Electronic Supplementary Information

Mimicking the bioelectrocatalytic function of recombinant CotA laccase through electrostatically self-assembled bioconjugates

David Alba-Molina,[a] Daily Rodríguez-Padrón,[b] Alain R. Puente-Santiago,[b*] Juan J. Giner-Casares,[a] María T. Martín-Romero,[a] Luis Camacho,[a] Lígia O. Martins,[c] Mario J. Muñoz-Batista,[b] Manuel Cano[a*] and Rafael Luqueb,[b,d*]

Experimental Part

Production of the recombinant CotA Laccase (CotALac)

Recombinant CotA Laccase from Bacillus subtilis was produced using a previously described protocol [1,2] Briefly, pLOM10 plasmid encoding the CotA-laccase gene was transformed into Escherichia coli Tuner (DE3) pLacI strain (Novagen) and cells were grown in Luria-Bertani medium supplemented with ampicillin (100 µg mL-1) at 37 ºC. Gene expression was induced at OD600nm≈0.6 with 0.1 mM isopropyl-D-thiogalactopyranoside, at that time 0.25 mM $CuCl_2$ was added for full copper loaded protein production and growth temperature was switched to 25 ºC. Agitation was stopped after 4 h and cells were harvested by centrifugation after 24 h of growth. Cells were disrupted using a French-Press apparatus and purification was performed using a two-step protocol with a cationic-exchange SP-Sepharose followed by a Superdex-200 size-exclusion column. Purified enzyme was stored at -20 ºC.

Synthesis of CotALac functionalized AuNPs

First, 15 nm and 95 nm monodisperse citrate-stabilized gold nanoparticles (AuNPs) with a uniform quasi spherical shape and a narrow size distribution were synthesized following a kinetically controlled seeded growth strategy via the reduction of $HAuCl_4$ by sodium citrate.[3] Both 15 nm (step 1) and 95 nm (step 11) AuNPs, the molar gold concentration was estimated from the absorbance at 400 nm, which is assumed to have a size independent absorption coefficient.[4]

Next, the resulting 15 nm AuNPs (step 1) and 95 nm AuNPs (step 11) were functionalized with the CotALac enzyme by exploiting the electrostatic self-assembly of the positively charged redox enzyme on the surface of the negatively charged citrate capped AuNPs. For this, 1 mg of CotALac was dissolved in 2 mL of 50 mM sodium phosphate buffer (PBS; pH 7.0; free chloride and fluoride) solution and subsequently added to 2 mL of AuNPs solution (~0.63 mM Au). This mixture was incubated for 24 hours at 4 ºC under gentle stirring. Then, both bioconjugates were collected by centrifugation at 5000 rpm for 5 min at 4 ºC. The supernatant was discarded, and the pellet was resuspended in 2 mL of 50 mM PBS (pH 7.0; free chloride and fluoride) solution. The resulting bioconjugates was stored in the dark at 4 ºC.

Material characterization

The electrostatic surface potential calculations were carried out using the AMBER algorithm to generate the PQR file (PDB2PKA package) and Adaptive Poisson-Boltzmann Solver (APBS package) to generate the DX file. Color representation of the molecules was performed employing the UCSF Chimera 1.11.2 software package and considering a color range from red to blue (from -10 to 10 kT/e, respectively).

UV-visible spectra were recorded on a Cary 100 Bio UV-Vis spectrometer in disposable polystyrene cuvettes with 1.0 cm path length.

Samples for transmission electron microscopy (TEM) were prepared by drying, under ambient conditions, a dispersion of the particles on 200 mesh copper grids coated with Formvar/Carbon film. TEM images were obtained in a JEOL JEM 1400 TEM microscope, operated at an accelerating voltage of 80 kV.

Dynamic light scattering (DLS) and Zeta-potential measurements were carried out using a Malvern Zetasizer Nano ZSP ZEN5600 analyzer equipped with 10 mW laser He-Ne (633 nm). Samples were dispersed in ultrapure water and the measurements were recorded in triplicate (n = 3) at 25 °C.

Fourier-transform infrared (FTIR) spectroscopy was performed in an ALPHA-T Bruker spectrometer. Spectra were recorded at room temperature in a 4000-600 cm^{-1} wavenumber range, using the OPUS software.

X-ray photoelectron spectroscopy (XPS) was carried out in a SPECS mod. PHOIBOS 150 MCD. Prior to the analysis, the sample was evacuated overnight under vacuum (<10-6 Torr). The experiment was accomplished at pressures <10-10 mbar, using a conventional X-ray source (XR-50, Specs, Mg-Kα, hv=1253.6 eV, 1 eV = 1.603 x 10^{-19} J) in a "stop and go" mode. The deconvolution of the obtained curves and element quantification was carried out using the XPS CASA program.

