Electronic Supplementary information (ESI)

Synthesis of a superparamagnetic ultrathin $FeCO_3$ nanorods-enzyme bionanohybrid as novel heterogeneous catalyst†

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Experimental Section

General

Candida antartica B lipase (CAL-B) solution was from Novozymes (Denmark). Ammonium iron(II) sulfate hexahydrate [$(NH_4)_2Fe(SO_4)_2$ x $6H_2O$ (Mohr´s salt)], hydrogen peroxide (33%), iodobenzene, toluene, p-nitro and p-aminoarenes, p- and m-phenyltoluene, bisphenol A, sodium bicarbonate and sodium borohydride were purchased by Sigma-Aldrich. Acetonitrile HPLC grade was purchased by Scharlab.

Inductively coupled plasma atomic emission spectrometry (ICP-AES) was performed on a Perkin Elmer OPTIMA 2100 DV equipment. The X-Ray diffraction (XRD) pattern was obtained using a Texture Analysis Diffractometer D8 Advance (Bruker) with Cu Kα radiation. X-ray photoelectron analysis (XPS) was carried out on SPECS GmbH spectrometer equipped with Phoibos 150 9MCD energy analyzer. A no monochromatic aluminum X-ray source with a power of 200 W and voltage of 12 kV was used using as reference standard the C1s adventitial carbon 284.8 ev. The transmission electron microscopy (TEM), high resolution TEM microscopy (HRTEM) analysis 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. To recover the biohybrids, a Biocen 22 R (Orto-Alresa, Spain) refrigerated centrifuge was used. 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.

The magnetic characterization of the samples was recorded in a vibrating sample magnetometer (MLVSM9, MagLab 9T, VSM, Oxford Instrument). The samples were accurately weighed and fitted into gelatin capsules for magnetic measurements. Hysteresis loops of the powdered samples were measured at room temperature applying a magnetic field of ± 3T.

General synthesis of CAL-B-$FeCO_3$NRs bionanohybrids.

3.6 mL of commercial *Candida antarctica* lipase solution (containing 4 mg lipase/mL) was added to 60 mL sodium bicarbonate buffer 0.1M pH =10 in a 100 mL glass bottle containing a small magnetic bar stirrer (12x4.5 mm). The solution was stirring in a magnetic agitator at 380 r.p.m. (*this is an important point for avoiding iron oxidation)* for 2 min. Then 600 mg of Fe $(NH_4)_2(SO_4)_2$* $6H_2O$ (10 mg/ml) was added to the protein solution and it was maintained for 16 hours at room temperature. After the first 30 min incubation, the solution turned cloudy (greenish gray) and the pH solution was measured indicating a decrease from 10 to 7. After 16 h incubation, the solution turned very dark green (If the color turns orange, this means that oxidation was produced).

In the cases were reduction was performed after incubation, 6 mL of $NaBH_4$ (300 mg) aqueous solution (1.2 M) was added to the cloudy solution (in two times of 3 mL) (obtaining a final concentration of 0.12 M of sodium borohydride in the mixture) stirring in a magnetic agitator at 380 r.p.m. The solution turned rapidly black and, the mixture

was reduced during different times depending on the bionanohybrid prepared (from 15, 30, 60, 90 and 360 minutes).

After the incubation (for the non-reduced catalyst) and this reducing time, in all cases, the mixture was centrifuged at 8000 rpm for 5 min, adding 11 mL per each 15 mL falcon type tube. The generated pellet was re-suspended in 15 mL of water. The pH of the supernatant solution was measured to be approximately 9. It was centrifuged again at 8000 r.p.m for 5 min and the supernatant removed. The pH of the supernatant solution was measured again, given a pH value of 7. The process was repeated once more. The pH of the final supernatant solution should be 5 to avoid residues of sodium borohydride and its interaction with the freeze-drying. Finally, the supernatant was removed and the pellet of each falcon was re-suspended in 2 mL of water, collected all solutions in a round-bottom flask, frozen with liquid nitrogen and lyophilized for 16 hours. Characterization of the different bionanohybrids was performed by XRD, XPS, SEM and TEM analysis. Also these were repeated after 30 days of preparation.

Catalytic reduction of 4-nitrophenol (pNP) to 4-aminophenol (pAP).

To an aqueous solution of p-nitrophenol (pNP) (1 mM; 2 mL), solid $NaBH_4$ (40 mM; 0.0302 g) was added to reach a final concentration of 0.4 M (The typical catalytic reaction was performed by adding an excess of $NaBH_4$ (0.4 M) to ensure its constant concentration throughout the reaction and, therefore, to apply a pseudo-first-order kinetic with respect to the pNP to an aqueous solution of the substrate in the presence of catalysts). 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, 3 mg of the different Fe bionanohybrid were added under gentle stirring at room temperature in an orbital shaker. The reaction progress

was monitored by taking out an aliquot of the solution (0.01 mL) at different times, diluting it with distilled water (2 mL) and measuring the absorption spectrum between 500 and 300 nm in a quartz cuvette.

Catalytic reduction of nitroarenes to aniline derivatives

To an aqueous solution of nitroarene (1 mM; 2 mL), solid 40 mM (in some cases 80 mM) $NaBH_4$ was added. After 30 second, 3 mg of bionanohybrids was added to the solution and the reaction process was followed at the same way than described above.

Reuse of CAL-B-$FeCO_3$NRs-2 bionanohybrid in the reduction of pNP

The CAL-B-$FeCO_3$NRs-2 bionanohybrid was reused six cycles in the reduction of pNP using the conditions described above. The catalyst was separated using a magnet and then rinsed with water once and centrifuged before the next reaction.

