Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supporting Information for the paper:

Enzymatic Self-Wiring in Nanopores and its Application in Direct Electron Transfer Biofuel Cells

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Determination of the active contents of GOx and BOD and their enzymatic activities on the electrode surfaces

The assessment of the active contents of GOx and BOD and their enzymatic activities on the electrode surfaces was carried out through a series of colorimetric measurements employing 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), $ABTS^{2-}$ as an enzymatic mediator and spectroscopic reporter. The BOD assay was based on the enzyme's catalytic reduction of O_2 to H_2O_2 in the presence of $ABTS^{2-}$, and the spectroscopic detection of the blue-green $ABTS^{-\cdot}$ radical, λ_{max}=422 nm, concurrently generated during the transformation. The GOx assay utilized the enzyme's catalyzed oxidation of glucose to gluconic acid with the concurrent reduction of O_2 to H_2O_2. In the presence of horseradish peroxidase, HRP, and $ABTS^{2-}$, the generated H_2O_2 was further reduced to H_2O, while simultaneously oxidizing $ABTS^{2-}$ to $ABTS^{-\cdot}$. Performing the assays at different stages of the electrode modification, as exemplified for GOx in Figure S1, allowed us to determine the total protein content (Γ_{ads}) as well as the activities of the enzymes adsorbed on the MPCNP electrodes. It should be noted that the calculations assume no loss of activity due to interactions with the MPCNPs matrix by the desorbed GOx.

The protein coverage values were calculated according to:

$$\Gamma_{ads} = [n_{tot} - (A_{des}/A_{tot})\cdot n_{tot}]/S_{electrode}$$

The activity of the enzymes on the surface relative to the solution is:

$$\%Act = 100 \cdot A_{ads}/(A_{tot}-A_{des})$$

Where n_{tot} represents the number of moles of the respectively assayed enzyme used in the modifying solution, A_{tot} is the absorbance of the modifying solution, A_{des} is the absorbance of the buffer solution that was allowed to interact with the enzymatically-adsorbed (and PtNCs-grown in the case of GOx) surface, and A_{ads} is the absorbance recorded for a buffer solution assayed in the presence of the enzymatically-adsorbed (and PtNCs-grown in the case of GOx) electrode. In all cases a constant 1.5 mL volume cuvette was used.

