Electronic Supplementary Information

Tailorable Synthesis of Heterogeneous Enzyme-Copper Nanobiohybrids and application in selective oxidation of benzene to phenol

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LPSGSDPAFSQPKSVLDAGLTCQGASPSSVSKPILLVPGTGTTGPQSF
DSNWIPLSTQLGYTPCWISPPPFMLNDTQVNTEYMVNAITALYAGS
GNNKLPVLTWSQGGLVAQWGLTFFPSIRSKVDRLMAFAPDYKGTV
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TDEIVQPQVSNSPLDSSYLFNGKNVQAQAVCGPLFVIDHAGSLTSQF
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Figure S1. Sequence of amino acids of the lipase from *Candida antarctica* B (CALB).

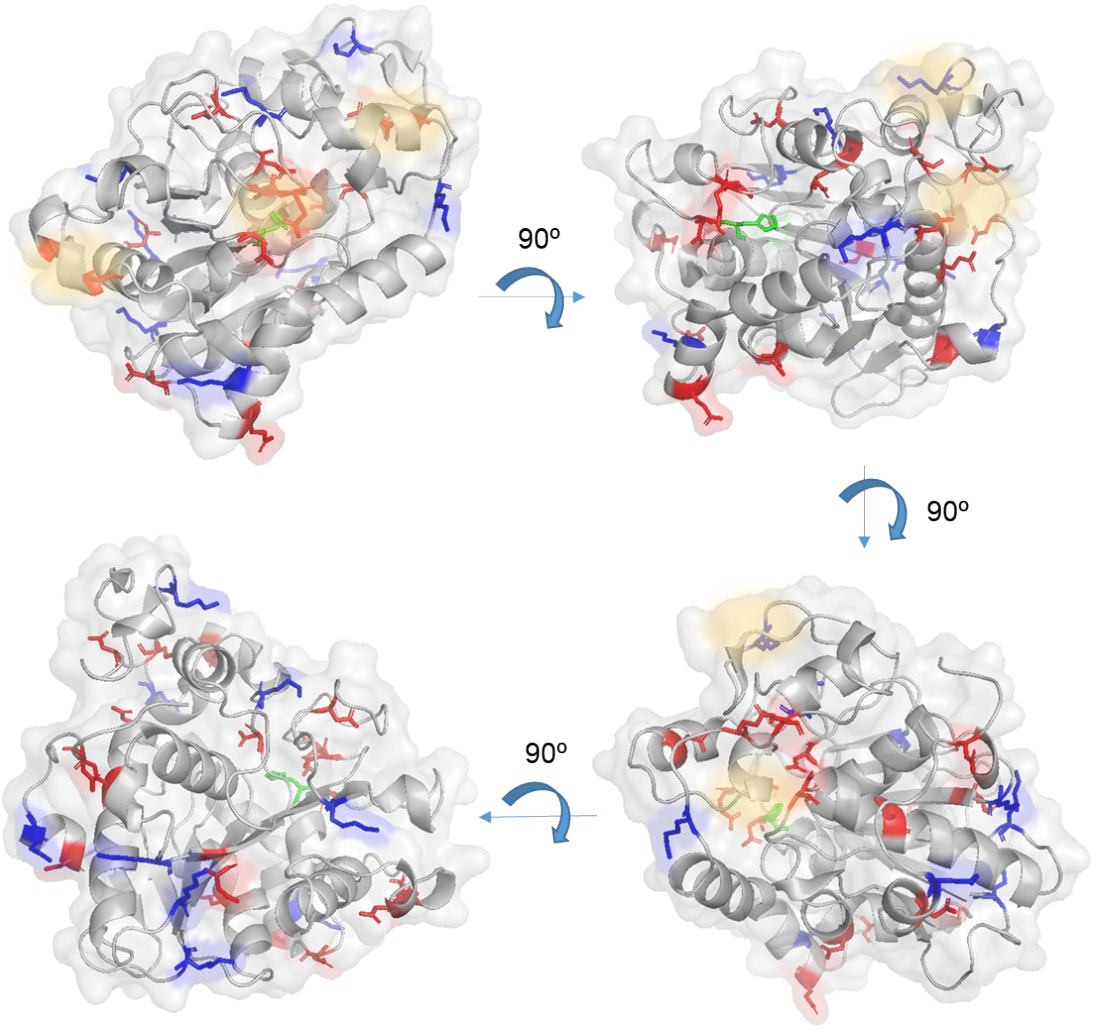
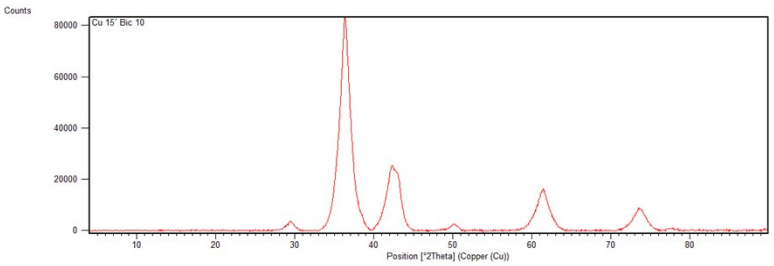