Electrochemical experiments

Cyclic and linear sweep voltammetries were recorded on an AUTOLAB PGSTAT30 electrochemical analyser using a three-electrode system. ITOs (Naranjo Substrates) were used as working electrodes. A drop of 25 µL of sample (i.e. AuNPs, CotALac@AuNPs or pristine CotALac) was loaded onto the clean surface of ITO or GC electrode and then dried overnight at 4 ºC. A platinum sheet and an Ag/AgCl electrode were used as counter and reference electrodes, respectively. A 50 mM phosphate buffer solution (PBS, pH 7.0) was used as supporting electrolyte. All the solutions were purged prior to electrochemical measurements using nitrogen (N_2) or oxygen (O_2) gas.

Results and discussion

Table S1. Particle-diameters determined by TEM and DLS measurements, and Z-potential values of the NPs under physiological conditions.

NPs	TEM size (nm)	Hydrodynamic size (nm) / PDI	Zeta-Potential (mV)
CotALac	-	19.7 / 0.249	-6.9 ± 2.8
15nmAuNPs	14.9 ± 2.4	29.4 / 0.158	-27.4 ± 4.7
CotALac@15nmAuNPs	20.4 ± 3.2	63.9 / 0.264	-9.2 ± 6.2
95nmAuNPs	95.3 ± 5.1	110.3 / 0.173	-30.3 ± 6.1
CotALac@95nmAuNPs	128.3 ± 8.5	141.2 / 0.244	-16.8 ± 8.3

Table S2. XPS binding energy and N/Au atomic ratio.

Sample	Position (eV)					N/Au (at.)
	C1s	N1s	O1s	Au 4f $_{7/2}$		
				Au(0)	Au(I)	
15nmAuNPs	284.6	-	530.8	82.8	84.3	-
95nmAuNPs	284.6	-	530.9	82.9	84.3	-
CotALac@15nmAuNPs	284.6	399.2	530.9	83.0	84.5	7.3
CotALac@95nmAuNPs	284.6	399.3	530.8	82.9	84.4	1.8