Catalytic Degradation of p-aminophenol by iron bionanohybrids

2 mg of pAP was dissolved in 18.88 mL solution of distilled water and 0.22 mL of hydrogen peroxide was added. To initialize the reaction, 2 mL of this solution was added to a 7 mL glass bottle containing 3 mg of bionanohybrid gentle stirring at room temperature in an orbital shaker (320 rpm). Samples (50 µl) at different times were taken and the reaction was followed by HPLC. The samples were diluted 40 times in distilled water before injection. The HPLC column was C8 Kromasil 150x4,6 mm AV-2059. The HPLC conditions used were: an isocratic mixture of 30% acetonitrile and 70% bi-distilled water, UV detection at 275 nm and 225 nm using a Diode array detector, and a flow rate of 0.5 mL/min. Under these conditions, the retention times of pAP was 4.65 min, and for H_2O_2 was 3.3 min.

Catalytic degradation of Bisphenol-A (BPA)

A solution of 10 mM of BPA in pure acetonitrile was prepared. 0.2 ml of this solution were dissolved in 10 mL of 100 mM sodium acetate buffer pH 4 up to achieving a 0.2 mM concentration of BPA. Hydrogen peroxide was added to this BPA solution to achieving different concentrations (50, 100 or 150 mM). To initialize the reaction, 2 mL of this solution (BPA and H_2O_2) was added to a 7 mL glass bottle containing 3 mg of CAL-B-$FeCO_3$NRs-2 bionanohybrid gentle stirring at room temperature in an orbital shaker (320 rpm). Samples (50 μl) at different times were taken and the reaction was followed by HPLC. The samples were diluted 5 times in a mixture of distilled water/acetonitrile 1/1 before injection. The HPLC column was C8 Kromasil 150x4,6 mm AV-2059. The HPLC conditions used were: an isocratic mixture of 50% acetonitrile and 50% bi-distilled water, UV detection at 225 nm using a Diode array detector, and a flow rate of 1 mL/min. Under these conditions, the retention times of BPA was 4.90 min, and for H_2O_2 was 1.57 min.

General procedure for Heck reaction

In a 20 mL screw-sealed vessel, to a solution containing iodobenzene **3** (0.0306 mL, 0.274 mmol) and ethyl acrylate **4** (0.059 mL, 0.55 mmol) in DMF or dry DMF (1 mL), 5 mg of CAL-B-$FeCO_3$NRs-2 bionanohybrid (0.04 mmol iron carbonate, 14.9 mol%) was added at 65 or 90ºC. After 5 min of preheating at the corresponding T, 2 equiv of base was added to initialize the reaction. The final suspension was left under vigorous magnetic stirring at the desired T for the indicated times.

The reaction outgoing was monitored by HPLC analysis of the reaction's samples withdrawn at different times. The analysis conditions were performed with a Kromasil-C8 (150 × 4.6 mm and 5 μm Ø), at a flow of 1.0 mL / min; λ: 250 nm; and a mobile phase:

50% (v/v) ACN in MilliQ water. In these conditions, **4** gets an R_t of 4.27 min and 3 an R_t of 13.66 min. The (E)-ethyl cinnamate product **5** gets an R_t of 11.32 min. This stereochemistry was confirmed by HPLC using the (E) and (Z)-**5** standards (Z gets an R_t of 10.38 min) . The yields were obtained extrapolating the values through a calibration curve of the product (R^2=0.9964).

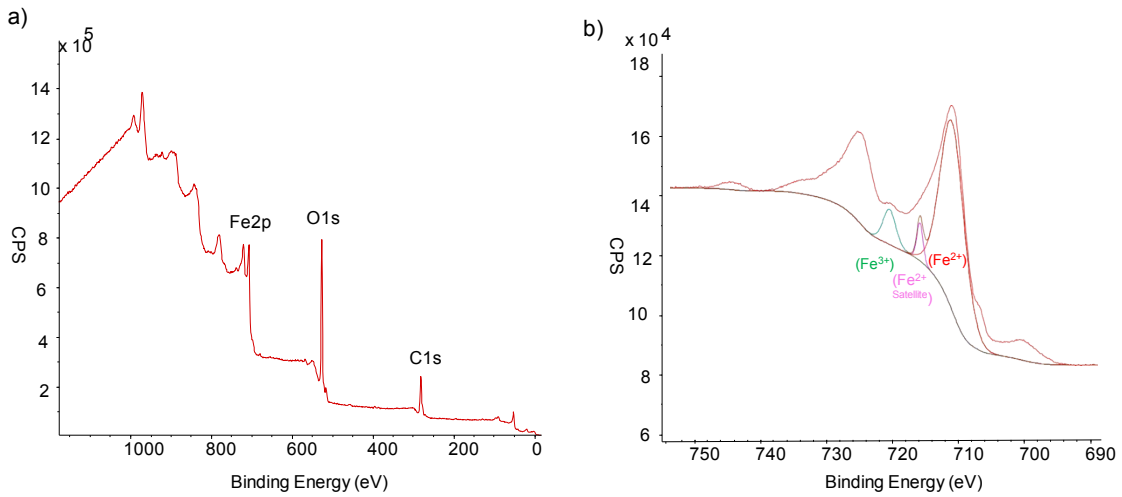


Figure S1. A. XPS full spectrum. B. XPS spectrum of Fe2p. of CAL-B-$FeCO_3$NRs-2 bionanohybrid.

