



HAL
open science

Organic β -cyclodextrin nanoparticle: an efficient building block between functionalized poly(pyrrole) electrodes and enzymes

Paulo Henrique M. Buzzetti, Marie Carrière, Monica Brachi, Karine Gorgy, Muhammad Mumtaz, Redouane Borsali, Serge Cosnier

► To cite this version:

Paulo Henrique M. Buzzetti, Marie Carrière, Monica Brachi, Karine Gorgy, Muhammad Mumtaz, et al.. Organic β -cyclodextrin nanoparticle: an efficient building block between functionalized poly(pyrrole) electrodes and enzymes. *Small*, 2022, 18 (10), pp.2105880. 10.1002/smll.202105880 . hal-03797790

HAL Id: hal-03797790

<https://hal.science/hal-03797790>

Submitted on 4 Oct 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Organic β -cyclodextrin nanoparticle: an efficient building block between functionalized**
2 **poly(pyrrole) electrodes and enzymes**

3 *Paulo Henrique M. Buzzetti¹, Marie Carrière^{1,2}, Monica Brachi¹, Karine Gorgy¹,*
4 *Muhammad Mumtaz², Redouane Borsali² and Serge Cosnier^{2*}*

5 Dr. Paulo Henrique M. Buzzetti, Dr. Marie Carrière, Monica Brachi, Dr. Karine Gorgy,
6 Dr. Serge Cosnier

7 ¹ Univ. Grenoble Alpes, CNRS, DCM, 38000 Grenoble, France

8 E-mail: serge.cosnier@univ-grenoble-alpes.fr

9 Dr. Muhammad Mumtaz², Dr. Redouane Borsali²

10 ² Univ. Grenoble Alpes, CNRS, CERMAV, F-38000 Grenoble, France

11 Keywords: (glyconanoparticles, β -cyclodextrin, host-guest interaction, nanomaterial design)

12 Glyconanoparticles (GNPs) made by self-assembly of carbohydrate-based polystyrene-block-
13 β -cyclodextrin copolymer were used as a building block for the design of nanostructured
14 biomaterials of electrode. The firm immobilization of GNPs was carried out on
15 electrochemically generated polymer, poly(pyrrole-adamantane), and copolymer,
16 poly(pyrrole-adamantane)/poly(pyrrole- lactobionamide) via host-guest interactions between
17 adamantane and β -cyclodextrin. The ability of GNPs for the specific anchoring of biological
18 macromolecules was investigated using glucose oxidase enzyme modified by adamantane
19 groups as a protein model (GOx-Ad). The immobilization of GOx-Ad was carried out by
20 incubation of an aqueous enzyme solution on a coating of GNPs adsorbed on a platinum
21 electrode. The presence of immobilized GOx-Ad was evaluated in aqueous glucose solution
22 by potentiostating the underlying platinum electrode at 0.7 V/SCE for the electro-oxidation of
23 H₂O₂ generated by the enzyme. The analytical performance of the bioelectrodes for the
24 detection of glucose was compared to control electrodes prepared without GNPs or without
25 electropolymerized films. The better permeability of copolymer compared to polymer and the
26 possibility to elaborate two alternating layers of GNPs and GOx-Ad were clearly observed.
27 The best amperometric response was recorded with a multilayered bioelectrode displaying a
28 wide linear range linear range of the calibration curve: 68 $\mu\text{mol L}^{-1}$ to 0.1 mol L⁻¹.

