Electronic Supplementary Information

Temperature-induced formation of Pd nanoparticles in heterogeneous

nanobiohybrids: application in C-H activation catalysis
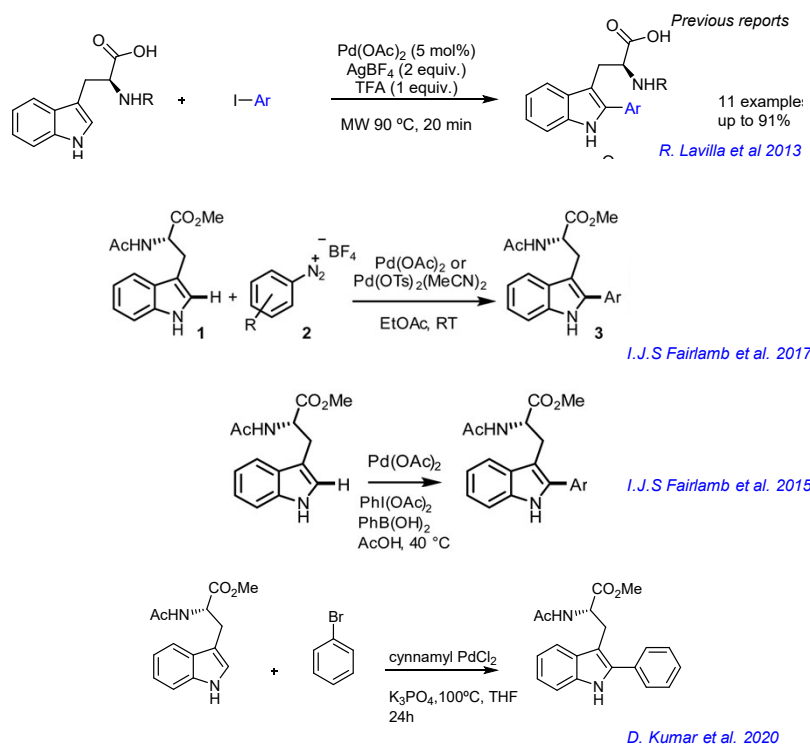
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[a] Instituto de Catálisis y Petroleoquímica (ICP), CSIC. C/Marie Curie 2. 28049 Madrid (Spain). *E-mail: josempalomo@icp.csic.es

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[†] Both authors contributed equally.