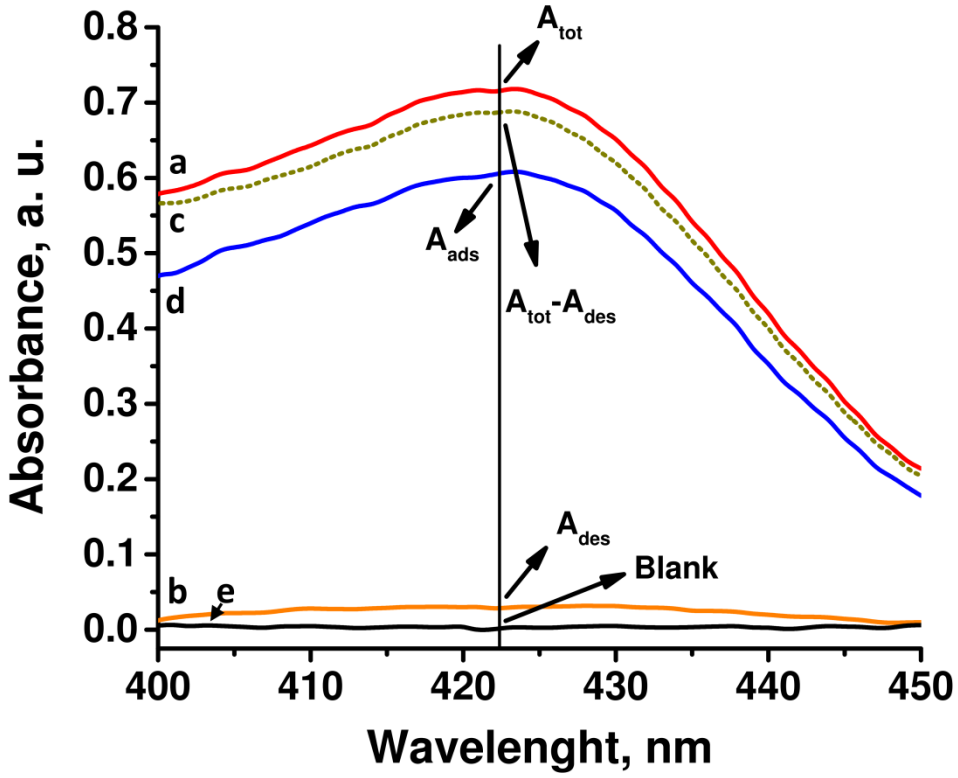

Figure S1. Absorbance spectra obtained by assaying: (a) The modifying solution - a buffer solution containing $3.75 \cdot 10^{-12}$ mole GOx, prior to interaction with the MPCNPs matrix. (b) A buffer solution in which the 60 minutes-grown PtNC@GOx/MPCNPs electrode was immersed for 5 minutes. (c) A spectrum corresponding to the difference in absorbance between (a) and (b), reflecting the equivalent absorbance by the total protein units on the surface. (d) The assay of a buffer solution in the presence of the GOx-adsorbed PtNC@GOx/MPCNPs electrode. The optical measurement was performed in the absence of the electrode in the cuvette. (e) The background absorbance of the buffer solution and all assay components excluding GOx. -Assay duration was in all cases 2 minutes. All measurements were performed in a HEPES buffer (0.1M, pH=7.0) containing 20 nmole HRP, 25 mM glucose and 3.5 mM $ABTS^{2-}$.

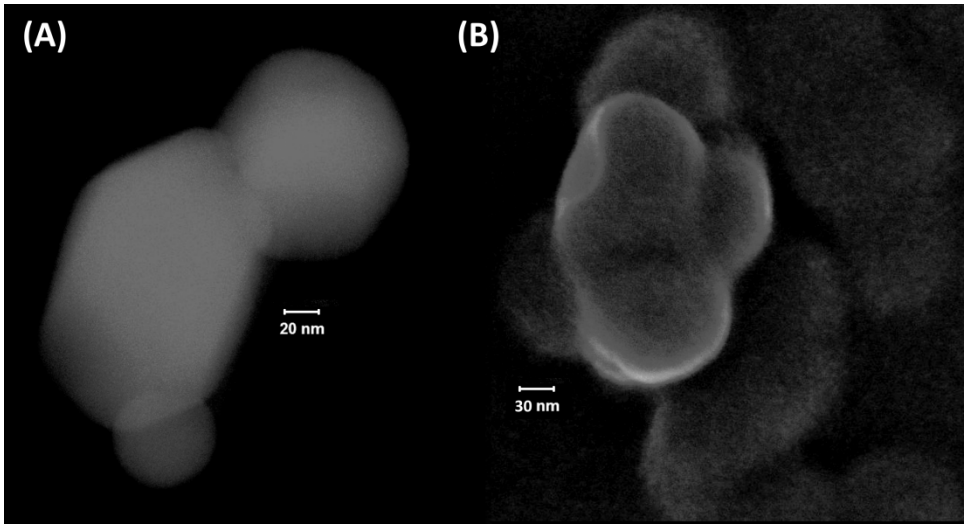

Figure S2. STEM images corresponding to attempts to: (A) enzymatically synthesize the PtNC@GOx/MPCNPs assembly for 60 minutes in the absence of glucose, and (B) enzymatically synthesize PtNC on MPCNPs in the presence of glucose, yet using an inert albumin enzymatic cap instead of GOx.

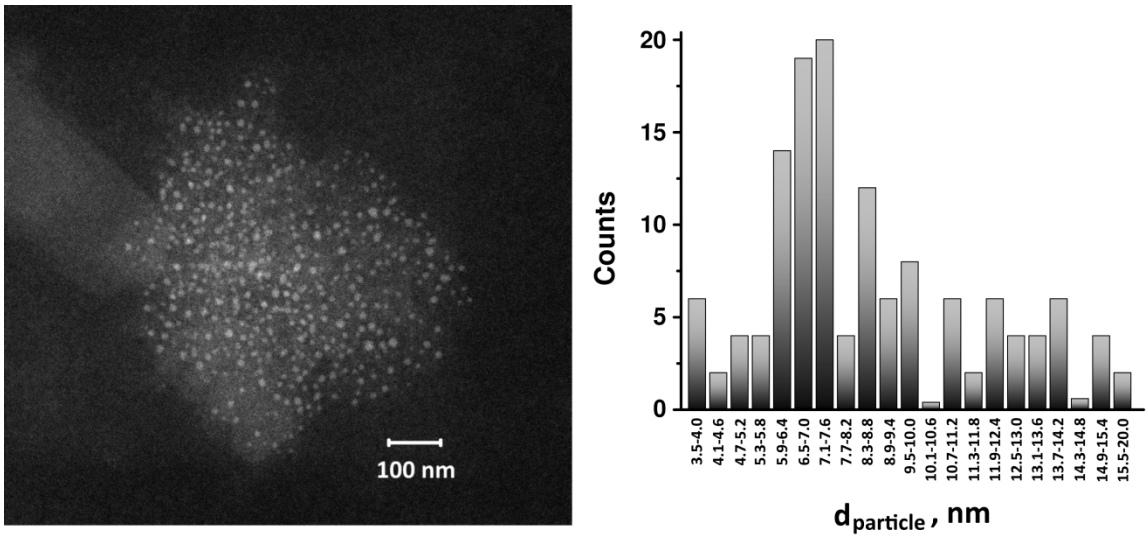

Figure S3. Left side: A STEM image corresponding to a cluster of PtNC@GOx hybrids synthesized in solution for 60 minutes. Right side: Histogram showing the size distribution of the PtNCs in the solution phase enzymatically grown assembly.

