Supplementary Information

Gold nanoparticles/silver-bipyridine hybrid nanobelts with tuned peroxidase-like activity

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Reagents and apparatus

All reagents were purchased from Sigma-Aldrich.

Transmission electron microscopy (TEM) and high resolution field emission scanning electron microscopy (FE-SEM) were performed with JEOL JEM 2100 and JEOL JSM-6335F microscopes, respectively. Amperometric measurements were performed with an Inbea potentiostat. Cyclic voltammetry (CV) were performed using a FRA2 μAutolab Type III potentiostat/galvanostat. A conventional three-electrode system was employed in the electrochemical studies, where the working electrode was a glassy carbon electrode (GCE, 3.0 mm diameter) modified with glucose oxidase and/or the AuNP-decorated hybrid material. An Ag/AgCl/KCl (3 M) and a Pt wire were used as reference and counter electrodes, respectively. The measurements with the biosensor were carried out at 25ºC in 0.1 M sodium phosphate buffer, pH 7.0 (working volume 10 ml).

Synthesis of the polyfunctionalized AuNP[1]

$HAuCl_4$ (50 mg) was dissolved in 12.7 mL of de-aerated DMSO. This solution was added dropwise to 12.7 mL of de-aerated DMSO containing 60 mg sodium borohydride, 10 mg 4-

mercaptopyridine and 36.2 mg 6-mercapto-1-hexanol under vigorous stirring. The reaction mixture turned deep brown immediately, but the reaction was continued for 24 h. The functionalized AuNPs were then precipitated by adding 25 mL CH_3CN, collected by centrifugation, and washed with 50 mL CH_3CN:DMSO (1:1 v/v), 50 mL ethanol and 10 mL diethyl ether. The nanoparticles were finally isolated by centrifugation and dried under N_2. The polyfunctionalized AuNP were characterized by TEM and FT-IR.

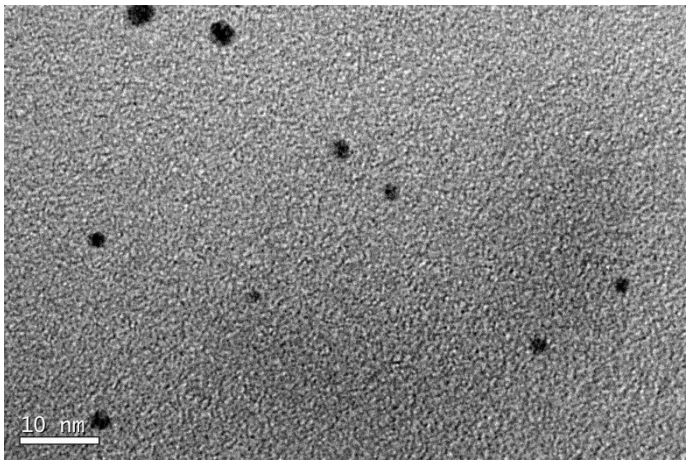

Fig. 1S TEM image of polyfunctionalized AuNP.

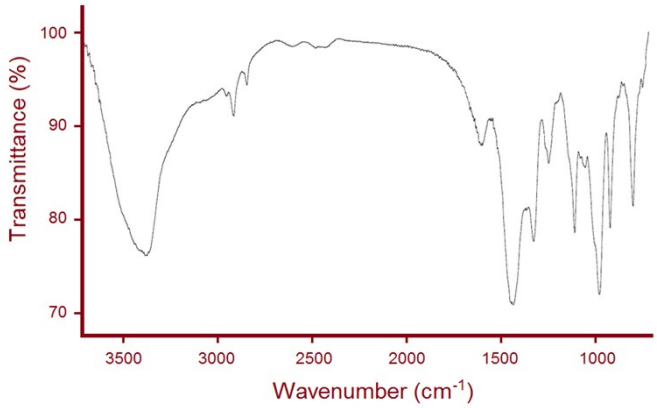

Fig. 2S FT-IR of polyfunctionalized Au nanoparticles.