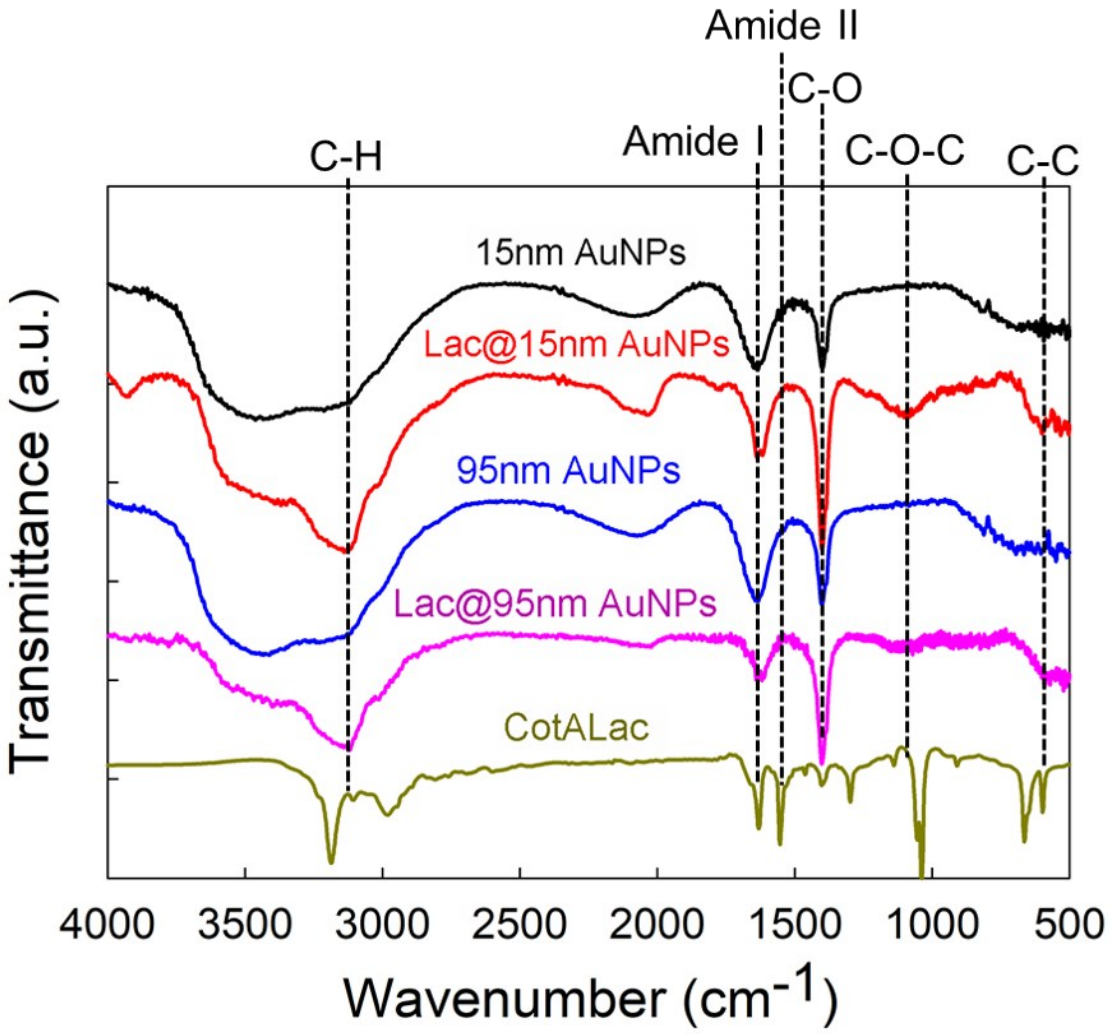

Fig. S1. FTIR spectra of 15nmAuNPs (black line), CotALac@15nmAuNPs (red line), 95nmAuNPs (blue line), CotALac@95nmAuNPs (pink line), and CotALac (dark-yellow line).

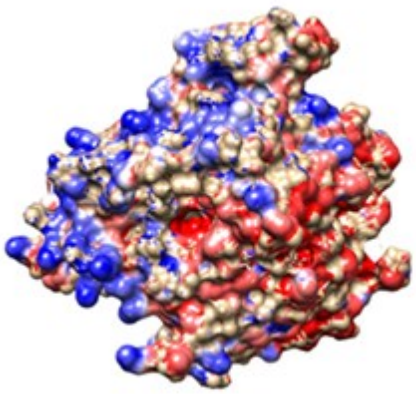

Fig. S2. Electrostatic surface distribution of CotALac at pH 7.0 (PDBID 4YVU) generated by the AMBER algorithm and Adaptive Poisson-Boltzmann Solver package. Blue and red areas represent the positively and negatively charged surface domains respectively.

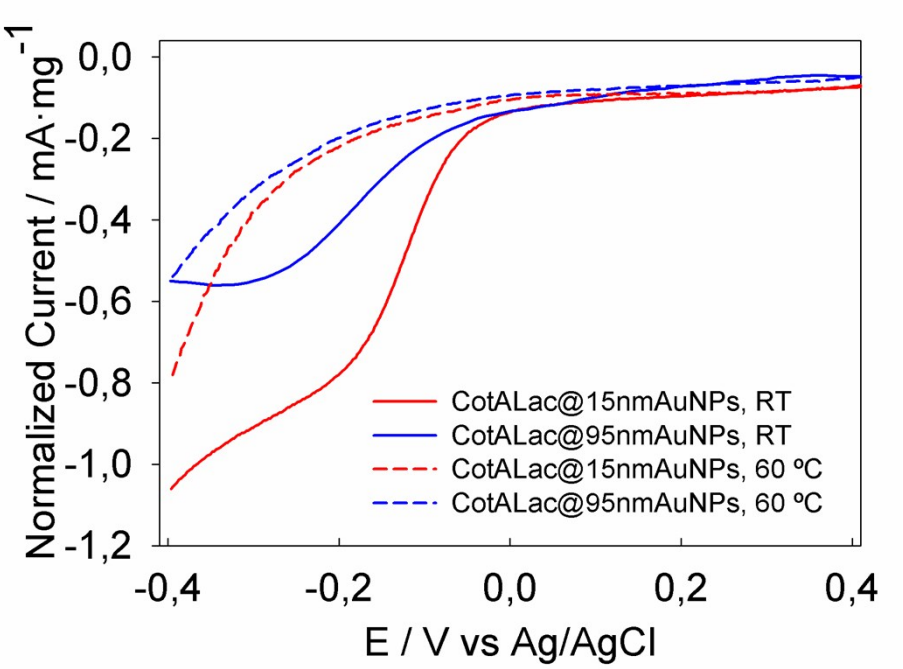

Fig. S3 CVs of ITO modified with CotALac@15nmAuNPs and CotALac@95nmAuNPs in O_2-saturated sodium phosphate buffer (50 mM, pH 7.0) at room temperature (RT) and at 60 ºC. Scan rate: 100 $mV \cdot s^{-1}$.

References

1. I. Bento, L. O. Martins, G. G. Lopes, M. A. Carrondo and P. F. Lindley, *Dalton Trans.*, 2005, 3507-3513.
2. Z. J. Chen, P. Durao, A. T. Fernandes, P. Hildebrandt, D. H. Murgida, S. Todorovic, M. M. Pereira, E. P. Melo and L. O. Martins, *J. Biotechnol.*, 2008, **136**, S320-S320.
3. N. G. Bastus, J. Comenge and V. Puntes, *Langmuir*, 2011, **27**, 11098-11105.
4. J. Rodriguez-Fernandez, J. Perez-Juste, P. Mulvaney and L. M. Liz-Marzan, *J.Phys. Chem. B*, 2005, **109**, 14257-14261.