Scheme S1. Selected examples of recent reports of C-H activation of tryptophan derivatives catalysed by Pd molecules.
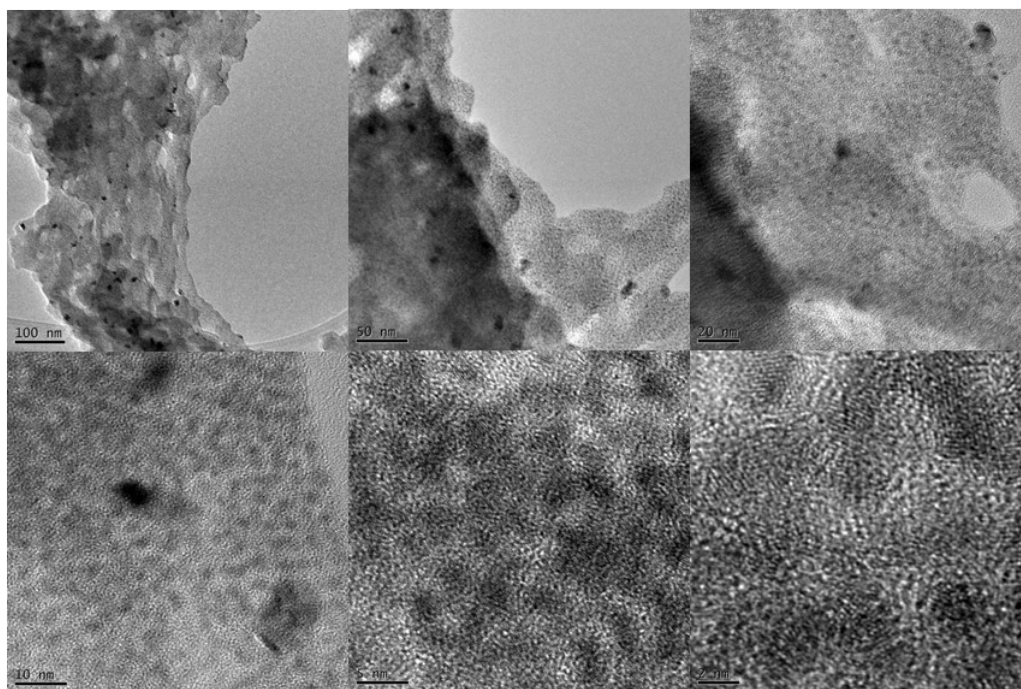
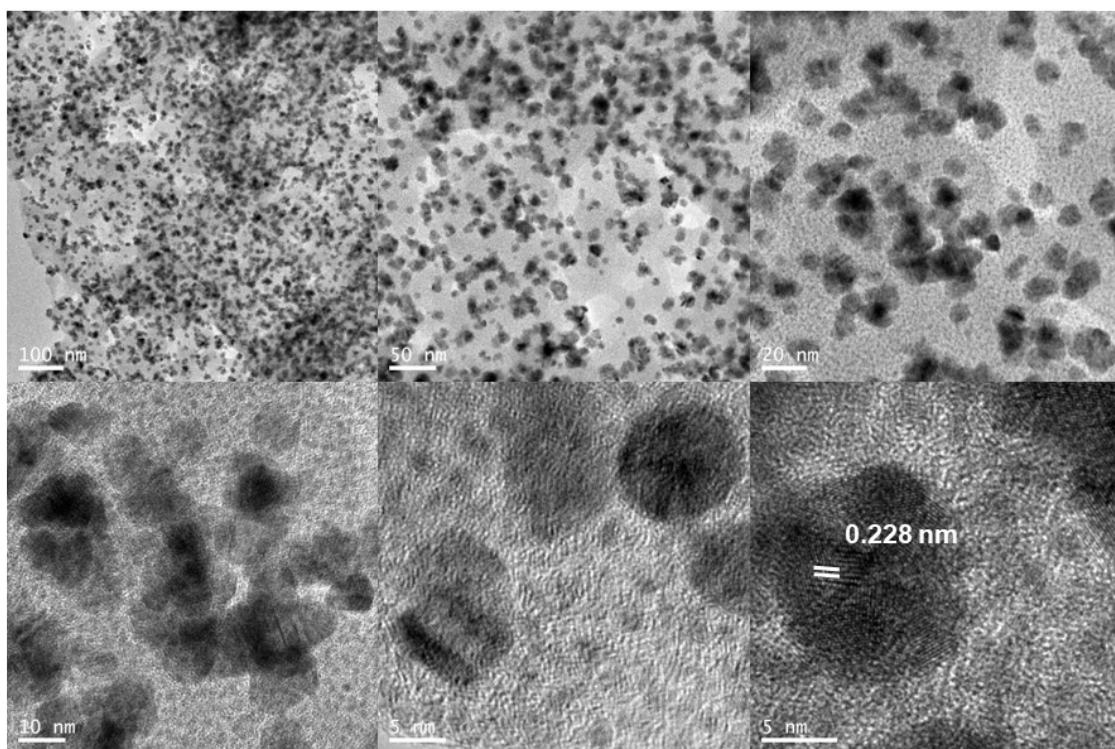**Figure S1.** TEM and HR-TEM images of **Pd@CALB4.**