29 **1. Introduction**

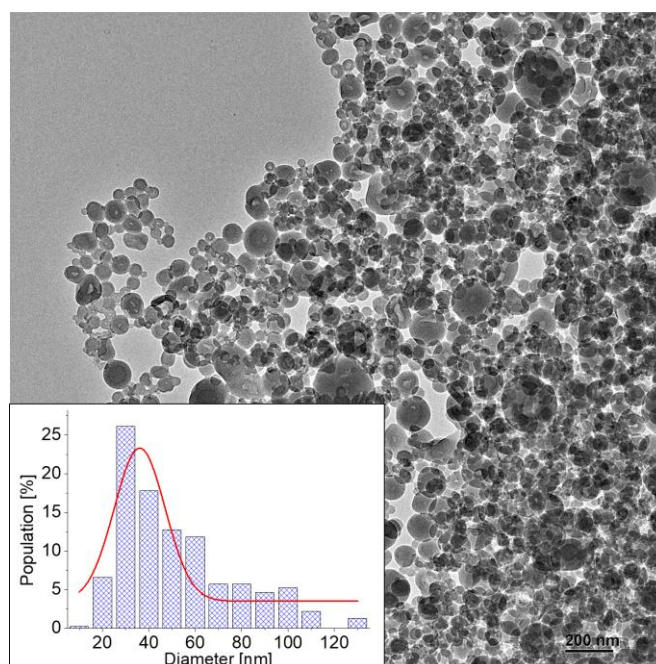
30 The intensive developments in the field of nanotechnologies have contributed to improve the
31 performances of various types of electrochemical devices such as biofuel cells,
32 supercapacitors or biosensors.^[1-4] Thanks to an increased surface area, nanomaterials such as
33 carbon nanotubes, graphene,^[5-13] semi-conductor quantum dots,^[14-15] have significantly
34 increased the power density of biofuel cells and capacitors as well as the analytical
35 performance of biosensors in terms of sensitivity and detection limit. In addition, metallic
36 nanoparticles^[16-18] have aroused great interest in the elaboration of electrode materials due to
37 their physical and chemical properties such as high surface / volume ratio and relatively high
38 electrical conductivity. Their main drawback lies in the post-chemical treatment of their
39 surface to obtain reactive groups such as carboxyl, thiol or amino groups, for example for
40 anchoring biomolecules. These steps are often complex and time consuming.^[19] Recently, an
41 attractive alternative to these metallic nanoparticles has been reported and consists of organic
42 nanoparticles generated by self-assembly of amphiphilic glycopolymers in water. In particular,
43 glyconanoparticles composed of polystyrene-block- β -cyclodextrin (PS-b- β CD) constitute in
44 aqueous medium, stable and polyvalent nanospheres easily functionalizable by inclusion of
45 hydrophobic compounds in the cavities of the β -cyclodextrin shell. These host-guest
46 interactions of the nanoparticle shell have been used successfully to immobilize redox
47 hydrophobic compounds such as tetrazine-naphthalimide,^[20] bispyrene-(2,2'-azino-bis(3-
48 ethylbenzothiazoline-6-sulfonic acid),^[21] phenanthrene-quinone,^[22] anthraquinone-
49 sulfonate.^[23] Some of these resulting redox nanoparticles were applied in suspension
50 successfully for efficient electrical connection of enzymes and the development of a
51 solubilized enzymatic fuel cells.^[22]

52 However, these glyconanoparticles (GNPs) have only been used in aqueous suspension and
53 have never been tested as a building block for the design of nanostructured (bio)materials of
54 electrode for biosensing application or energy conversion. In this context, we report here the
55 possibility to attach these nanoparticles on an electrode surface by specific host-guest
56 interactions and compared this approach to a simple adsorption. Owing to the high affinity of
57 adamantane to form inclusion complexes with β -cyclodextrin (association constant $K = 10^5$
58 $\text{mol}^{-1} \text{L}$),^[24] the immobilization of GNP was carried out on an electropolymerized film of
59 poly(pyrrole-adamantane). The possibility of functionalizing the shell of nanoparticles by
60 proteins via host-guest interactions was also investigated with a glucose oxidase modified by
61 adamantane groups (GOx-Ad) as protein model. The ability to immobilize the nanoparticles
62 and combine them with enzyme molecules has been described through various electrode
63 configurations based on GOx-Ad with and without GNPs. The performances of the resulting

64 nanostructured bioelectrodes as amperometric biosensors for the detection of glucose at 0.7
65 V/SCE were evaluated.