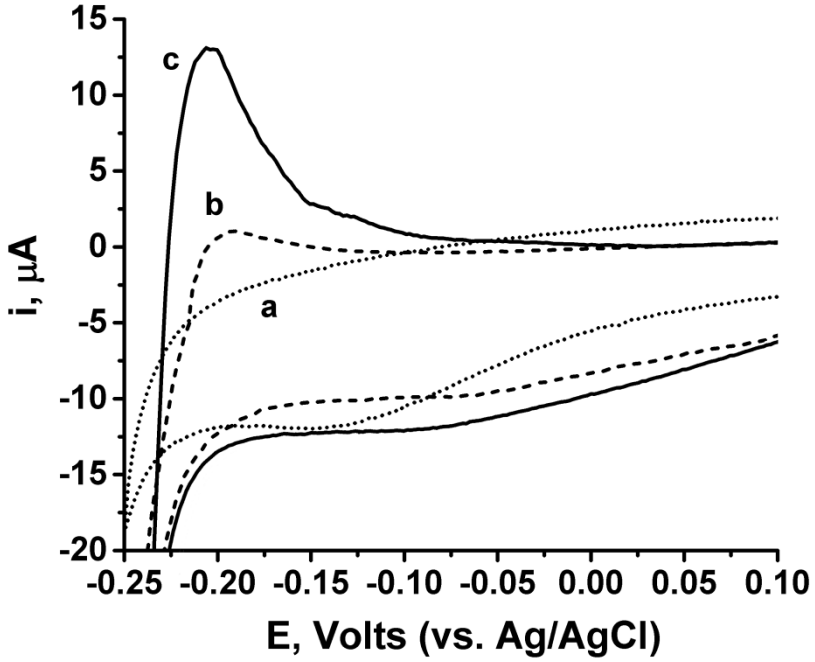

Figure S4. Cyclic voltammograms corresponding to: (a) An attempt to enzymatically synthesize the PtNC@GOx/MPCNPs assembly for 60 minutes in the absence of glucose, (b) 15 minutes enzymatically grown PtNC@GOx/MPCNPs assembly, and (c) 60 minutes enzymatically grown PtNC@GOx/MPCNPs assembly. All samples were recorded in a N_2-purged 0.2 M H_2SO_4 at a scan rate of 10 $mV \cdot s^{-1}$.