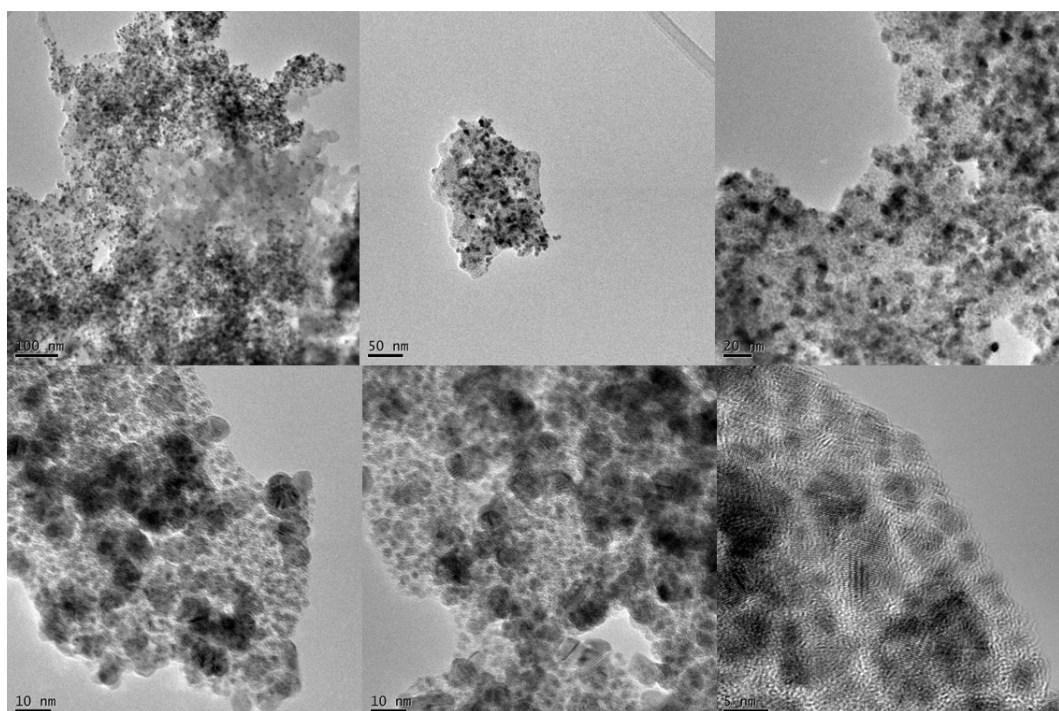**Figure S2.** TEM and HR-TEM images of **Pd@CALB50.**
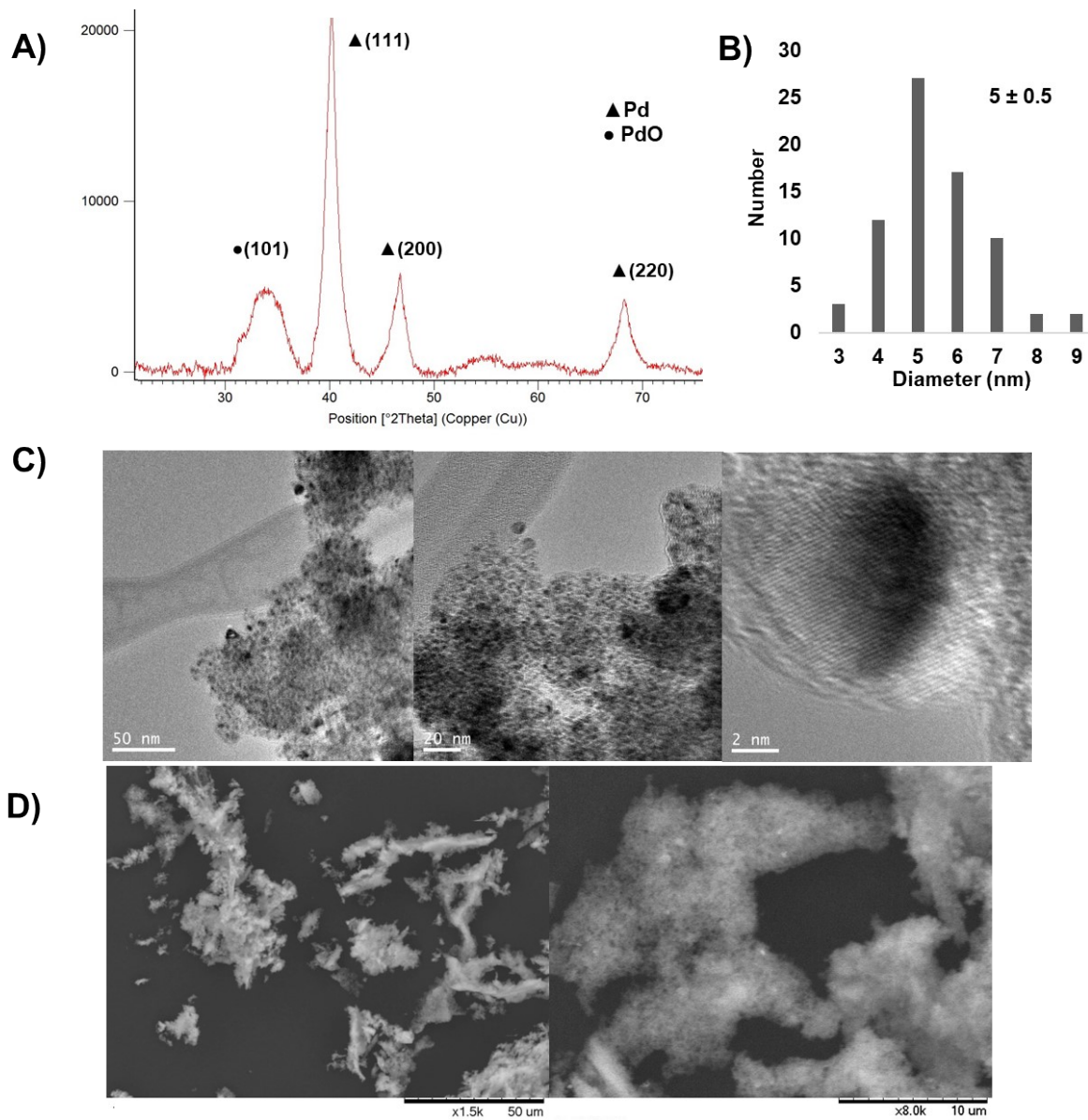
Figure S3. TEM and HR-TEM images of **Pd@CALB60.**

Figure S4. Characterization of **Pd@TLL25. A)** XRD spectrum; **B)** Particle size distribution; **C)** TEM and HR-TEM images; **D)** SEM images.