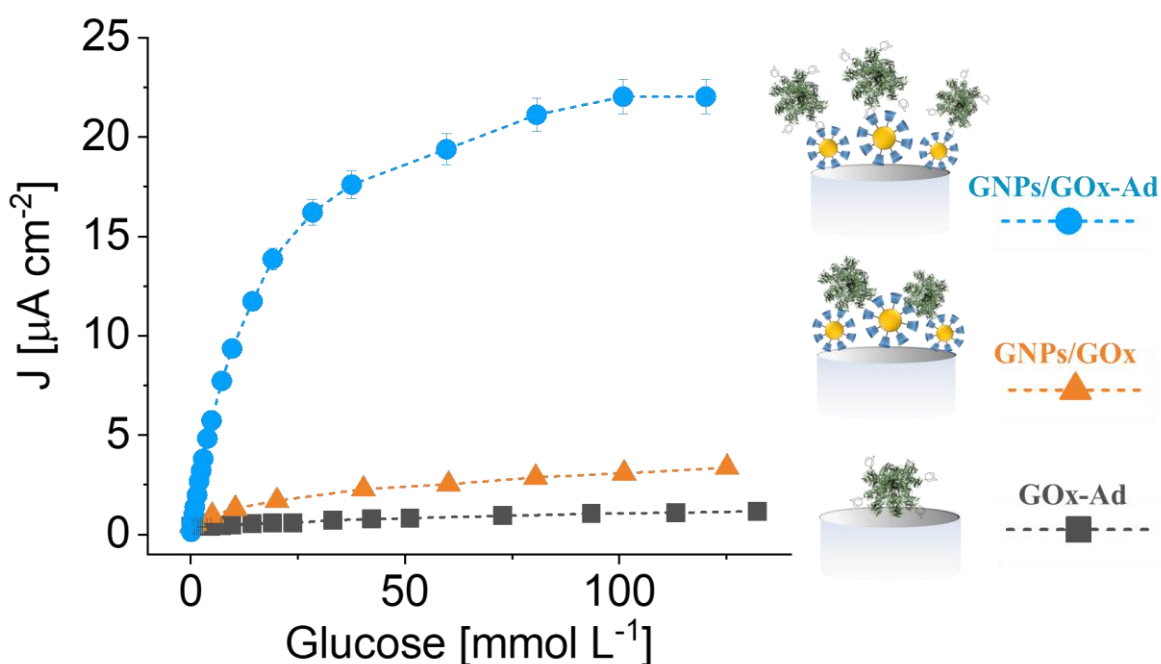
66 2. Results and discussion

67 The nanoprecipitation technique to prepare using PS-b- β CD block copolymer led to stable
68 GNPs, which in their adsorbed state have a spherical morphology with average diameters
69 between ranging from 30 to 60 nm (Figure 1).



70
71 **Figure 1.** TEM images showing the resulting assembly to obtain the GNPs; inset: histogram
72 showing the size distribution of the GNPs.

73 With the aim to design new biological three-dimensional architectures based on GNPs, we
74 first examined the ability of GNPs to adsorb on an electrode surface to anchor an enzyme
75 labeled with adamantane groups. In order to demonstrate the ability of GNPs to fix GOx-Ad,
76 an aqueous suspension of GNPs ($50 \mu\text{L}$, 0.187 mg L^{-1}) was spread and dried on a platinum
77 disk electrode. The electrode modified by adsorbed GNPs was then incubated with GOx-Ad
78 solution ($25 \mu\text{L}$, 0.5 mg L^{-1}) for 7 hrs at $4 \text{ }^\circ\text{C}$ and then washed thoroughly with water. Since
79 GOx-Ad catalyzes the aerobic oxidation of glucose with the concomitant production of H_2O_2 ,
80 the potential presence of immobilized enzyme was evaluated via the electro-oxidation of the
81 enzymatically generated H_2O_2 at the platinum underlying electrode potentiostated at 0.7
82 V/SCE. The amperometric response of the modified electrode towards glucose was thus
83 recorded in 0.1 mol L^{-1} phosphate buffer and compared to a control electrode made by direct
84 adsorption of the enzyme onto the surface of the platinum. Figure 2 illustrates the variation of
85 the anodic current response of the bioelectrodes as a function of glucose concentration.