Figure S2. 3D-structure of CALB. Asp,Glu (red), Lys(blue), His (Green), potential metal-binding area (yellow). The 3D structure was obtained from the Protein Data Bank (PDB) using Pymol vs 0.99. The pdb code for CAL-B is TCA.

A



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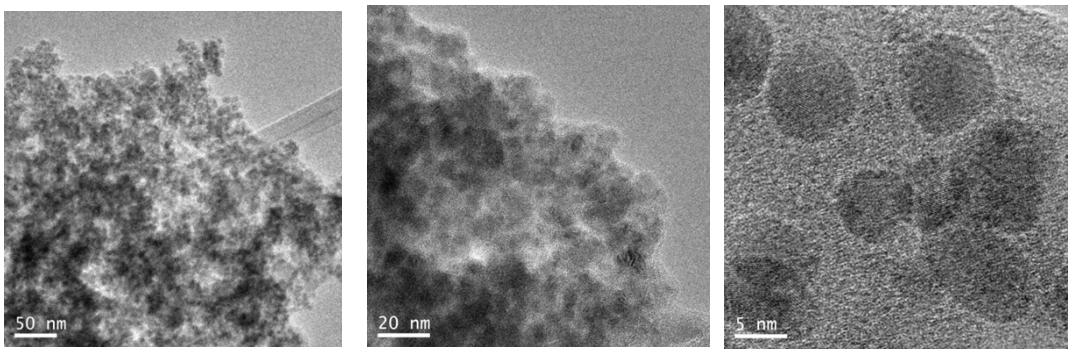


Figure S3. Characterization of the **Cu-CALB-BIC** nanobiohybrid drying at 100ºC. A) XRD spectrum, B) TEM images.

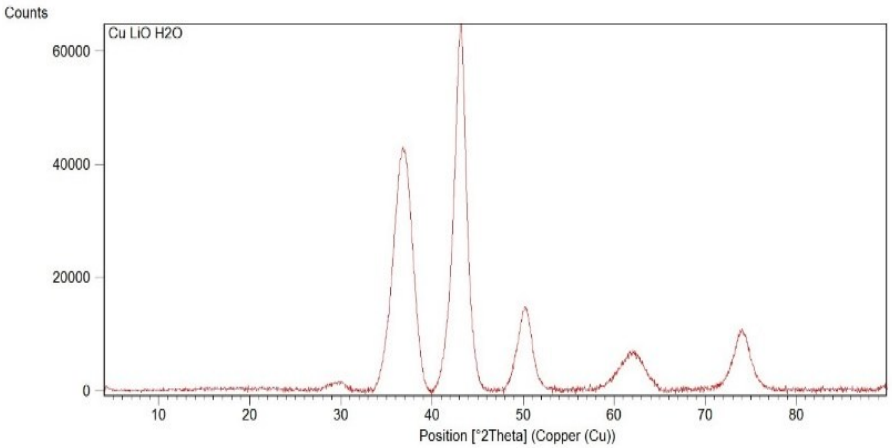


Figure S4. XRD pattern of **Cu-CALB** nanobiohybrid synthesized in water.