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           10              20              30              40              50
MRSSLVLFFV  SAWTALASPI  RREVSQDLFN  QFNLFAQYSA  AAYCGKNNDA
           60              70              80              90             100
PAGTNITCTG  NACPEVEKAD  ATFLYSFEDS  GVGDVTGFLA  LDNTNKLIVL
          110             120             130             140             150
SFRGSRSIEN  WIGNLNFDLK  EINDICSGCR  GHDGFTSSWR  SVADTLRQKV
          160             170             180             190             200
EDAVREHPDY  RVVFTGHSLG  GALATVAGAD  LRGNGYDIDV  FSYGAPRVGN
          210             220             230             240             250
RAFAEFLTVQ  TGGTLYRITH  TNDIVPRLPP  REFGYSHSSP  EYWIKSGTLV

PVTRNDIVKI  EGIDATGGNN  QPNIPDIPAH  LWYFGLIGTC  L
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Length:291

Mass (Da):31,807

Figure S5. Amino acids sequence of TLL. Asp, Glu and His in bold. Sequence was obtained from https://www.uniprot.org/uniprot/O59952
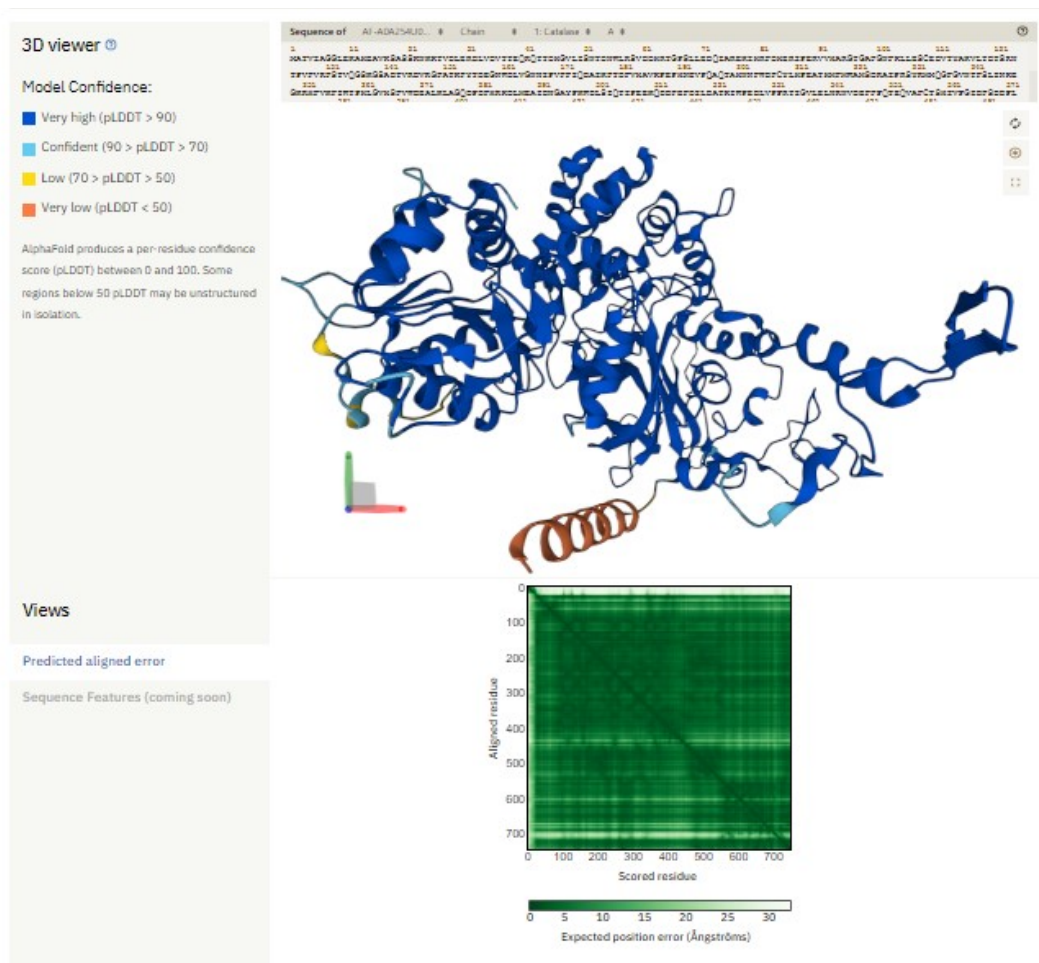
Figure S6. Predicted structure of catalase by AlphaFold, artificial intelligent system developed by DeepMind.
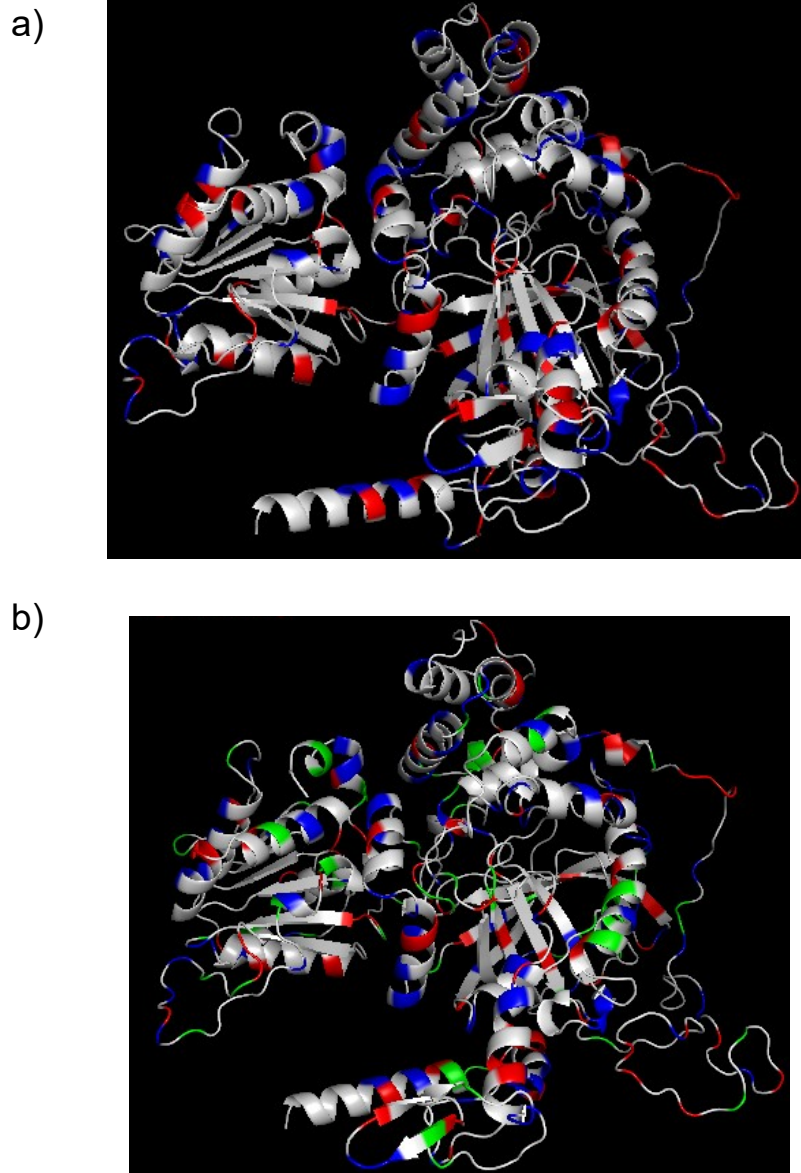
a)

