Electronic Supplementary Information

Measurement of adsorption constants of laccase on gold nanoparticles to evaluate the enhancement in enzyme activity of adsorbed laccase

Miguel Peixoto de Almeida, Pedro Quaresma, Susana Sousa, Cláudia Couto, Inês Gomes, Ludwig Krippahl, Ricardo Franco, and Eulália Pereira *

1. Transmission Electron Microscopy and size histogram of the as-synthesized AuNPs

Figure S1 – TEM micrograph of a representative area of the TEM grid and resulting size histogram showing an average diameter of 13.6 nm with a standard deviation of 2.2 nm.

2. XPS of AuNP-CALNN

Figure S2 – XPS analysis of AuNP-CALNN, showing the areas corresponding to Au, N and S.
3. Dynamic Light Scattering (DLS) measurements for BNC at different AuNP concentrations

Binding constants of laccase to CALNN-functionalized AuNPs (obtained by DLS) are proportional to AuNP concentration (Figure S3), whereas the volumes of the fully packed bionanoconjugates are mostly independent on AuNP concentration (Table 1).

![Graph showing the variation of $1/K_L$ as a function of the concentration of AuNPs. Bars represent the standard error calculated from the fitting of DLS data. Linear fitting of these data is $1/K_L = 30.7 \text{ [AuNP]}$, with $R^2=0.990$.](image)

**Figure S3** – Variation of $1/K_L$ as a function of the concentration of AuNPs. Bars are the standard error calculated from the fitting of DLS data. Linear fitting of these data is $1/K_L = 30.7 \text{ [AuNP]}$, with $R^2=0.990$. 
4. Analysis of Agarose Gel Electrophoresis data

The migration distances for each concentration ratio were computed from the digital image of the electrophoresis gel by fitting Gaussian curves to the image intensity profiles averaged for each lane. This allowed a more reliable quantification of band migration, since the most relevant bands were quite broad.

Figure S4—Processed digital image of the gel and migration quantification. The black lines show the average brightness intensity measured along each lane. The red lines show the fitted Gaussian curves and the peak positions for each lane, with lane 1 corresponding to the leftmost lane on the gel. The horizontal scale is in image pixels.

The Langmuir equation was fit considering that the occupation factor is:

$$\Theta = \frac{\mu_{\text{free}} - \mu}{\mu_{\text{free}} - \mu_{\text{min}}}$$  \hspace{1cm} (S1)

where \( \mu \) is the mobility of each peak; \( \mu_{\text{free}} \) is the maximum mobility, obtained directly from the position of the peak at ratio 0; and \( \mu_{\text{min}} \) is the minimum mobility at full occupation, obtained from the fitting of the curve. The reason for fitting \( \mu_{\text{min}} \) instead of measuring it directly is that a direct measure would make this value too sensitive to the exact placement of the last bands. The curve fitted is the Langmuir isotherm:

$$\Theta = \frac{K_L [LAC]}{1 + K_L [LAC]}$$ \hspace{1cm} (S2)
where $K_L$ is the binding constant and [LAC] the concentration of laccase. Fitting the curve to both $\mu_{\text{min}}$ and $K_L$ gave a $K_L$ of $(0.6 \pm 0.1) \times 10^8 \text{ M}^{-1}$, with a 95% confidence interval determined by residual bootstrapping using 500 replicas.

This analysis was done using eReuss, a gel analysis application currently under development and freely available at https://github.com/lkrippahl/eReuss.

Figure S5 – Langmuir isotherm plot, obtained by fitting $\mu_{\text{min}}$ and $K_{eq}$ to the band migration values at different concentrations of laccase.
5. Fluorescence quenching

![Figure S6](image)

**Figure S6.** Fluorescence emission spectra for laccase fluorescence quenching (excitation at 280 nm) by increasing concentrations of AuNPs (A); and respective Stern-Volmer plot (emission at 330 nm) (B).

6. Calculation of the contributions to the enzyme activity of laccase in solution and adsorbed laccase in the bionanoconjugates

In order to evaluate the molar activity of free laccase and adsorbed laccase, it is necessary to evaluate the concentration of free laccase in the BNC solution. We started by using a simple geometrical model of packing of spheres on a sphere (D. Kottwitz, *Acta Crystallographica Section A* **1991**, **47** (3), 158). Using the hydrodynamic diameter of the AuNPs and the diameter of a globular protein with the laccase molecular weight, a maximum of 30 protein molecules per particle was calculated (see main text). We then calculated the contribution of each laccase adsorbed to the increase in the hydrodynamic volume of bionanoconjugates as:

$$ V_{LAC} = \frac{\Delta V_{H,max}}{30} $$

(S3)
where $\Delta V_{H,\text{max}}$ is the maximum increase in hydrodynamic volume obtained by fitting DLS data to a Langmuir equation (Table 1, main text). The average number of laccase bound per AuNP (N) was then calculated, for each bionanoconjugate studied, using the equation:

$$V_{H,BNC} = V_{H,AuNPS} + N \times V_{LAC}$$  \hspace{1cm} (S4)

where $V_{H,BNC}$ is the hydrodynamic volume of the bionanoconjugates calculated from fitting of the experimental DLS data to equation 1 (main text); $V_{H,AuNPS}$ is the hydrodynamic volume of the parent AuNPs alone.

The amount of laccase adsorbed was then calculated by:

$$n_{LAC,ads} = N \times [AuNP] \times V_{sol}$$  \hspace{1cm} (S5)

where $[AuNP]$ is the concentration of AuNPs and $V_{sol}$ is the volume of the solution.

The amount of free laccase was calculated by:

$$n_{LAC,free} = n_{LAC,\text{total}} - n_{LAC,ads}$$  \hspace{1cm} (S6)

where $n_{LAC,\text{total}}$ is the total amount of laccase in solution.

The contribution of free laccase to the enzymatic activity was then calculated by:

$$act_{LAC,free} = act_{LAC,\text{control}} \times \frac{n_{LAC,free}}{n_{LAC,\text{total}}}$$  \hspace{1cm} (S7)

where $act_{LAC,\text{control}}$ is the enzymatic activity in the control with laccase only.

The enzymatic activity of adsorbed laccase was calculated as follows:

$$act_{LAC,ads} = act_{BNC} - act_{LAC,free}$$  \hspace{1cm} (S8)

where $act_{BNC}$ is the experimental enzymatic activity of solutions containing bionanoconjugates and free laccase.
7. Enzymatic assay

Figure S7 – Enzymatic activity of laccase solutions in the concentration range 15-250 nM. The inset contains the results of the linear correlation. Data in red corresponds to the experimental points in Figure 4.

To evaluate the colloidal stability of the nanoparticles during the enzymatic assay, we have taken UV/vis spectra (Figure S8) and determined the hydrodynamic diameter of the bionanoconjugates before and after the enzymatic essay. The hydrodynamic diameter before the assay was (21.6 ± 6.2) nm and after the assay was (19.8 ± 6.0) nm, showing no evidences for significant aggregation. UV/vis spectra shown in Figure S8 show an increase of the absorbance at 530 nm, due to the formation of the oxidation product of syringaldazine, but no major increase of the bandwidth, nor increase of light scattering at lower wavelengths, the two major changes expected in case of aggregation of the gold nanoparticles.
**Figure S8**: UV/vis spectra of a solution containing bionanoconjugates in the experimental conditions for the enzymatic assay. Curve in green is the spectrum before addition of the substrate syringaldazine, and the curve in blue is after 5 minutes of reaction. The increase in absorbance at 530 nm arises from the oxidation product of syringaldazine and it was used to follow enzymatic kinetics.