

Supplementary Information

Physicochemical Study of the Interaction between Gold Nanoparticles and Lipase from *Candida sp.* (CALB): Insights into the Nano-Bio Interface

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SPR band in function of pH and time

The following SPR spectra correspond to the samples AuNPs@CALB and s_A CALB/AuNPs synthesized in water and PBS buffer at pH 6.8. The spectra were acquired soon after the synthesis (0 h) and after 24 h storage in the fridge. As can be observed, SPR band profiles are very similar for the samples prepared in water and pH 6.8 for both samples, even after 24 h. Besides, the spectrum acquired after 24 h, at pH 6.8, slightly differs from the other samples.

In addition, it is important to mention that for all the results showed in the present article, the samples were always prepared in the same day for all analyses (i.e., were examined in less than 12 h) to avoid enzyme denaturation. In this way, even though buffer medium is more frequently used for enzyme experiments, the observations presented herein show that the use of water has not compromised our results.

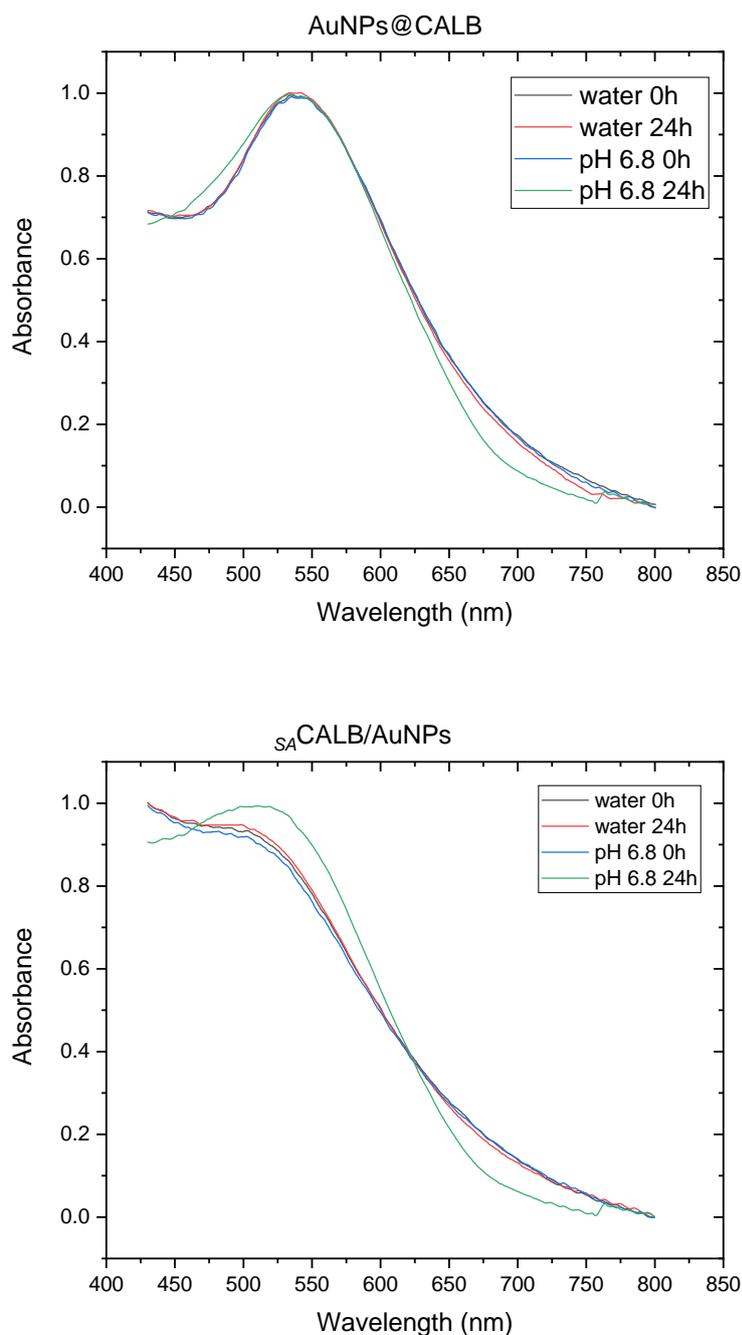


Figure S1. SPR band monitoring of AuNPs@CALB and s_A CALB-AuNPs in water and pH 6.8, recorded at 0 h and after 24 h.

SDS page

To perform the SDS page characterization, electrophoresis was made in a polyacrylamide gel electrophoresis (PAGE) and stained with Coomassie Blue. SDS page was also used to collect information about the nanoparticle-protein interactions (Figure S2) and can be associated with zeta potential results. Lanes A, C, and E correspond, respectively, to AuNPs@CALB, s_A CALB/AuNPs and CALB. Different profiles for CALB and CALB in the presence of AuNPs are clearly observed. For AuNPs@CALB and s_A CALB/AuNPs no protein fraction was noticed in the gel. On the other hand, CALB presented the characteristic band at 33 KDa with few bands at lower molecular weight.

Therefore, these results suggest that the interactions of AuNP and CALB promoted a complex structure, which promoted to the increase of the overall size of these bio-hybrid materials in relation to CALB alone, hindering the run in the gel. We can associate these observations with the variation of zeta potential from -5.27 mV for CALB to -17.5 mV and -27.9 mV in the presence of AuNPs, confirming the changes in the surface surrounding AuNPs and CALB, building up a new kind of bio-hybrid materials.

As a proof of concept, SDS page was carried out for denatured samples. The sample were denatured by boiling, breaking down the enzyme into fragments. The lanes B, D, and F are the respective runs for the denatured samples AuNPs@CALB, s_A CALB/AuNPs and CALB. The profiles of the denatured samples were different from the former ones. Upon boiling, AuNPs@CALB and s_A CALB/AuNPs presented the characteristic band of CALB at 33 KDa. Therefore, suggesting that the complex AuNP-CALB structures can be affected by boiling the samples, releasing CALB from their structure. Furthermore, CALB presented news and more intense bands after denaturation (lane F) indicating other protein fragments. In addition, these other bands were not evidenced in presence of AuNPs (lanes B and D) which suggests that the interactions AuNP/CALB are efficient at the point of somehow protecting CALB from denaturation.

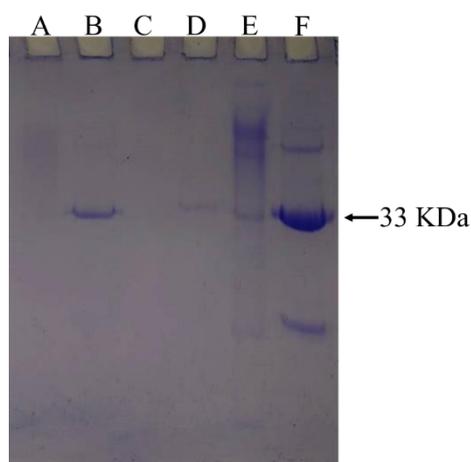


Figure S2. SDS page of AuNPs @CALB (lane A), s_A CALB/AuNPs (lane C) and CALB (lane E) and the respectively denatured sample after boiling AuNPs @CALB (lane B) and s_A CALB/AuNPs (lane D) and CALB (lane F).