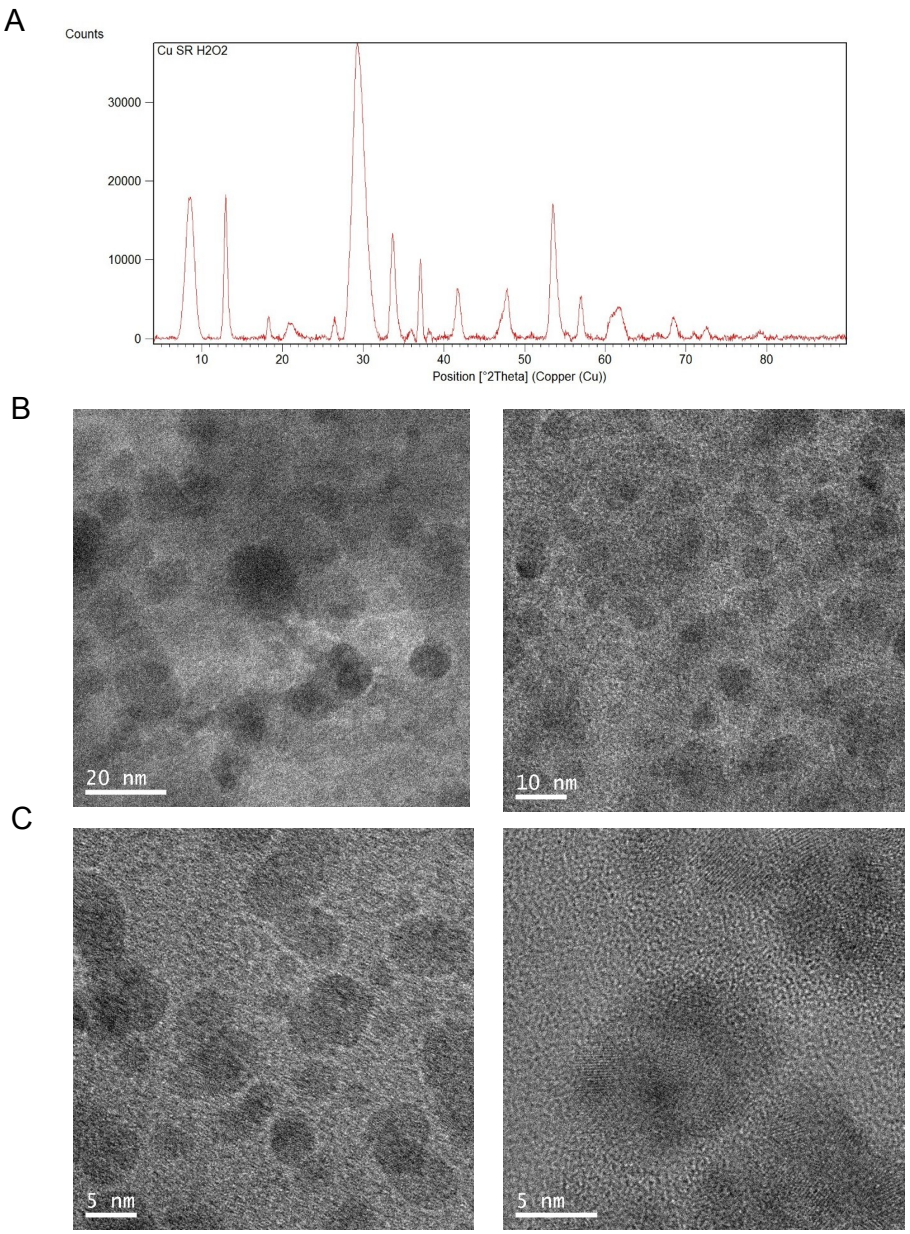


Figure S5. Characterization of the **Cu-CALB-PHOS-NRH$_2$O$_2$** nanobiohybrid. A) XRD spectrum, B) TEM images, C) HTEM images.