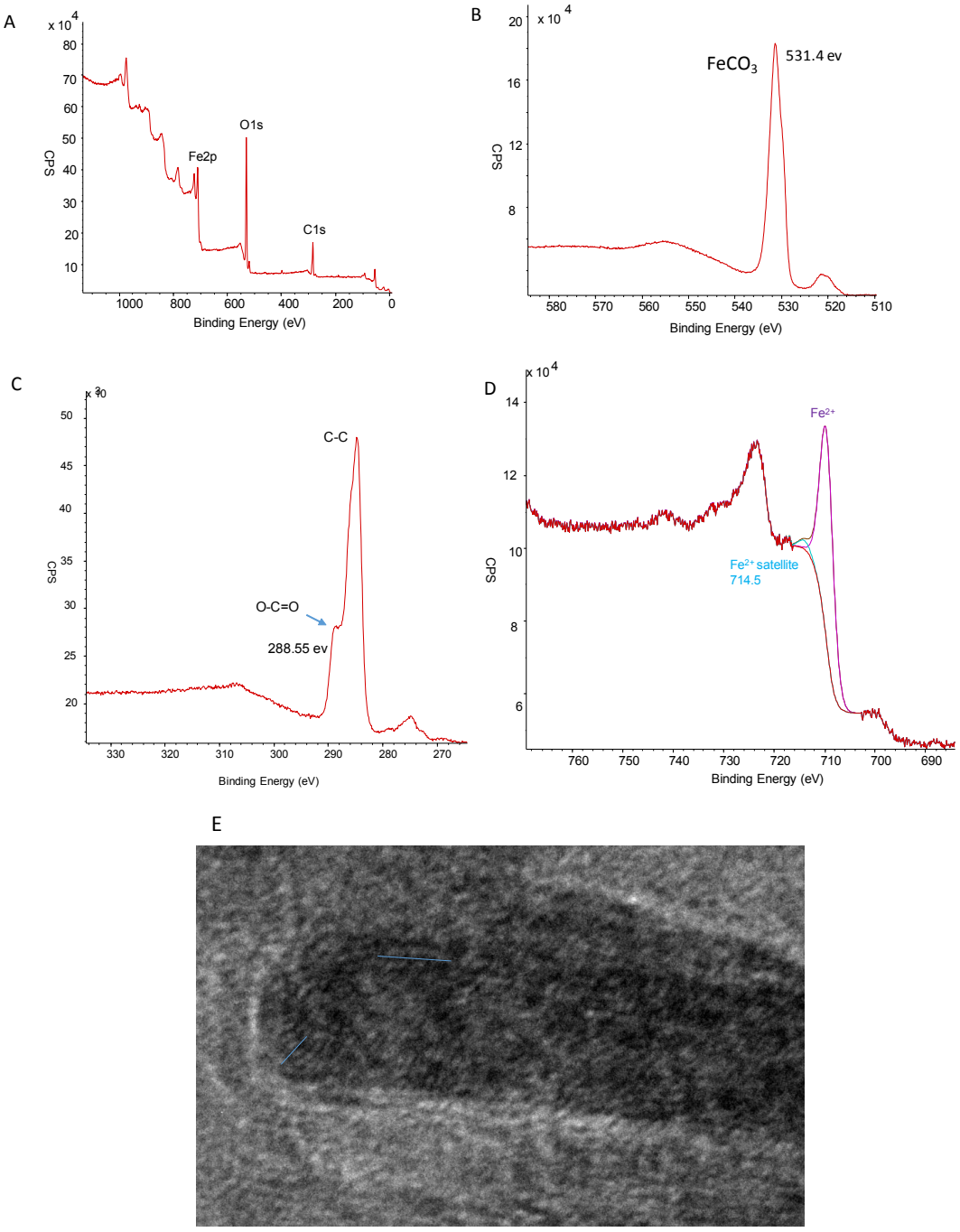


Figure S2. A. XPS full spectrum. B. XPS spectrum of O1s. C. XPS spectrum of C1s. D. XPS spectrum of Fe2p. E. HRTEM for iron carbonate in CAL-B-$FeCO_3$NRs-2 bionanohybrid.