86
 87 **Figure 2.** Calibration curves for glucose obtained at Pt electrode modified (dark grey) by
 88 adsorption of GOx-Ad, (orange) by successive adsorption of GNPs and GOx, and (blue) by
 89 successive adsorption of GNPs and GOx-Ad and their respective schematic representation.
 90 Applied potential, 0.7 V/SCE in 0.1 mol L⁻¹ phosphate buffer (pH 7, 25°C).

91 As expected, the GNP/ GOx-Ad electrode presents a calibration curve following Michaelis-
 92 Menten kinetics. The sensitivity of the bioelectrode and its maximum current density (J_{\max}) at
 93 saturating glucose condition are 1.32 mA M⁻¹ cm⁻² and $22 \pm 5 \mu\text{A cm}^{-2}$, respectively (Table 1).
 94 In contrast, the control experiment carried out without adsorbed GNPs led to markedly lower
 95 performance. In particular, the J_{\max} value for the GNP/ GOx-Ad electrode which represents
 96 the quantity of immobilized enzyme is markedly higher than that observed for the control
 97 electrode, namely $1.3 \pm 0.1 \mu\text{A cm}^{-2}$. In addition, the control experiment based on an electrode
 98 with adsorbed GNPs incubated with regular GOx presents also a low J_{\max} value, namely 3.36
 99 $\mu\text{A cm}^{-2}$. These comparisons highlight the efficient specific immobilization of GOX-Ad and
 100 therefore the availability of βCD groups from the shell of the GNP adsorbed on the Pt
 101 electrode to anchor labelled enzyme via host-guest interactions.

102 Besides, the experimental value of the apparent Michaelis-Menten constant K_m^{app} (14 mmol L⁻¹)
 103 (Table 1), that reflects the kinetics of the immobilized enzyme, is lower than the K_M of free
 104 enzymes (33 mmol L⁻¹) [25] indicating that the enzyme maintains an excellent catalytic activity
 105 despite its binding.

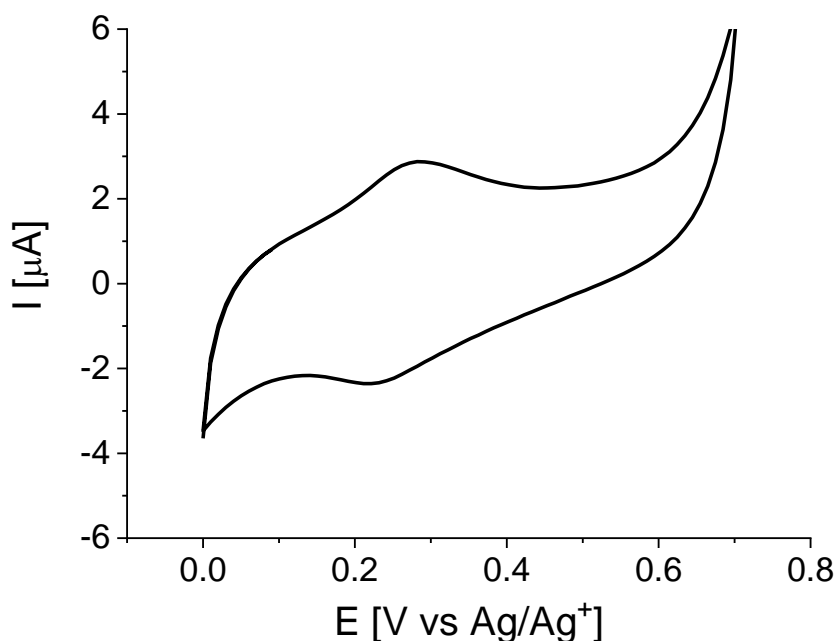
106 **Table 1:** Comparison of different electrode biomaterials based on glyconanoparticles GNPs
 107 and GOx-Ad for the amperometric detection of glucose.