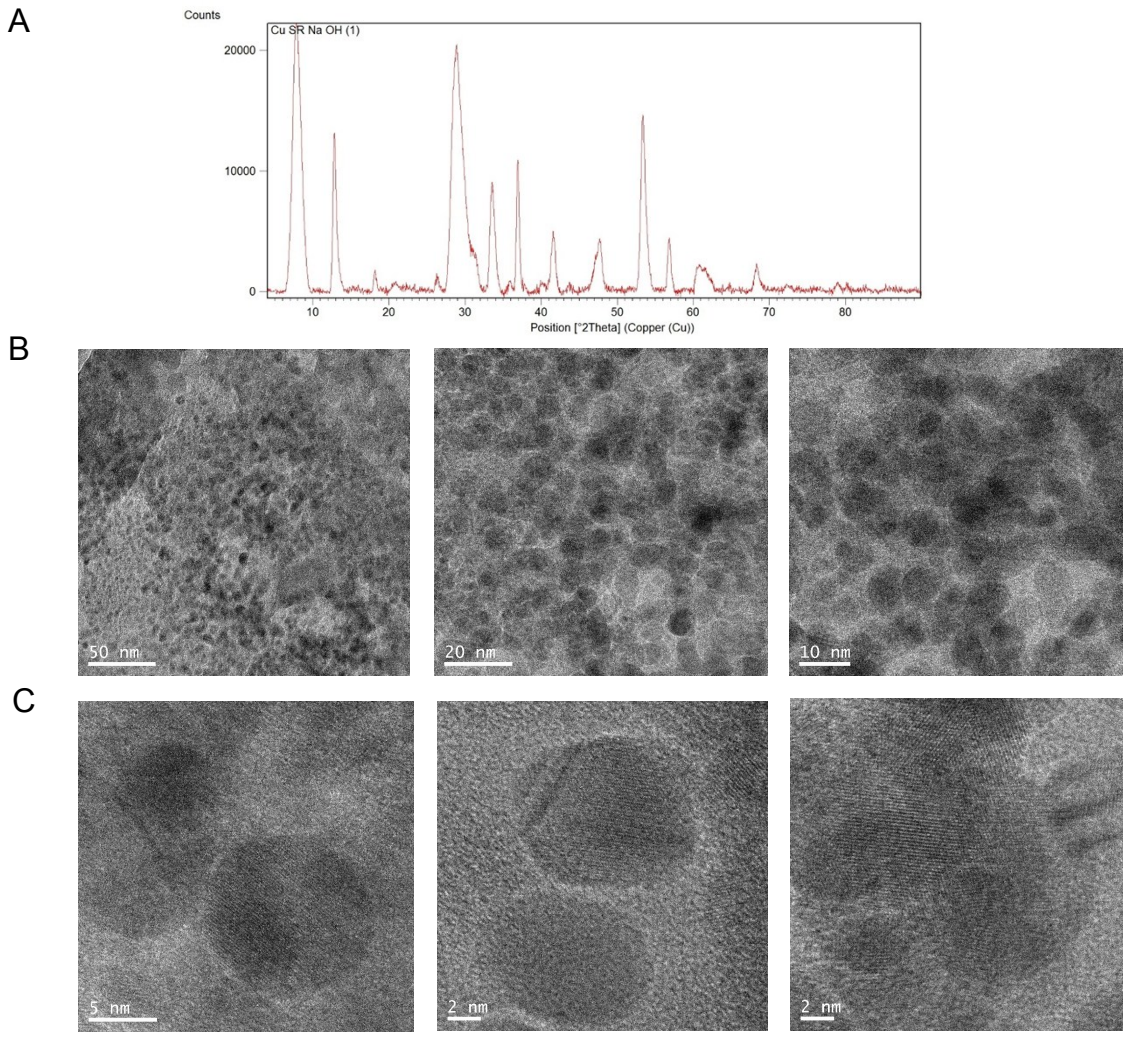
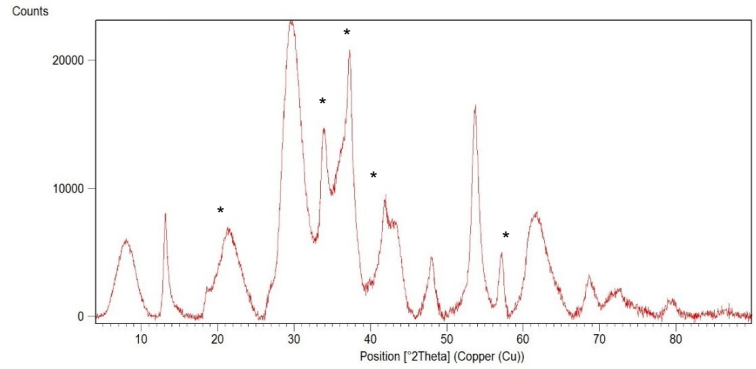