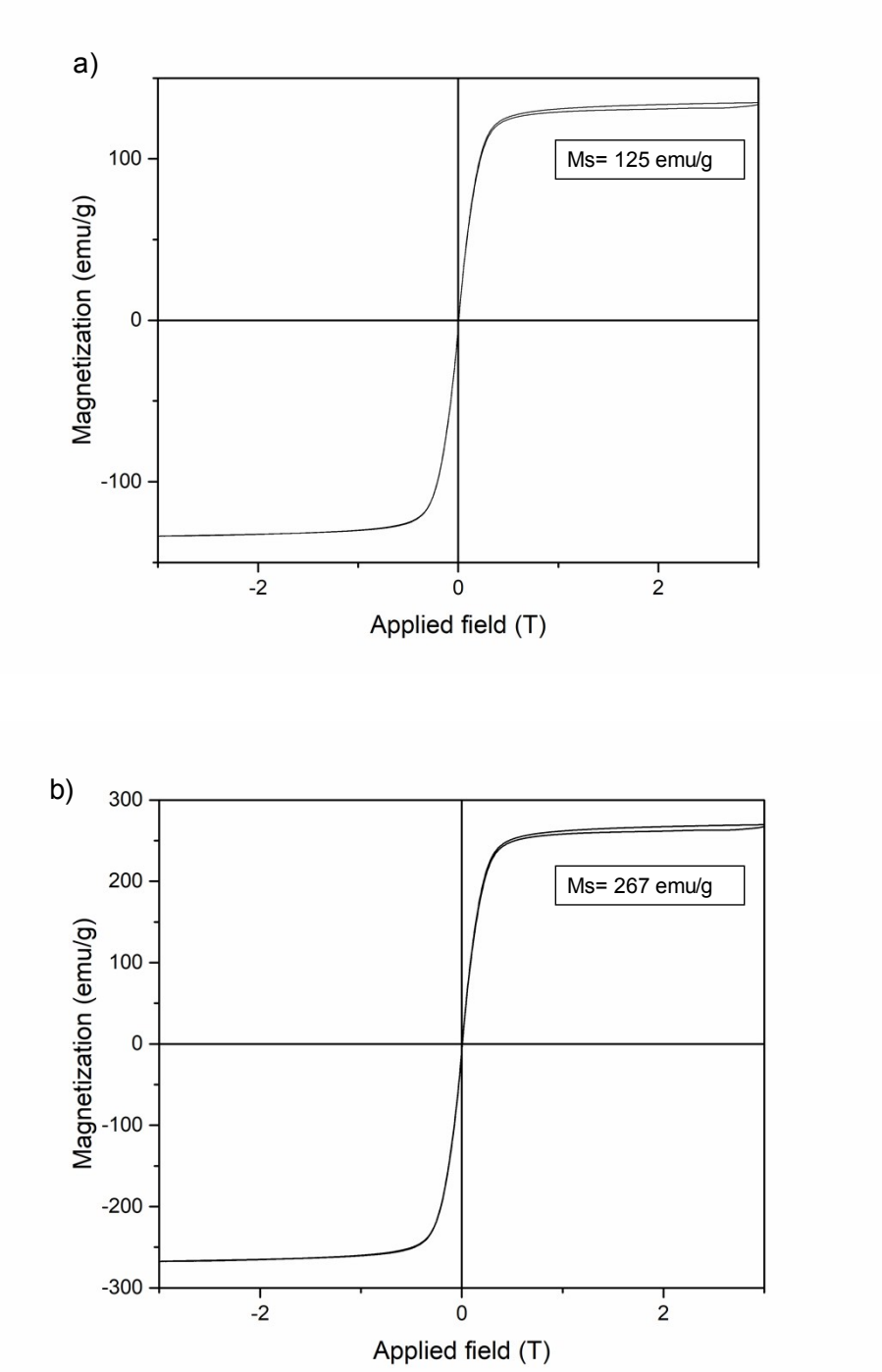


Figure S3. Magnetization hysteresis loops of CAL-B-$FeCO_3$NRs-2 at room temperature. T:334K. A) Normalized at total mass of the sample (inset- saturation magnetization (Ms) value). B) Normalized at content of iron in the sample (inset- saturation magnetization (Ms) value).

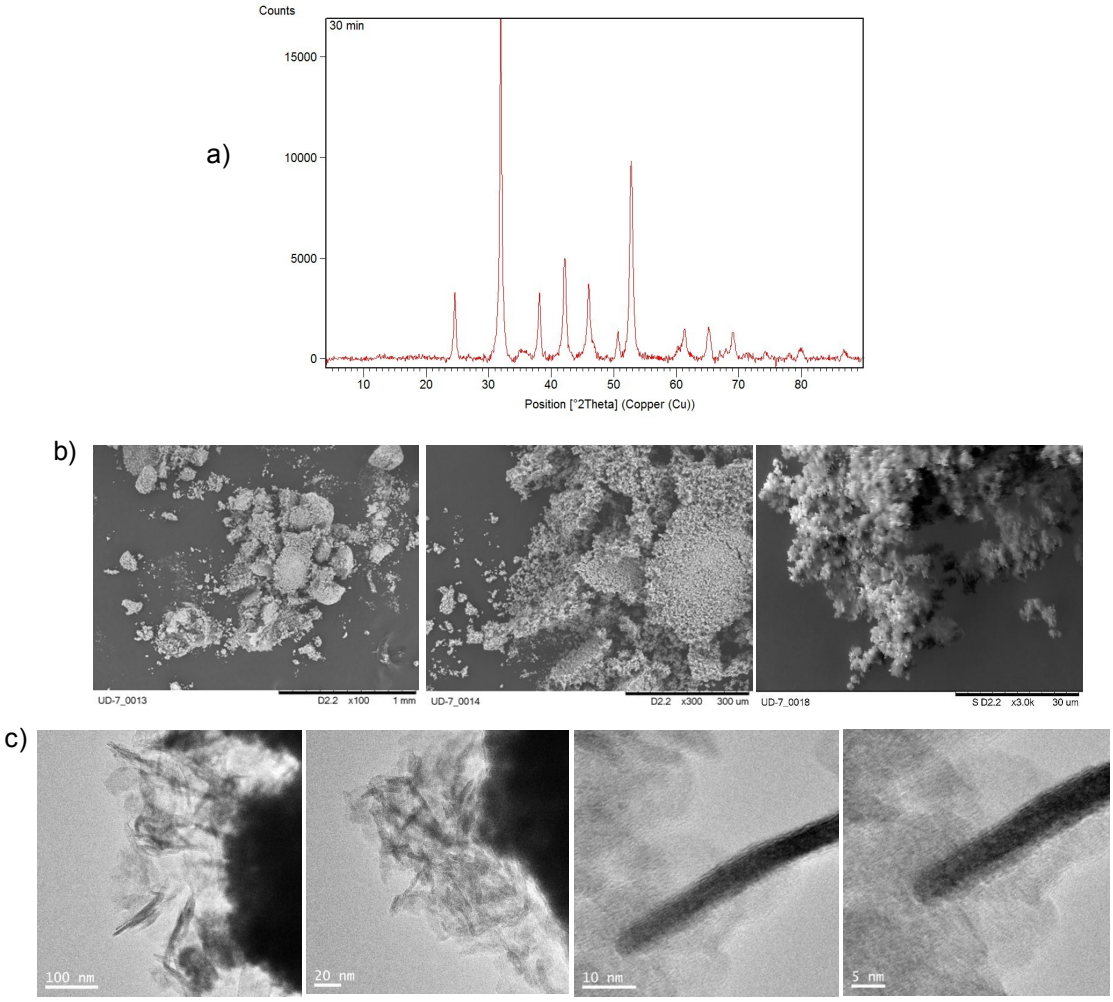


Figure S4. Characterization of CAL-B-$FeCO_3$NRs-3 bionanohybrid. A) XRD. B) SEM, C) TEM.