Electrode material	GNPs/GOx-Ad	^{a)} polymer / GNPs/GOx-Ad	^{b)} copolymer / GNPs/GOx-Ad	^{b)} copolymer / (GNPs/GOx-Ad) ₂
Limit Detection [$\mu\text{mol L}^{-1}$]	80	44	84	68
Sensitivity [mA mol L ⁻¹ cm ⁻²]	1.32 ± 0.04 (R ² = 0.996)	0.85 ± 0.05 (R ² = 0.990)	3.3 ± 0.2 (R ² = 0.996)	2.5 ± 0.2 (R ² = 0.996)
Linear Range [mmol L ⁻¹]	0.08 - 2.89	0.044 - 60.5	0.084 - 72.1	0.068 - 100.6
J_{max} [$\mu\text{A cm}^{-2}$]	22 ± 5	87 ± 6	144 ± 10	272 ± 12
K_m^{app} [mmol L ⁻¹]	14	47	22	61

108 ^{a)} polymer is poly(pyrrole-adamantane); ^{b)} copolymer is poly(pyrrole-adamantane) / poly(pyrrole-lactobioamide).

109 In order to obtain a more stable and active biomaterial, a more sophisticated approach consists
 110 to specifically anchoring of GNPs on the electrode surface instead of just adsorbing them. To
 111 achieve this goal, the Pt electrode was modified by electrogenerating a poly(pyrrole
 112 adamantane) film capable of offering host-guest interactions with GNPs. The
 113 electropolymerization of the pyrrole-adamantane (3 mmol L⁻¹ in CH₃CN + 0.1 mol L⁻¹
 114 LiClO₄) was performed by controlled potential oxidation at 0.79 V vs. Ag⁺ 10⁻² mol L⁻¹/ Ag in
 115 CH₃CN. The electrolysis charge was limited to 2.5 mC cm⁻² to form a thin and compact
 116 polymer layer [26]. The cyclic voltammogram of the resulting electrode, transferred in a
 117 solution exempt of monomer, exhibits a reversible peak system at E_{1/2} = 0.27 V, reflecting the
 118 electroactivity of the polypyrrolic skeleton (Figure 3). The apparent surface coverage of the
 119 electropolymerized adamantane derivative, $\Gamma = 4.4 \cdot 10^{-7} \text{ mol m}^{-2}$, was determined from the
 120 charge integrated under the oxidation wave of the poly(pyrrole) backbone by taking into
 121 account that 0.33 electron molecule⁻¹ is involved in the electrooxidation process. As indicated
 122 previously for the bare electrode, the poly(pyrrole-adamantane) electrode was then incubated
 123 successively with identical amounts of GNP and GOx-Ad. The analytical performance of the
 124 resulting bioelectrode for the determination of glucose was investigated. The calibration curve
 125 for the polymer/GNPs/GOx-Ad exhibits a J_{max} value (87 ± 6 $\mu\text{A cm}^{-2}$) almost four times
 126 higher than that of similar bioelectrode without polymer (Figure 4). Taking into account the
 127 fact that J_{max} is directly proportional to the quantity of immobilized enzyme, the remarkable

128 increase in J_{\max} highlights the beneficial effect of the polymer which reinforces the GNPs
 129 adsorption by specific interactions and prevents their desorption during the washing steps and
 130 therefore the loss of enzymes. It should be noted that this J_{\max} value is significantly higher
 131 than those ($3.4\text{--}23 \mu\text{A cm}^{-2}$) previously reported for electrodes modified by a compact
 132 enzyme monolayer formed by affinity interactions with underlying functionalized
 133 poly(pyrrole) films.^[27,28] This clearly illustrates the increase in surface immobilization of
 134 enzymes provided by the spherical shape of the nanoparticles and the nanostructuring of the
 135 interface.