Figure S6. Characterization of the **Cu-CALB-PHOS-NRNaOH** nanobiohybrid. A) XRD spectrum, B) TEM images, C) HTEM images.

A



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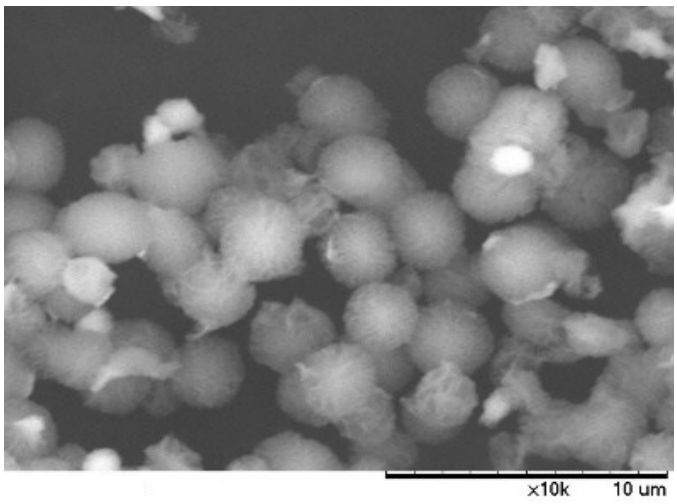

Figure S7. Characterization of the **Cu-CALB-PHOS-10** nanobiohybrid, A) XRD, B) SEM.

