde in presence of H_2O_2 catalyzed by CRL–PdNPs bionanohybrids.

Catalyst[a]	H_2O_2 [v/v, µl]	Time [h]	Conversion[b] [%]	Selectivity[c] [%]
CRL-PdNPs-1	10	24	46	>99
CRL-PdNPs-1	20	24	51	>99
CRL-PdNPs-1	50	2	42	>99
CRL-PdNPs-2	10	24	48	>99
CRL-PdNPs-2	20	24	52	>99
CRL-PdNPs-2	50	2	66	>99
CRL-PdNPs-3	10	24	33	>99
CRL-PdNPs-3	20	24	68	>99
CRL-PdNPs-3	50	24	59	>99

[a] Conditions used were: 1mM of benzyl alcohol, 3 mL THF, 50ºC, 5 mg of catalyst.
[b] Conversion of product was determined by TLC and HPLC.
[c] Selectivity to benzaldehyde as unique product, determined by HPLC.

Reusability of the catalysts

Considering the results obtained the robustness of the two best catalysts CRL-PdNPs-1 and CRL-PdNPs-2 was tested by recycling experiments. Both catalysts were used at least 5 times in the oxidation reaction at 50ºC in THF in absence of any oxidant, and the conversion and selectivity was evaluated in each step. As shown in Figure 5, both nanocatalysts kept the initial catalytic activity and selectivity producing exclusively aldehyde.

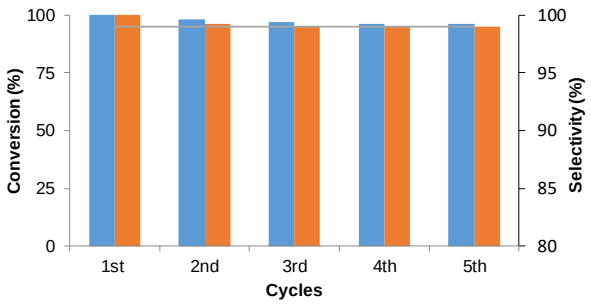

Figure 5. Comparison in reuse of CRL-PdNPs-1 and CRL-PdNPs-2 in the oxidation of benzylalcohol. CRL-PdNPs-1 (blue) and CRL-PdNPs-2 (orange).

Aromatic Alcohol oxidation catalyzed by CRL-PdNPs bionanohybrid

In order to expand the applicability of these nanobiohybrids, the oxidation to other aromatic alcohols was evaluated (Table 4).

For example, the application of CRL-PdNPs-1 to p-methoxybenzyl alcohol, 54% conversion was obtained in 96 h. Interesting was the application on a racemic secondary alcohol. 76% conversion was achieved at that time. Also, the oxidation result for the two enantiomeric alcohols were surprising, the conversion of ketone was higher for the S-isomer, 84% conversion against 68% for R-isomer. This is quite interesting because a selectivity of the lipase against S-enantiomer of this type of substrate has been described [35], which could indicate that protein tridimensional structure is involved on the final catalytic capacity of the palladium [36].

Table 4. Selective Oxidation of different aromatic alcohols catalyzed by CRL–PdNPs-1 bionanohybrid.[a]

Entry	Substrate	Product	Conversion[b] [%]
1	4-methoxybenzyl alcohol	4-methoxy aldehyde	54[c]
2	4-nitrobenzylalcohol	4-nitroaldehyde	0
3	R/S-phenylethanol	acetophenone	76
4	S-phenylethanol	acetophenone	84
5	R-phenylethanol	acetophenone	68

[a] Conditions used were: 1mM of benzyl alcohol, 2 mL THF, 50ºC, 5 mg of catalyst, 96h .
[b] Conversion of product was determined by TLC and HPLC.
[c] Selectivity to corresponding aldehyde were >99%.

Enzymatic activity of the bionanohybrids

Together to the excellent palladium catalytic activity, the enzymatic activity of the different bionanohybrids was evaluated (Table 5). The analysis was performed by the hydrolysis of a model substrate such as p-nitrophenyl propionate (pNPP). The CRL-PdNPs-1 showed the highest enzymatic activity (0.125 U), 65% higher activity than that of CRL-PdNPs-2. At these conditions, the enzymatic activity of CRL-PdNPs-3 was negligible. Both catalysts,

CRL-PdNPs-1 and CRL-PdNPs-2 conserved full enzymatic activity after five times recycling.