b)

Figure S7. Three-dimensional structure of monomer of tetrameric catalase from *Aspergillus niger*. Structure predicted by AlphaFold and PDB was from AlphaFold. https://alphafold.ebi.ac.uk/entry/A0A254U0S5. Jumper, J *et al*. Highly accurate protein structure prediction with AlphaFold. *Nature* (2021). Image was prepared using Pymol 2.0 software. A) Image structure with Asp and Glu (blue) and Lys and Arg (red). C) Image structure with Asp and Glu (blue), Lys and Arg (red) and Cys, Ser and Tyr (green).
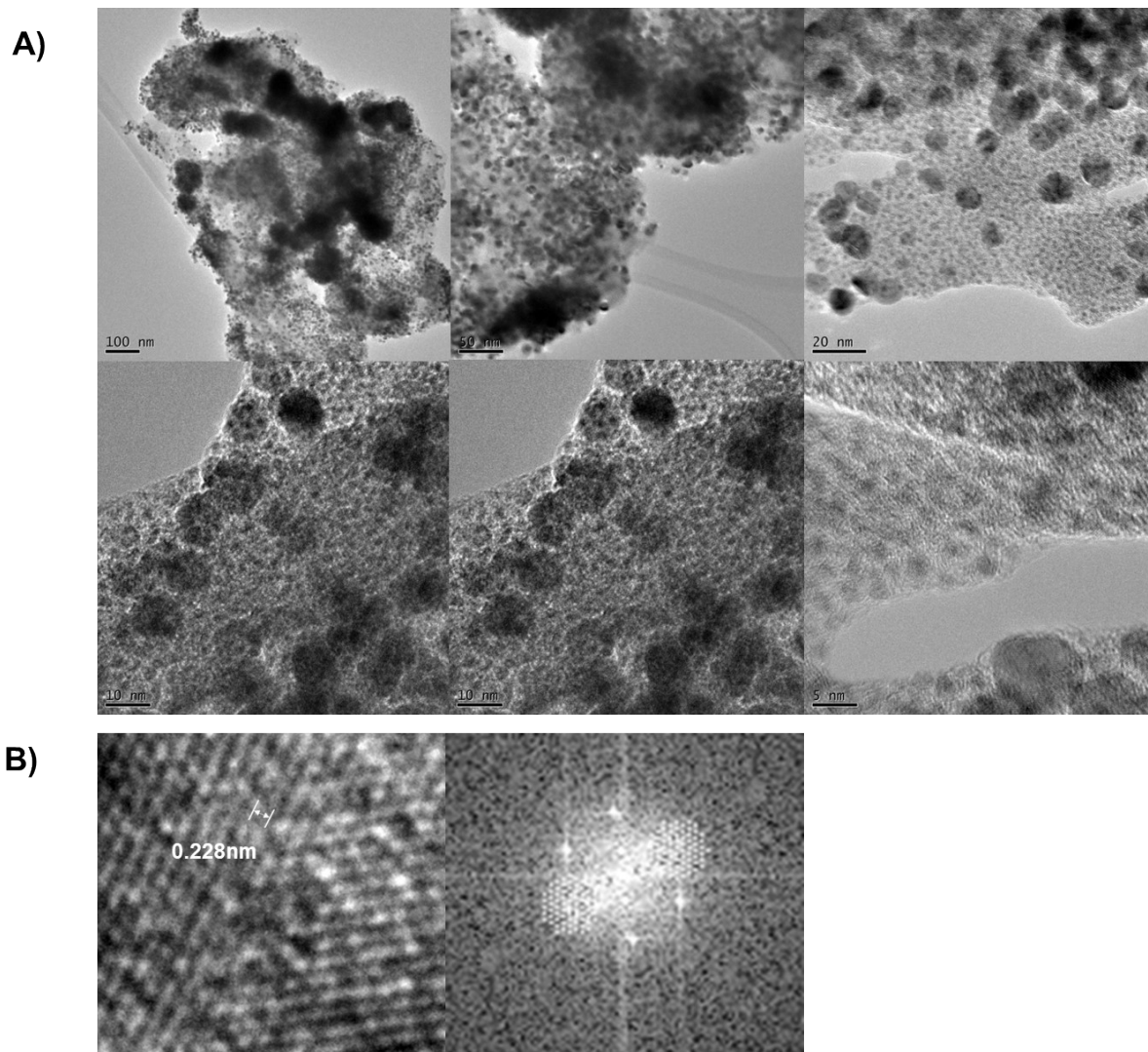
A)