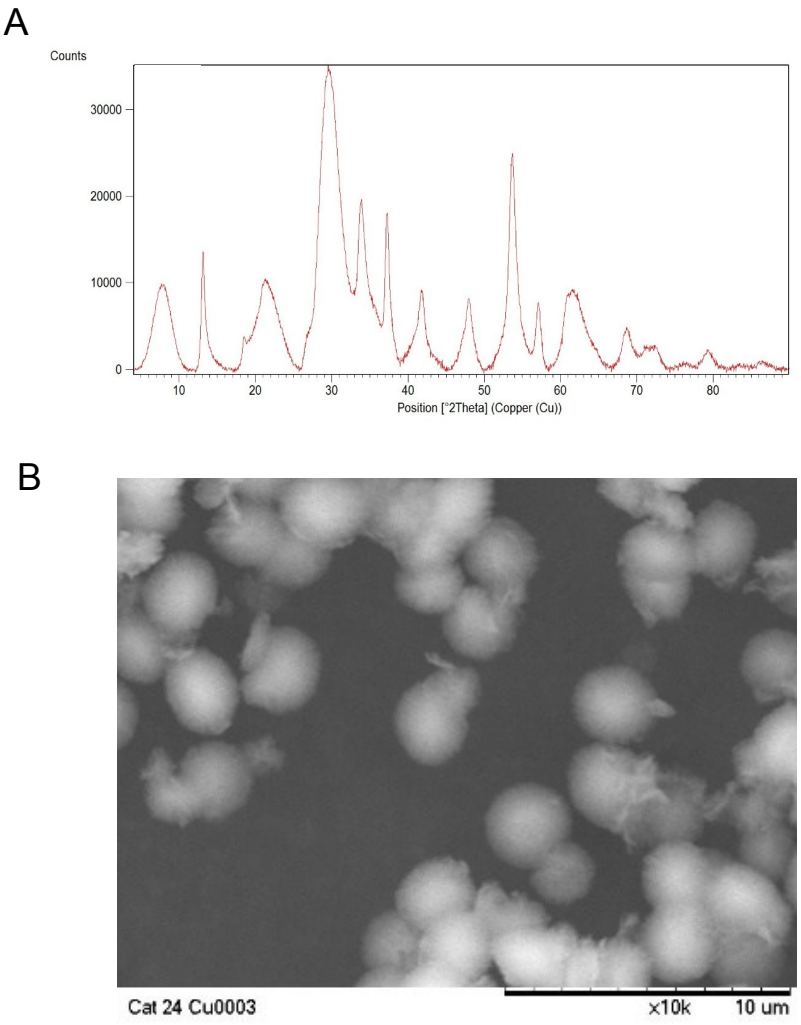

Figure S8. Characterization of the **Cu-CALB-PHOS-NR-10** nanobiohybrid, A) XRD, B) SEM.

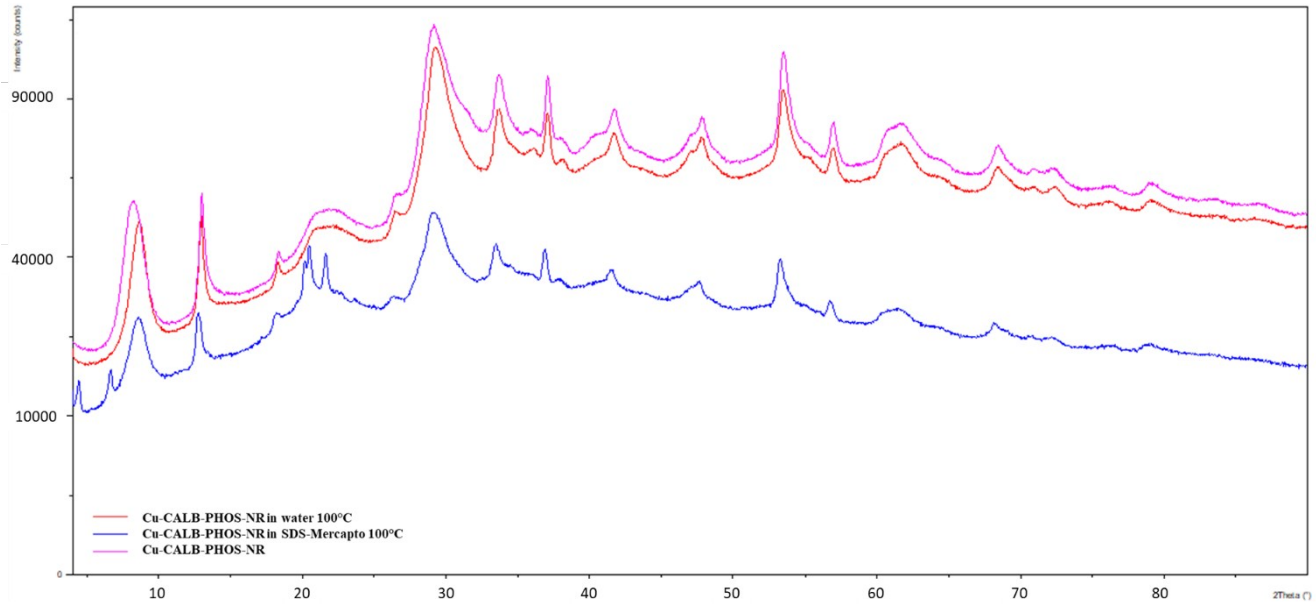

Figure S9. Comparative between XRD of Cu-CALB-PHOS-NR nanobiohybrid after heat treatment in water (red) and in SDS-Mercapto (blue) and XRD original of Cu-CALB-PHOS-NR nanobiohybrid (pink).