FT-IR analysis revealed the presence of 4-mercaptopyridine moieties on the Au nanoparticles surface by the peaks at 16255 cm^{-1}, 14554 cm^{-1} and 13478 cm^{-1}, which can be ascribed to the symmetric wag-stretch, symmetric C-H wag and C-H wag in the aromatic pyridine ring, respectively. On the other hand, 6-mercapto-1-hexanol residues were identified by the broad peak at 3380 cm^{-1} corresponding to the stretching of the O-H groups, as well as by the peaks at 29212 cm^{-1} and 1003 cm^{-1}, which can be ascribed to the stretching of the C-H and C-O groups, respectively.

Preparation of the AuNP-decorated hybrid nanomaterial

The AuNP-decorated nanohybrids were prepared by modification of the method reported by Luo *et al.* for silver:bipyridine nanobelts.[2] Aqueous solutions of AuNP (10 mg/mL) and $AgNO_3$ (34 mg/mL), and ethanolic solution of 4,4´-bipyridine (16 mg/mL) were freshly prepared. Nanohybrids with different content of AuNP were prepared by mixing different volumes of AuNP stock solution (0, 50, 100, 150, 200 and 300 μL) with 1 mL $AgNO_3$ and 2 mL 4,4´-bipyridine stock solutions under gentle continuous stirring at room temperature. After 10 min, the resulting precipitate was centrifuged, repeatedly washed with double distilled water and ethanol and finally dispersed in ethanol up to 14 mg/mL final concentration.

The Au/Ag molar ratio in these hybrid nanomaterials was determined by X-ray fluorescence spectroscopy, and the results are shown in Table 1S.

Table 1S Au/Ag ratio in the hybrid nanomaterials

Sample	Au/Ag (mol/mol)
AuNP/$AgNO_3$-0	-
AuNP/$AgNO_3$-50	0.043
AuNP/$AgNO_3$-100	0.093
AuNP/$AgNO_3$-150	0.151
AuNP/$AgNO_3$-200	0.188
AuNP/$AgNO_3$-300	0.287

Enzymatic assay

The peroxidase-like activity of the nanomaterials was evaluated by recording the changes in A_{405nm} at 25ºC in 40 mM sodium phosphate buffer, pH 7.0, using 9 mM ABTS and 3 mM H_2O_2 as substrates, according to the protocol previously reported by Jiang & Penner for peroxidase enzyme.[3]

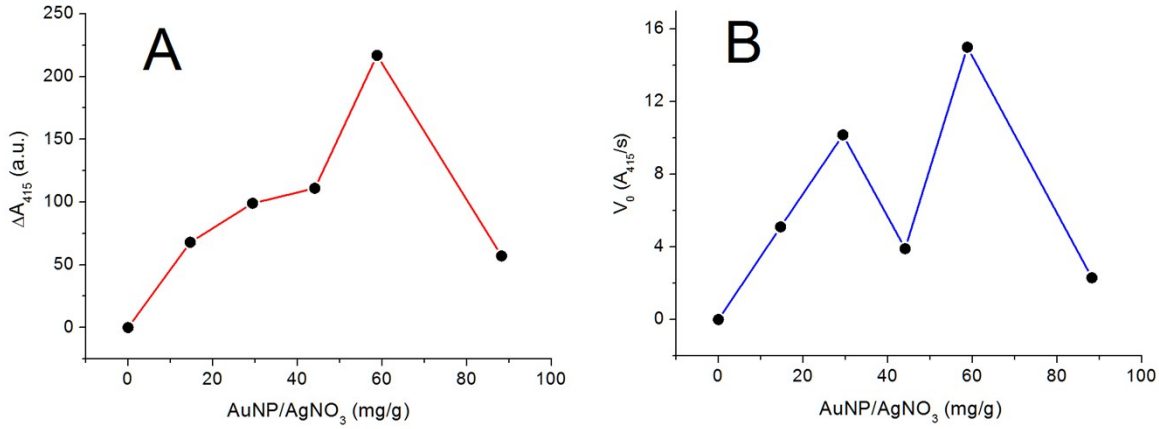

Fig. 3S Effect of AuNP-decorated nanohybrid composition on the maximum increase of absorbance at 405 nm (A) and initial velocity (B).