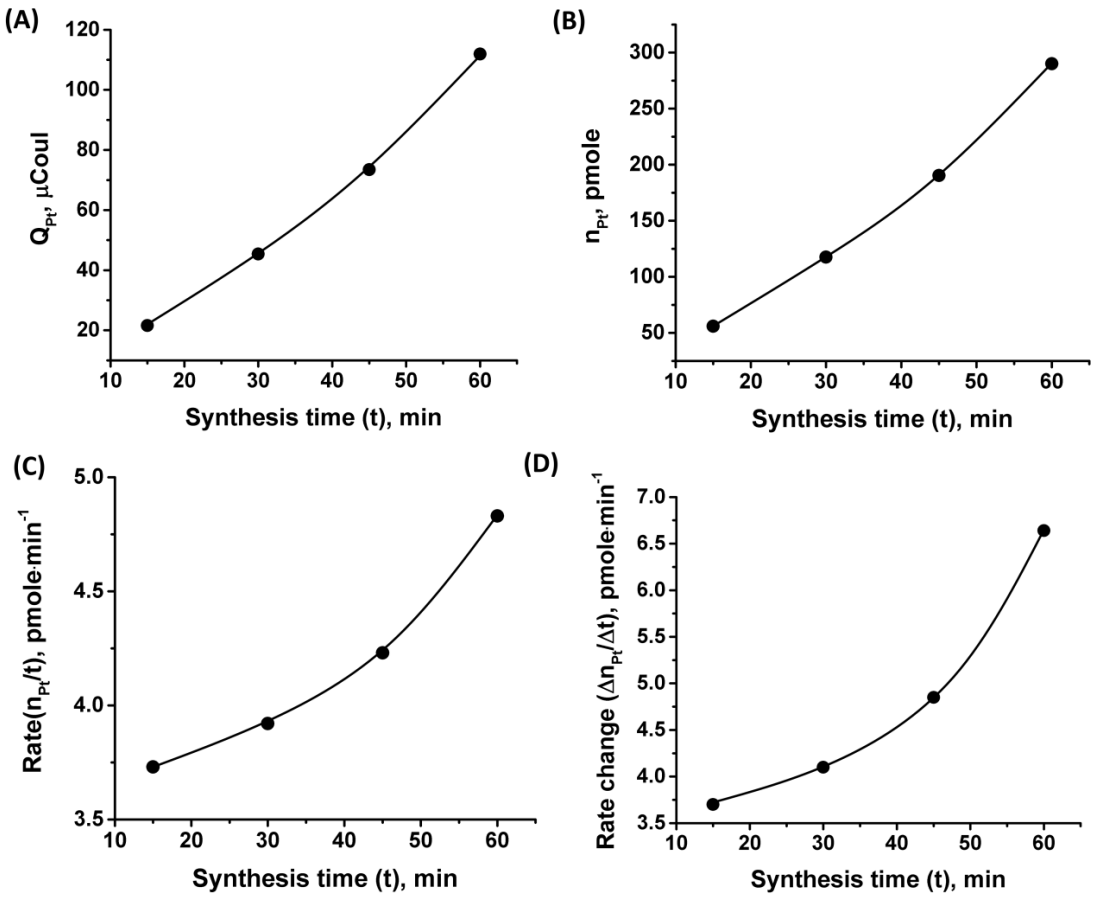

Figure S5. Monitoring the enzymatic growth of the PtNCs based on the time-dependent coulometric responses recorded following the synthesis of the different PtNC@GOx/MPCNPs assemblies. (A) The coulometric responses, at -0.1<E<-0.23 V vs. Ag/AgCl, of Pt-adsorbed H, recorded for the different time-grown assemblies. (B) The equivalent moles of Pt generated in the different time-grown assemblies. (C) Rate of Pt generation in the different assemblies. (D) Rate of change in Pt generation between the different assemblies. The coulometric values were evaluated from cyclic voltammograms recorded in a N_2-purged 0.2 M H_2SO_4 at a scan rate of 10 mV·s^{-1}.