B)

0.228nm

Figure S8. Characterization of **Pd@CAT50. A)** TEM and HR-TEM images; **B)** FFT and IFFT analysis.
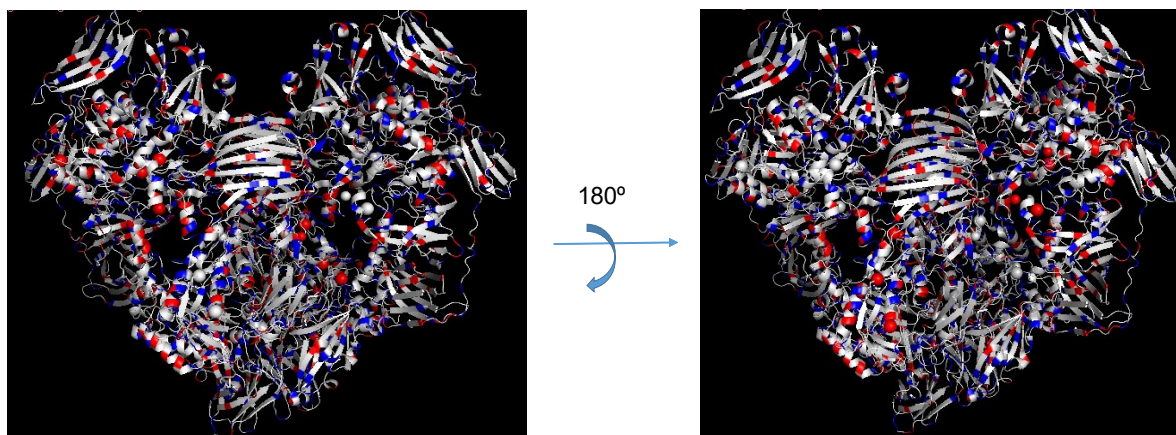
180º