Table 5. Catalytic characterization of different CRL-PdNPs Biohybrids.

Catalyst	Enzyme activity (U)[a] [%]
CRL-PdNPs-1	0.125
CRL-PdNPs-2	0.08
CRL-PdNPs-3	0

[a] Activity was calculated using 1.2 mM pNPP hydrolytic assay as described in experimental part. (U was defined as µmol x min^{-1} x mg $hybrid^{-1}$).

Domino reaction by dual activity of the bionanohybrids

In order to evaluate both activities (palladium and enzyme activities) in the bionanohybrids for cascade process application, the domino reaction of transformation of p-nitrophenyl propionate (pNPP) to p-aminophenol (pAP) was performed (Figure 6a). Both PEI modified hybrids CRL-PdNPs-1 and CRL-PdNPs-2 were selected. In both cases, previous to the domino process, considering the enzymatic activity determined above, pNP reduction assay was performed. Both catalysts were extremely rapid and complete reduction of pNP to pAP was obtained in 5 min (Figure 6b-c), although CRL-PdNPs-1 showed a TOF value six times higher than CRL-PdNPs-2, 135 min^{-1} against 20 min^{-1} respectively.

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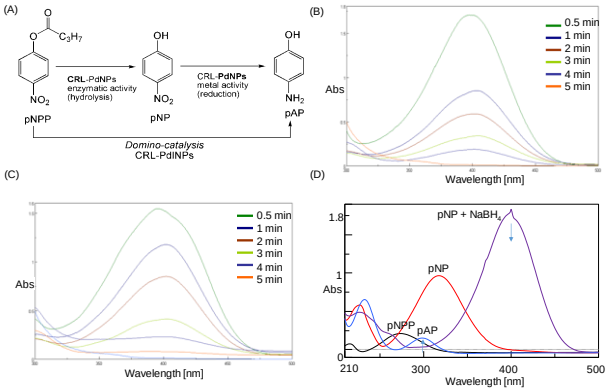

Figure 6. Domino synthesis of pAP from pNPP. a) Reaction scheme.
B) UV absorption spectra of the pNP reduction catalyzed by CRL-PdNPs-1.
C) UV absorption spectra of the pNP reduction catalyzed by CRL-PdNPs-2.
D) UV absorption spectra of the reaction. The adsorption peak at 400 nm (corresponding to the concentration of pNP, time-depending quickly decreased in intensity with a contemporary appearance of an increasing shoulder at 300 nm, indicating the reduction of pNP to pAP.

Then, the direct domino transformation of pNPP to pAP was performed using both biohybrids confirming the good results previously achieved for separate. The reaction was performed using 1.2 mM of pNPP which was completely transformed in pNP after 50 min and immediately transformed in pAP after adding sodium borohydride (Figure 6d).

Conclusions

We have described the preparation of a new type of bionanohybrids containing fine Pd nanoparticles, whose synthesis is induced by a protein, lipase from *Candida rugosa*. The effect on the enzyme structure by the presence of different additives such as PEI polymer or Triton X-100 influenced the final size of the PdNPs. PEI maintained the enzyme mainly in the closed form protecting it from the solvent and giving it stability. Triton X-100 permits the open conformation of the lipase although this can be worse for stability. The bionanohybrids synthesized with PEI gave the more homogeneous size of NPs being smaller while the detergent resulted in higher particle sizes. In all cases, it was possible to synthesize heterogeneous catalysts in very mild conditions. These new heterogeneous bionanohybrids

resulted in excellent selective oxidative catalysts producing exclusively aldehydes from aromatic primary alcohols. The two biohybrids created in the presence of PEI were the best with excellent recyclability, conserving complete Pd activity and selectivity after 5 cycles. Also the lipase activity of both hybrids was conserved, which is a valuable capacity for applying the dual activity in cascade processes. A practical application of that was demonstrated by the one-pot transformation of p-nitrophenylpropionate (pNPP) in p-aminophenol (pAP) catalyzed by CRL-PdNPs-2 bionanohybrid.

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