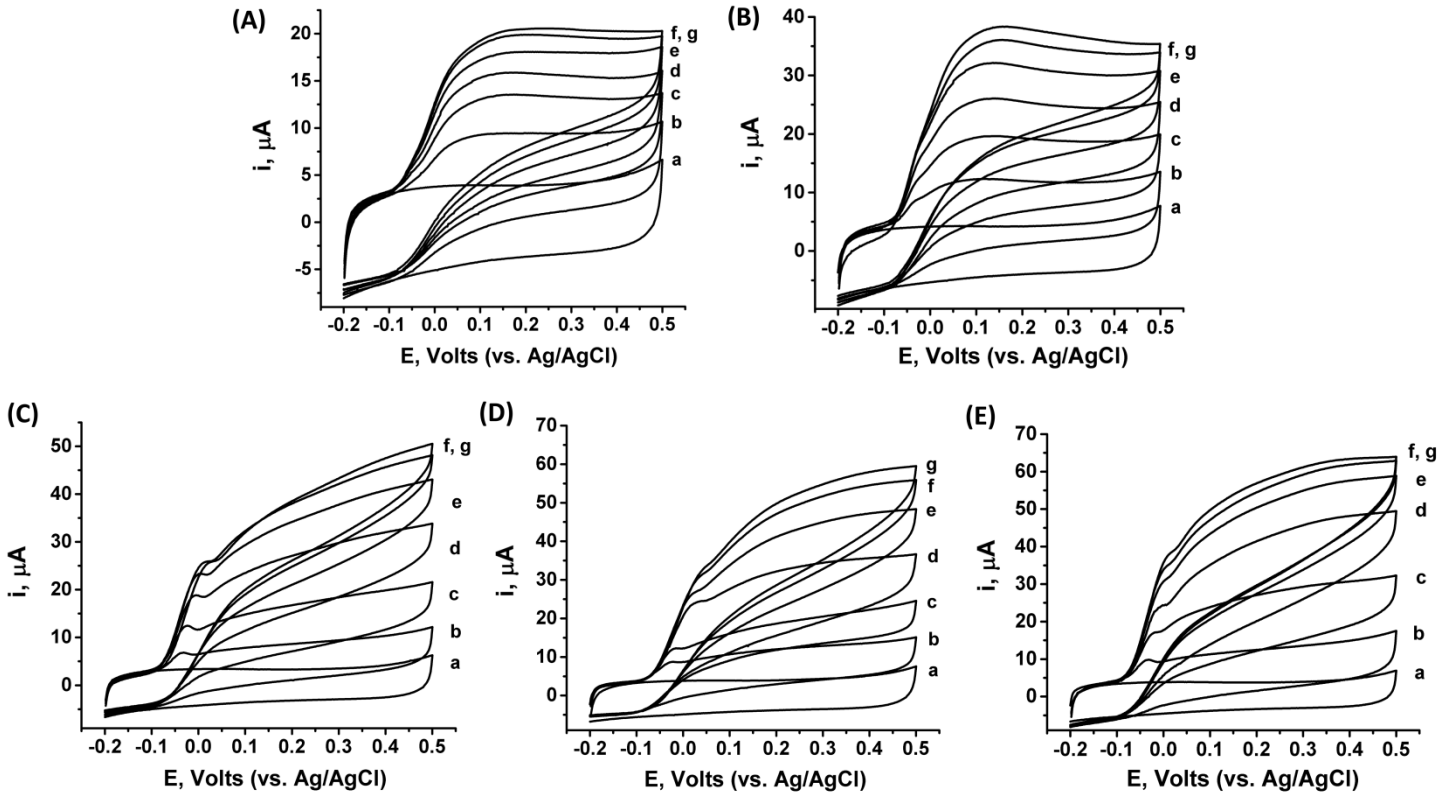

Figure S6. Cyclic voltammograms showing bioelectrocatalytic currents on inside-out-synthesized PtNC@GOx/MPCNPs assemblies that were enzymatically grown for variable time-intervals: (A) 15, (B) 30, (C) 45, (D) 60, and (E) 90 minutes. The curves correspond to a gradual increase in the glucose concentration in the electrolyte from 0 mM to saturation in 10 mM steps. All measurements were performed in a N_2-saturated HEPES buffer (0.1 M, pH=7.0) at a scan rate of 10 $mV \cdot s^{-1}$.

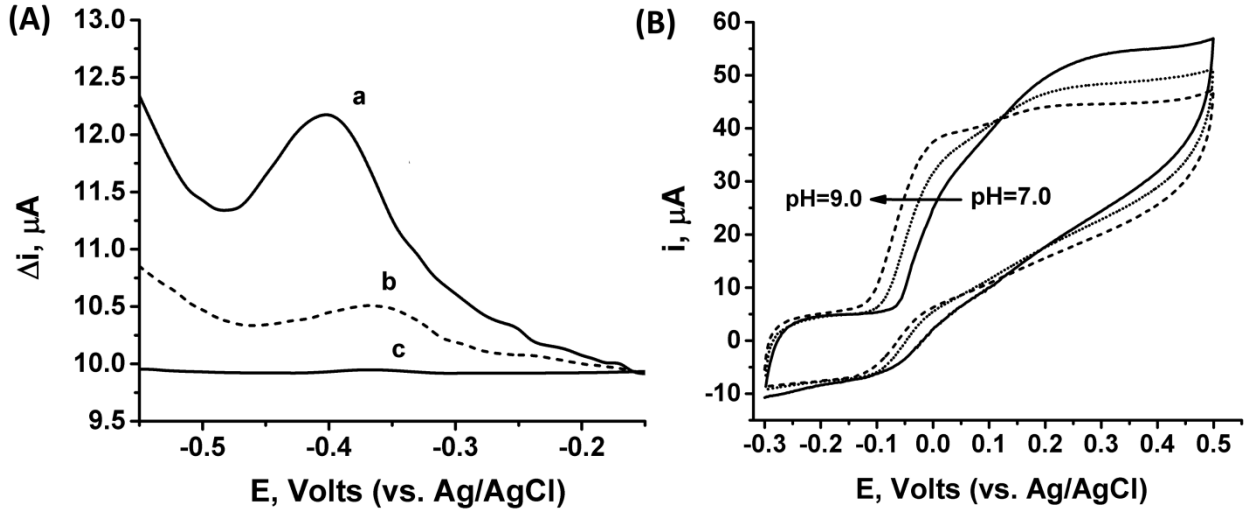

Figure S7. (A) Differential pulse voltammograms, DPVs, corresponding to: (a) the enzymatically inside-out-grown PtNC@GOx/MPCNPs assembly, (b) the electrochemically outside-in-grown PtNC-GOx/MPCNPs assembly, and (c) the base MPCNPs matrix. Measurements were performed in a N_2-saturated HEPES buffer (0.1 M, pH=7.0). (B) Cyclic voltammograms corresponding to bioelectrocatalytic currents by the 60 minutes enzymatically grown PtNC@GOx/MPCNPs assembly in N_2-saturated HEPES buffer containing 60 mM glucose at three different pHs: 7.0, 8.0, and 9.0. All measurements were performed at a scan rate of 10 $mV \cdot s^{-1}$.