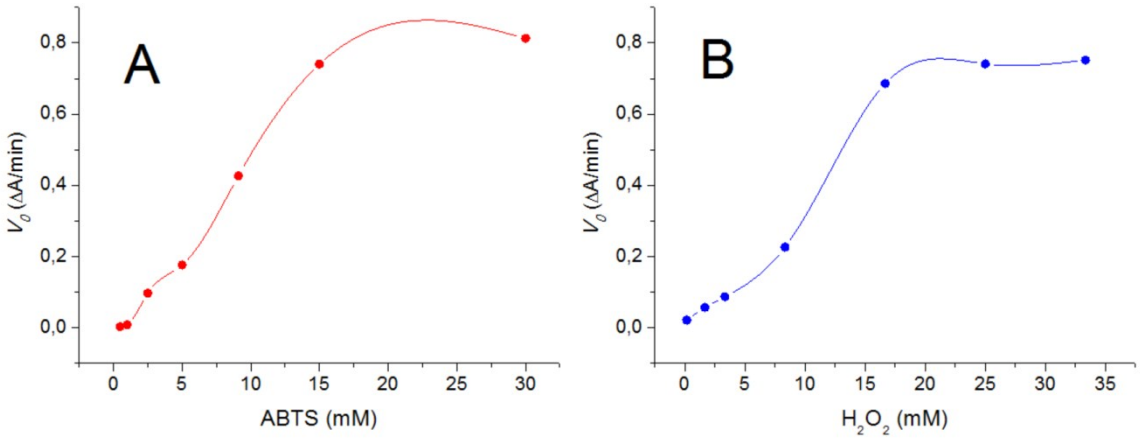

Fig. 4S Effect of ABTS (A) and H_2O_2 (B) concentration on the initial rate for the nanohybrid-catalyzed reaction. Fixed H_2O_2 concentration in (A) = 3 mM; fixed ABTS concentration in (B) = 9 mM.

Preparation of the electrodes

To construct the amperometric sensor for H_2O_2, a polished GCE was coated with 20 µL of the ethanolic dispersion of the nanohybrid and allowing drying. To construct the enzyme biosensor for glucose, glucose oxidase was further immobilized on the nanostructured electrode by dropping 10 µL of a 10 mg/mL enzyme solution in 50 mM sodium phosphate buffer, pH 7.0, and mixed with 2 µL of 25% (v/v) glutaraldehyde. The electrode was kept at 4ºC for 1 h, then washed several times with cold 50 mM sodium phosphate buffer, pH 7.0, dried and finally stored in refrigerator until use.

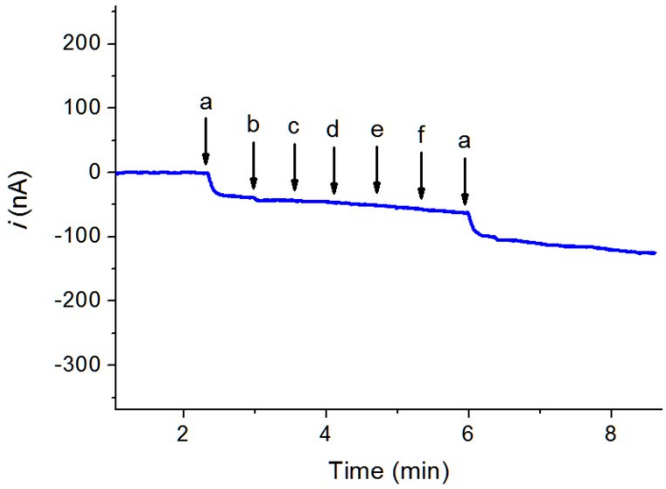

Fig. 5S Amperometric response of the the glassy carbon electrode coated with the AuNP-decorated nanohybrid toward H_2O_2 (a), ascorbic acid (b), uric acid (c), glucose (d), L-tyrosine (e) and sucrose (f) at 100 µM final concentration. E_{app} = -100 mV, stirring condition: 300 rpm.

References

[1] R. Villalonga, P. Díez, M. Eguílaz, P. Martínez, J.M. Pingarrón, *ACS Appl. Mater. Interfaces,* 2012, **4,** 4312.

[2] Y. Luo, W. Lu, G. Chang, F. Liao, X. Sun, *Electrochim. Acta*, 2011, **56**, 8371.

[3] S. Jiang, M.H. Penner, *Anal. Biochem.*, 2015, **476**, 20.