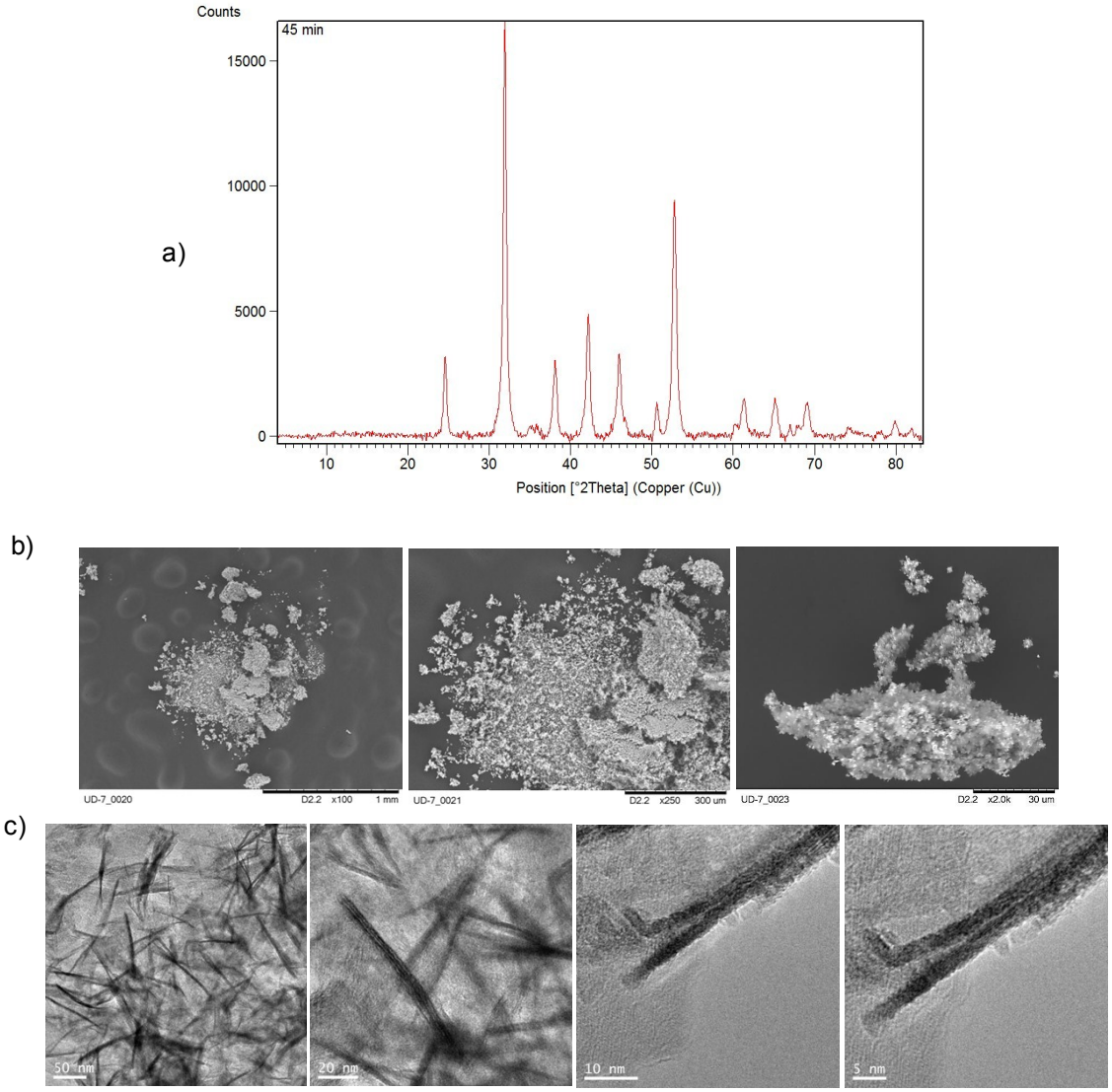


Figure S5. Characterization of CAL-B-$FeCO_3$NRs-4 bionanohybrid. A) XRD. B) SEM, C) TEM.

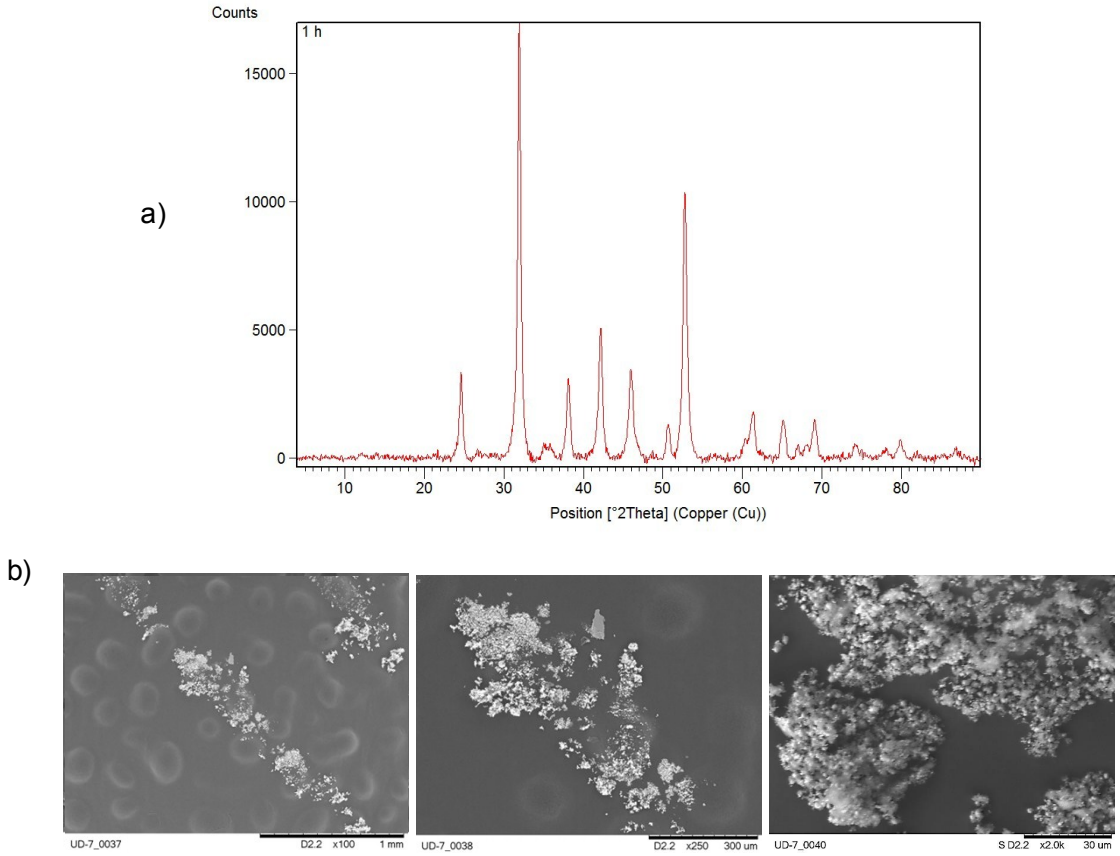


Figure S6. Characterization of CAL-B-$FeCO_3$NRs-5 bionanohybrid. A) XRD. B) SEM, C) TEM.