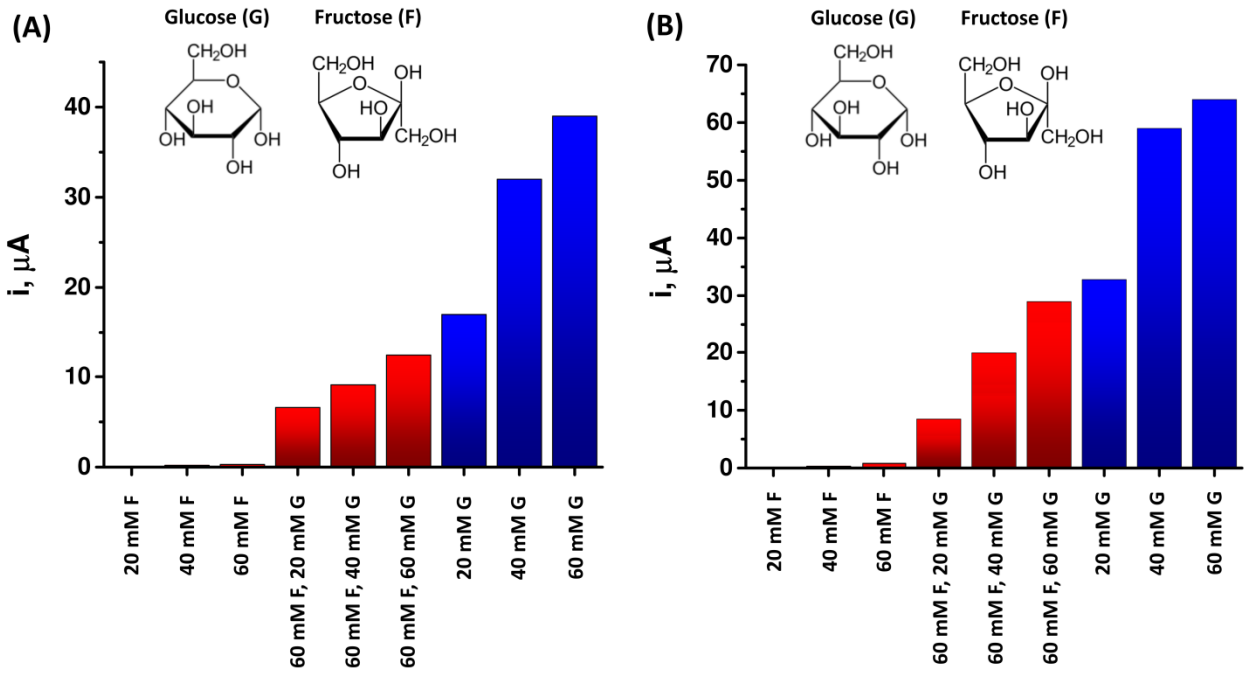

Figure S8. Comparative experiments showing the current responses for a 90-minutes grown PtNC@GOx/MPCNPs assembly in the presence of fructose (F), glucose (G), and their mixtures. (A) Measurement of the pre-catalytic peak at ca. E=0.0 V and (B) Measurement of the bioelectrocatalytic current at E=0.5 V vs. Ag/AgCl. All measurements were performed in a HEPES buffer (0.1 M, pH=7.0) at a scan rate of 10 $mV \cdot s^{-1}$.