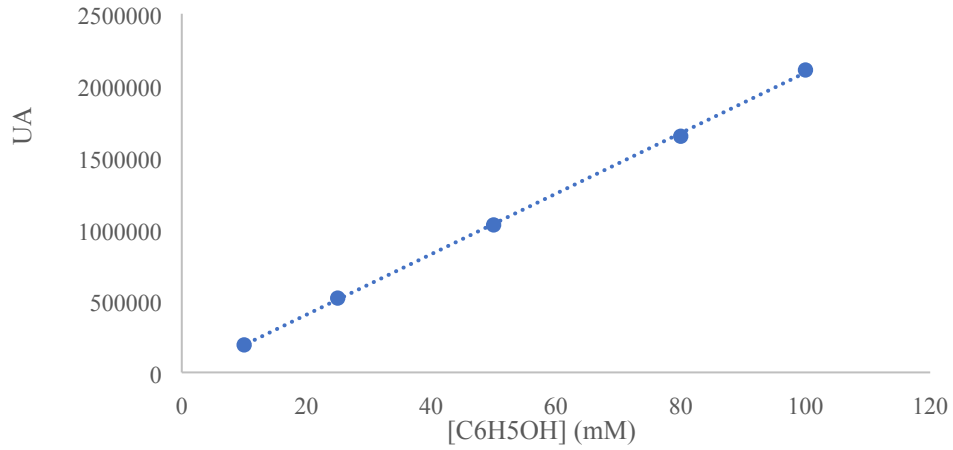

Figure S10. Phenol calibration curve.

Table S1. Content of Cu in the different nanobiohybrid determined by ICP-OES.

Cu- nanobiohybrid	*Amount of Cu by ICP-OES (%)*[a]
Cu-CALB-PHOS	81
Cu-CALB-BIC	84
Cu-CALB-PHOS-2	60
Cu-CALB-BIC-2	93
Cu-CALB-PHOS-NR	32
Cu-CALB-PHOS-NRNaOH	35
Cu-CALB-PHOS-NRH_2O_2	22
Cu-CALB-PHOS10%R	48
Cu-CALB-PHOS10	50
Cu-CALB-PHOS-NR10	50

[a]The measurement was performed of the solid material. 10 mg of the solid powder was treated with 5 mL of HCl (37% v/v) for digestion. Then, it was added with 5 mL of water, centrifuged and the clear solution analyzed by Cu content.

Table S2. Solubility of benzene at different concentrations [a].

$[C_6H_6]_{inicial}$ (mM)	Solubility[b] C_6H_6 in water a r.t
50	20
100	62
200	71

[a] 33% H_2O_2 (1.25 mmol), catalyst (5 mg), 10 mL solution (99%water, 1%ACN), 30ºC, 24 h. [b]Benzene solubility was calculated by HPLC quantification using standard concentrations in pure acetonitrile.

Table S3. Solubility of benzene at different amount of co-solvent.

$[C_6H_6]_{inicial}$ (mM)	Co-Solvent [b] (%)	Solubility [c] C_6H_6 in water a r.t
100	1	62
100	10	68
100	20	72
100	50	85
100	100	100

[a]Benzene (1 mmol), 33% H_2O_2 (1.25 mmol), catalyst (5 mg), 10 mL aqueous medium, 30ºC, 24 h. [b]Amount of acetonitrile as co-solvent in water (v/v). [c] Benzene solubility was calculated by HPLC using standard concentrations in pure acetonitrile.