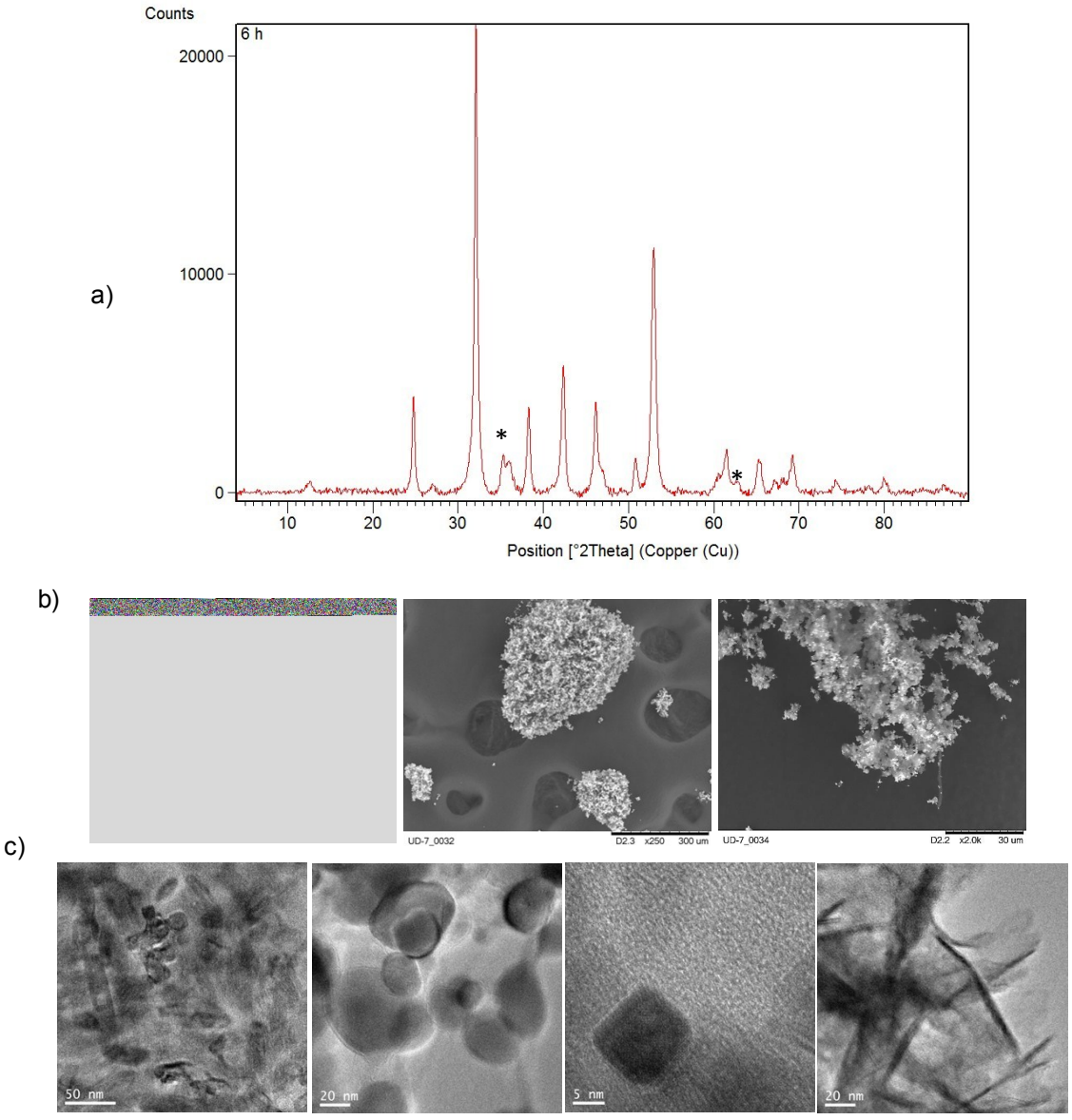


Figure S7. Characterization of CAL-B-$FeCO_3$NRs-6 bionanohybrid. A) XRD, * impurity of iron oxide. B) SEM, C) TEM.