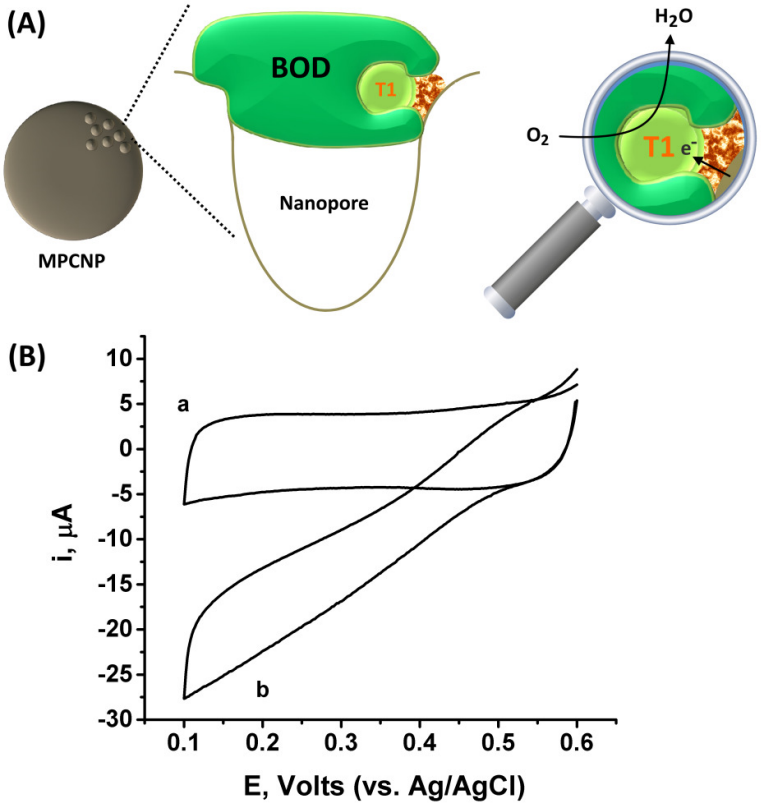

Figure S9. (A) Illustration of the BOD-capped MPCNPs cathode system. (B) DET bioelectrocatalysis by the BOD-capped MPCNPs cathode. Curve (a) is recorded in a N_2-saturated buffer and curve (b) is recorded in an O_2-saturated HEPES buffer (0.1 M, pH=7.0).

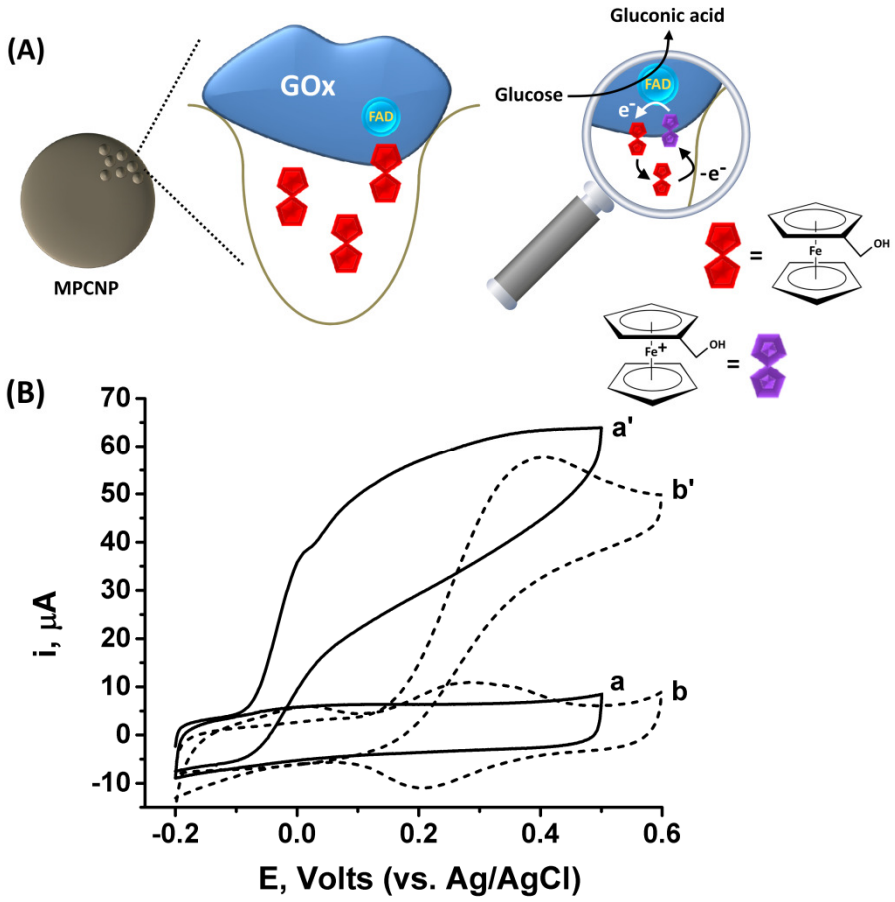

Figure S10. (A) Illustration of the FcMe entrapped, GOx-capped MPCNPs anode system. (B) Cyclic voltammograms corresponding to: (a) and (a') the enzymatically grown PtNC@GOx/MPCNPs assembly in the absence and the presence of 60 mM glucose, respectively, and (b) and (b') the FcMe entrapped, GOx-capped MPCNPs assembly in the absence and the presence of 60 mM glucose, respectively. All measurements were performed in a N_2-saturated HEPES buffer (0.1 M, pH=7.0) at a scan rate of 10 $mV \cdot s^{-1}$.