Figure S9. Three-dimensional structure of beta-galactosidase from *Kluyveromyces lactis.* 3OB8 from PDB bank. Structure determined in ref: Pereira-Rodriguez, A., Fernandez-Leiro, R., Gonzalez-Siso, M.I., Cerdan, M.E., Becerra, M., Sanz-Aparicio, J. *Structural basis of specificity in tetrameric Kluyveromyces lactis* beta-galactosidase. J Struct Biol. 2012, 177, 392-401. Asp and Glu (blue), Lys, Arg (red). Mn, Mg (grey balls), Na ions (red balls).
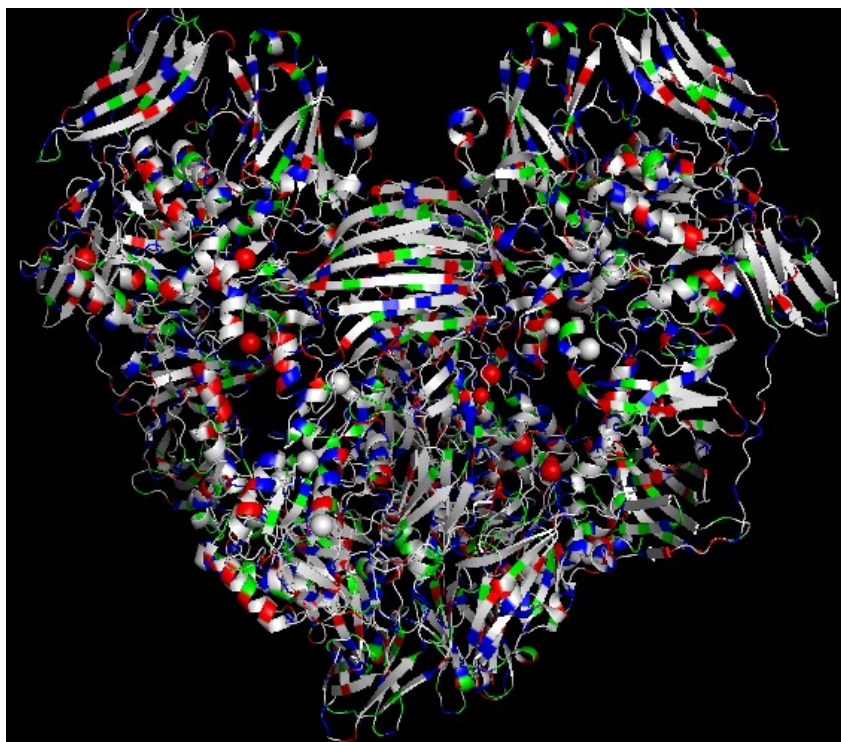
Figure S10. Three-dimensional structure of beta-galactosidase from *Kluyveromyces lactis*. 3OB8 from PDB bank. Structure determined in ref: Pereira-Rodriguez, A., Fernandez-Leiro, R., Gonzalez-Siso, M.I., Cerdan, M.E., Becerra, M., Sanz-Aparicio, J. Structural basis of specificity in tetrameric *Kluyveromyces lactis* beta-galactosidase. J Struct Biol. 2012, 177, 392-401. Asp and Glu (blue), Lys, Arg (red) and Cys, Ser and Tyr (green). Mn, Mg (grey balls), Na ions (red balls).