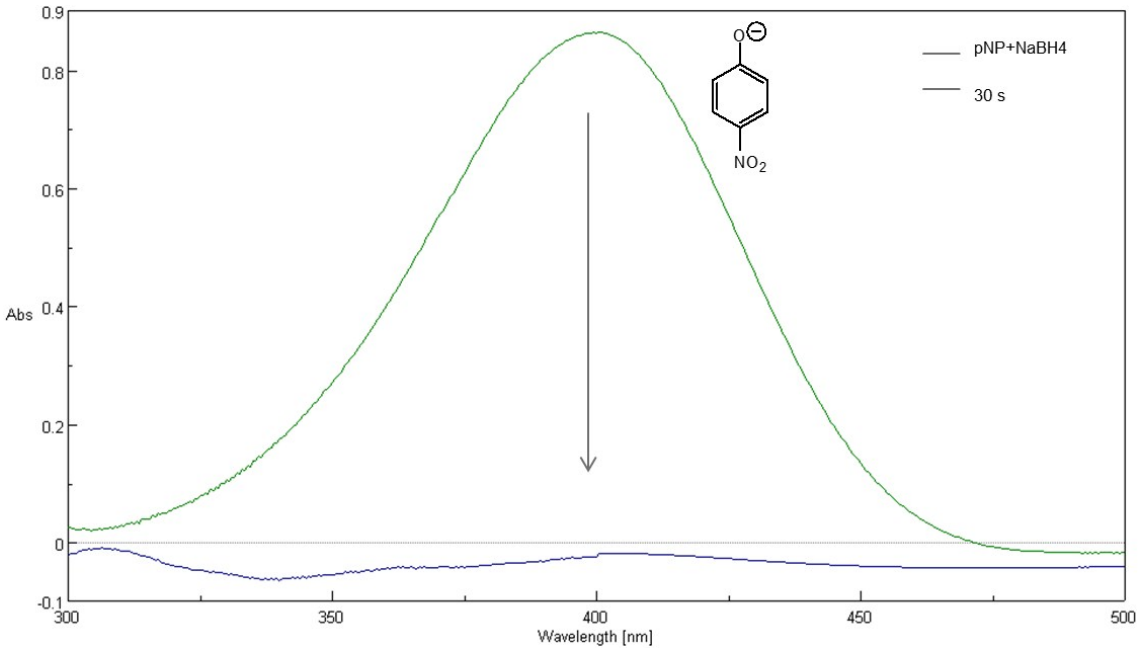


Figure S8. Reduction of pNP catalyzed by CAL-B-$FeCO_3$NRs-2 bionanohybrid in aqueous media.

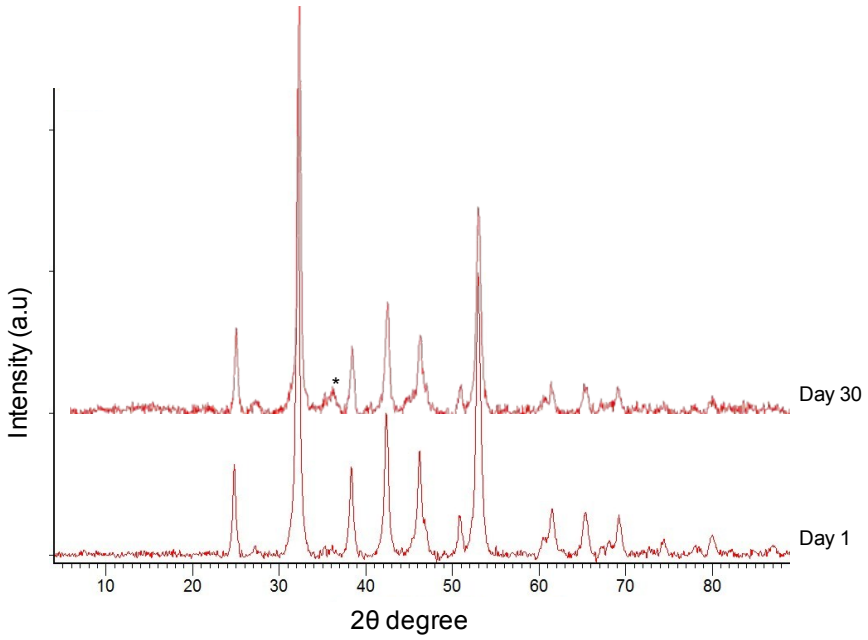
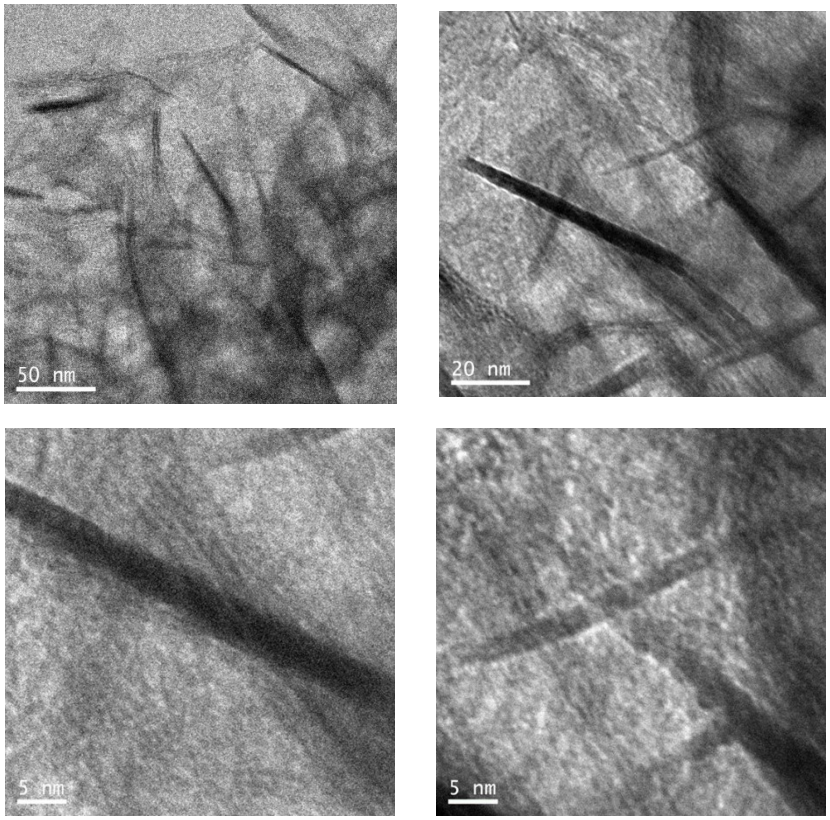


Figure S9. Comparison of the $FeCO_3$ XDR spectrum of CAL-B-$FeCO_3$NRs-2 bionanohybrid from the preparation (day 1) to 30 days after. (*= residual impurity)



Diameter of nanowires/nm	5±1
Length of nanowires/nm	60±3

Figure S10. TEM analysis of the CAL-B-$FeCO_3$NRs-2 bionanohybrid after 30 days.