(1) $[glucose] + [FAD/Pt] \underset{k_{des}}{\overset{k_{ads}}{\rightleftarrows}} [FAD/Pt\ (glucose)]$

(2) $[FAD/Pt(glucose)] \xrightarrow{k_{cat}} [FADH_2/Pt] + [gluconic\ acid]$

Following the Michaelis-Menten kinetics $K_M = \frac{k_{cat} + k_{des}}{k_{ads}}$

$k_{cat} = \frac{V_{max}}{[FAD/Pt]_{tot}}$, when $[FAD/Pt(glucose)] = [FAD/Pt]_{tot}$

Under steady state conditions, the rate thus becomes:

$$v = \frac{k_{cat}[FAD/Pt]_{tot}[glucose]}{K_M + [glucose]}$$

The current density of the bioelectrocatalytic process, which corresponds to the electrochemical regeneration of the cofactor, is proportional to the rate of equation (3):

(3) $[FADH_2/Pt] \xrightarrow{k_{electrochem}} [FAD/Pt]$

$k_{electrochem}$ reflects the wiring efficiency in the system.

The enzymatic turnover rate of electron transfer, k_{et}, values reported in the manuscript for the anodic assemblies reflect the overall kinetics involved in the processes described by equations (1)-(3) per single enzyme on the electrode surface. k_{et} is determined experimentally and evaluated by:

(4) $k_{et} = i_{max} / nFA\Gamma_{eff}$

Where i_{max} refers to the bioelectrocatalytic saturation current at E=0.5 V vs. Ag/AgCl, n is the number of electrons involved in the bioelectrocatalytic process, F is Faraday constant, A is the surface area of the electrode, and Γ_{eff} is the effective surface coverage corresponding to the loading of the active biocatalyst on the electrode surface.

Scheme S1. Kinetic scheme describing the adsorption-assisted bioelectrocatalysis facilitated by the PtNC@GOx/MPCNPs assembly. Please note that due to the fact that no diffusional mediation is used, no correction is needed for the classical Michaelis-Menten model.

As the $FADH_2$/Pt concentration relies on the adsorption stage in (1), $k_{ads}>k_{des}$, and consequently on the surface area provided by the PtNCs, the bioelectrocatalytic responses increase with the time allowed for the enzymatic synthesis, Figure S6.

Growth time, min	I_{max}, μA	Γ_{eff}, mole·cm^{-2}	k_{et}, e$^-$·s^{-1}
15	20.4	$1.85 \cdot 10^{-12}$	1039
30	38.2	$1.95 \cdot 10^{-12}$	1362
45	52.8	$1.95 \cdot 10^{-12}$	1914
60	60.5	$1.90 \cdot 10^{-12}$	2255
90	68.3	$1.95 \cdot 10^{-12}$	2578

Table S1. Enzymatic turnover rates evaluated for the different inside-out-synthesized PtNC@GOx/MPCNPs assemblies which were enzymatically grown for variable time-intervals.

Configuration	Fuel and oxidizer	Mediators	OCV, Volt	Power, $\mu W \cdot cm^{-2}$	Reference
Mediated systems					
GOx/FcMeOH/CNPs // BOD/$ABTS^{2-}$/CNPs	Glucose, O_2	FcMe $ABTS^{2-}$	0.50	95	60
GOx-LOx/FcMeOH/CNPs // BOD-CAT/$ABTS^{2-}$/CNPs	Glucose + lactate, O_2 + H_2O_2	FcMe $ABTS^{2-}$	0.50	90	61
GO_x/MSU-F-C // Pt	Glucose, O_2	FcCA	-	66	67
GOX/HCMSC-PAH/Fc // Pt	Glucose, O_2	Fc	0.38	-	68
Non mediated systems					
OMC // Pt	Glucose, O_2	DET	1.2	110	69
GIOMC/Au NPs/MSA/GDH // IOMC-$PhSO_3H$/AuNPs/BOD	Glucose, O_2	DET	0.51	33	70
GDH/OMCs-MDB // LAC/OMCs	Glucose, O_2	DET	0.82	39	71
PtNC@GOx/MPCNPs // BOD/MPCNPs	Glucose, O_2	DET	0.57	45	Current work

Table S2. Performance of reported mediated and non-mediated biofuel cells which are based on mesoporous carbon materials. LOx - lactate oxidase, CAT - Catalase, GDH - glucose dehydrogenase, Fc - ferrocene, FcCA - ferrocene carboxylic acid, FcMe - ferrocene methanol, OMC - ordered mesoporous carbon, IOMC - ionic liquid derived

ordered mesoporous carbon, GIOMC - guanine-ionic liquid derived ordered mesoporous carbon, MSA - mercaptosuccinic acid. MDB - Meldola's blue. MSU-F-C - mesocellular carbon foam, HCMSC - hollow core mesoporous shell carbon, PAH - poly(allylamine hydrochloride).