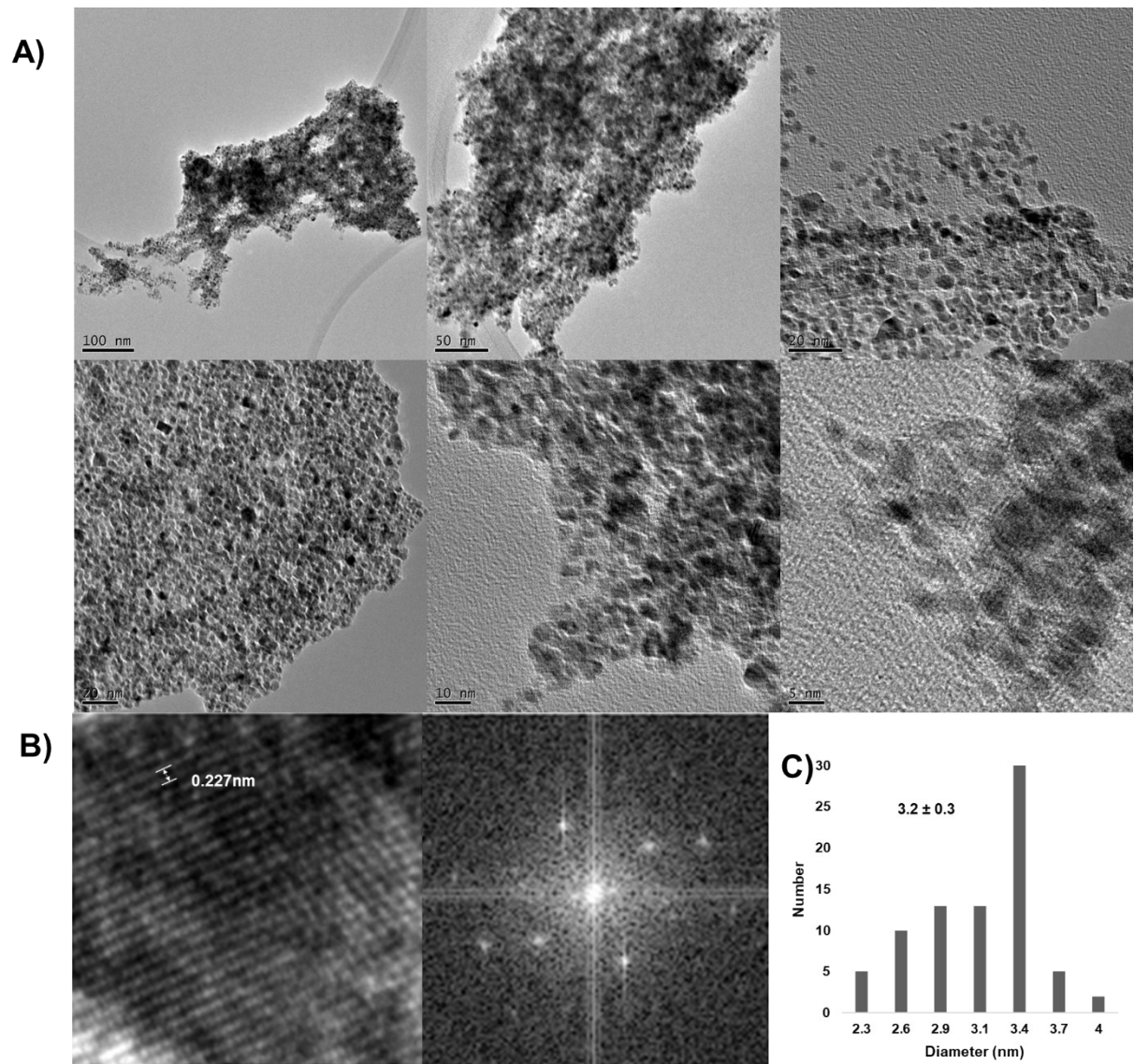
Figure S11. Characterization of **Pd@LAC70. A)** TEM and HR-TEM images; **B)** FFT and IFFT analysis; **C)** Particle size distribution.
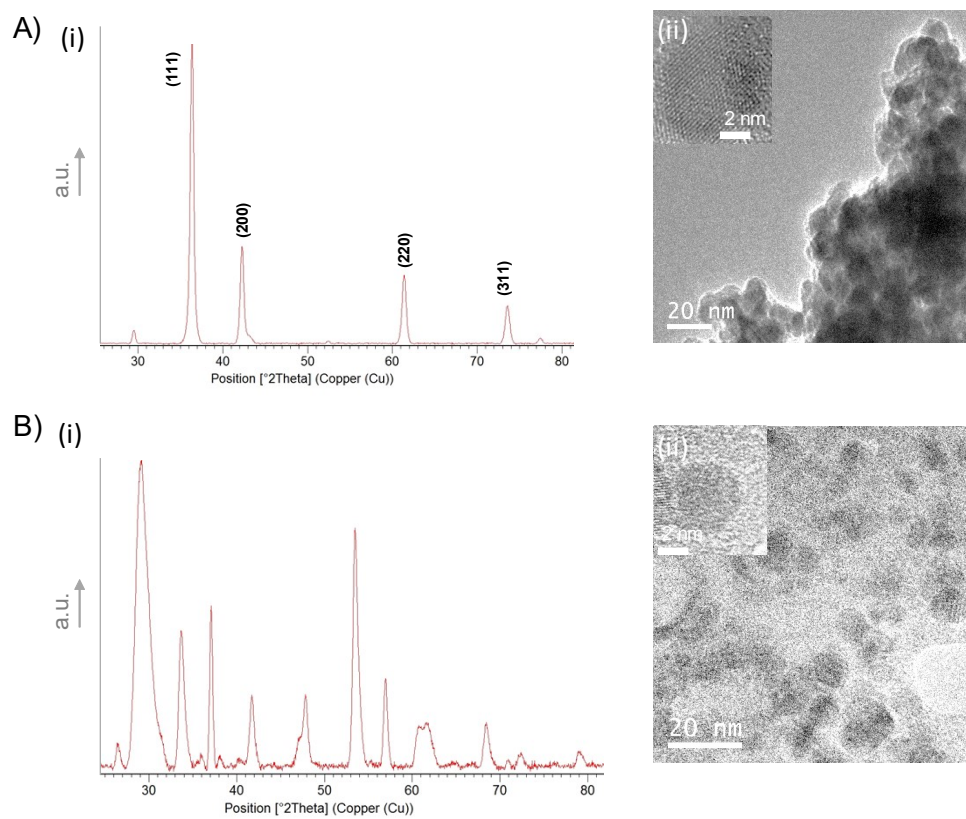
Figure S12. Characterization of Cu hybrids. A) Cu-FOS2. B) Cu-NR. i) XRD analysis, ii) TEM image (inset HRTEM).

Table S1. Determination of Pd% and yield of Pd deposition in the different Pd@enzyme hybrids.

Pd Hybrids	Pd employed (μmol)	Pd content (w/w %)[a]	Amount of Hybrid (mg)	Pd Deposited (μmol)	Pd Deposition yield (%)
Pd@CALB4	188	15	23	32.43	17
Pd@CALB25	188	26	24	58.65	31
Pd@CALB50	188	22	16	33.08	18
Pd@CALB60	188	22	23	47.56	25
Pd@TLL25	188	35	14	46.06	24
Pd@CAT50	188	33	0.6	1.86	1
Pd@LAC70	188	64	9.4	56.55	30

[a] Determined by ICP_OES analysis. The measurement was performed of the solid material. 1 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 Pd content.