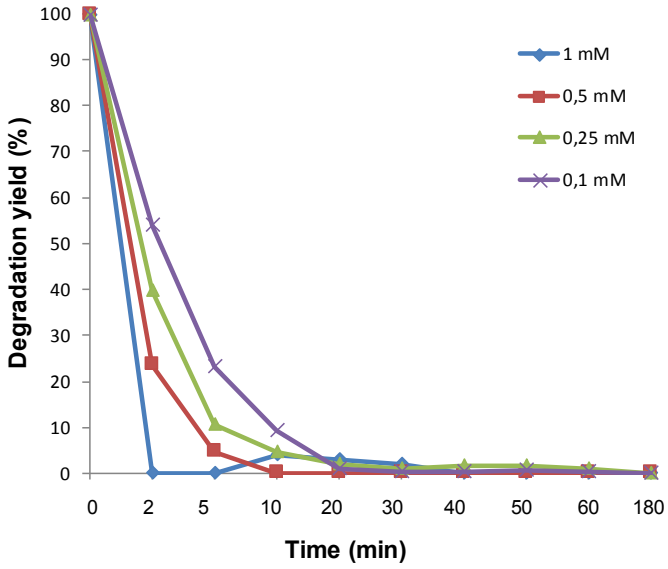


Figure S11. Degradation of PAP in the presence of different concentrations of hydrogen peroxide catalyzed by CAL-B-$FeCO_3$NRs-2 nanobiohybrid in acetate buffer pH 4.

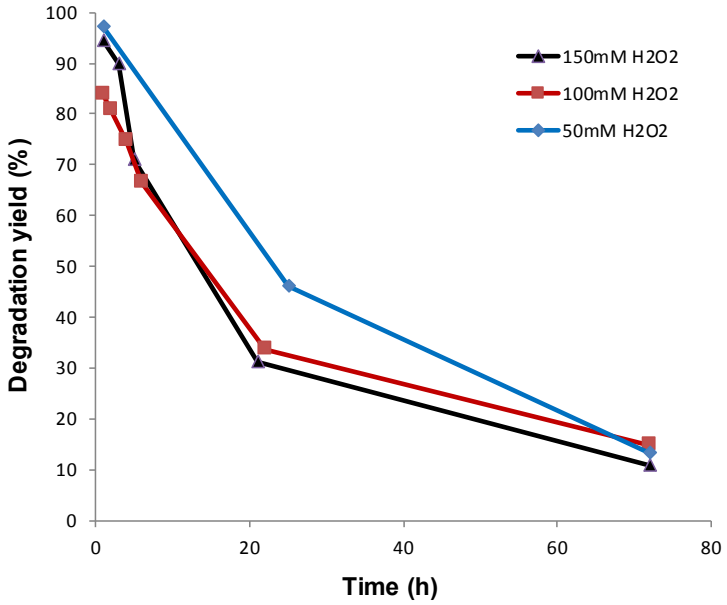


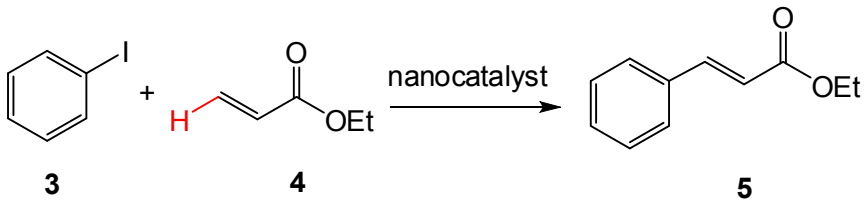
Figure S12. Degradation of BPA in the presence of hydrogen peroxide catalyzed by CAL-B-$FeCO_3$NRs-2 nanobiohybrid in acetate buffer pH 4.

Table S1. The dependence of $FeCO_3$ nanorod size on different reducing times in the presence of sodium borohydride.

Reducing time (min)	Without reduction	15	30	45/60	360 [a]
Diameter of nanorods/nm	7±3	5±1	5±1	5±1	5±1
Length of nanorods/nm	59±9	40±5	55±5	90±7	93±8

[a] iron nanoparticles were also observed.

Table S2. Heck coupling of aryl iodide with ethyl acrylate catalyzed by CAL-B-$FeCO_3$NRs-2 biohybrids as catalyst.



3 **4** **5**

Entry	Solvent	Additive	T [ºC]	Base	Time [h]	Yield of **5** [%]
1	DMF	-	65	NEt_3	24	0[a]
2	DMF (dry)	-	65	NEt_3	24	0[a]
3	DMF(dry)	-	90	NEt_3	24	traces [a]
4	DMF (dry)	-	90	NEt_3	24	traces [b]
5	DMF (dry)	-	90	NEt_3	24	traces[c]
6	DMF (dry)	-	90	Na_2CO_3	24	2.2[a]
7	DMF (dry)	-	90	^tBuOOK	24	0[a]
8	DMF (dry)	-	90	DIPEA	24	0[a]
9	DMF (dry)	DMAP	90	^tBuOOK	24	0[d]
10	DMF (dry)	Molecular sieves 3Å[e]	90	NEt_3	24	20[c]
11	DMF (dry)	Molecular sieves 3Å[e]	90	NEt_3	72	56[c]
12	DMF (dry)	Molecular sieves 3Å[e]	90	Na_2CO_3	24	<1[c,f]
13	DMF/H_2O (90/10)	Molecular sieves 3Å[e]	90	NEt_3	24	1.84 [c]

[a]Conditions: **3** (0.274 mmol), 4 (0.55 mmol), 1 mL solvent, 2 equiv base, 5 mg of CAL-B-$FeCO_3$NRs-2.
[b] 5 equiv base.
[c] **3** (0.0274 mmol), 4 (0.055 mmol), 1 mL solvent, 2 equiv base, 5 mg of CAL-B-$FeCO_3$NRs-2 bionanohybrid.
[d] **3** (0.091 mmol), 4 (0.45 mmol), 1 mL solvent, 4 equiv base, 5 equiv DMAP, 5 mg of CAL-B-$FeCO_3$NRs-2
[e] 18 mg
[f] mainly produce the *cis*-**5** (total conversion 3%)