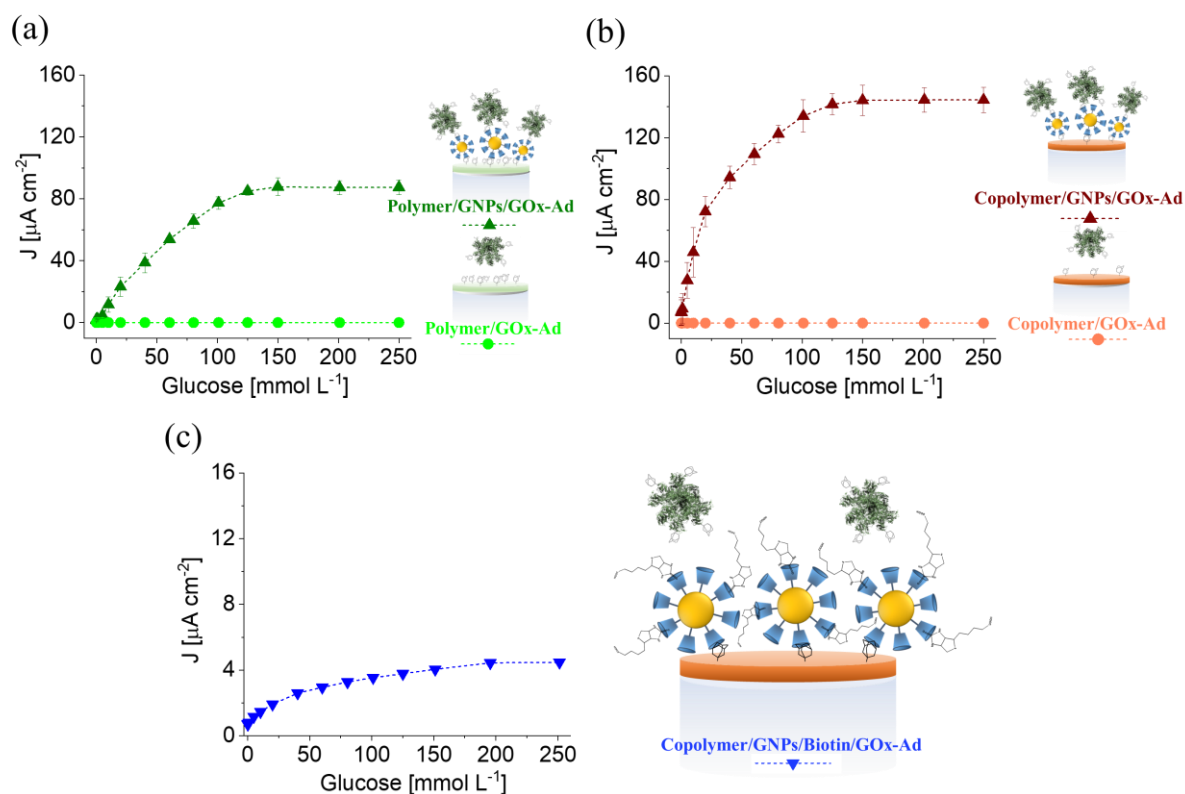


136
 137 **Figure 3.** Voltamperogram recorded at Pt electrode (5mm diameter disk) after
 138 electropolymerization of pyrrole-adamantane (3 mmol L^{-1}) (Electrolysis charge = 2.5 mC cm^{-2})
 139 2) in $\text{CH}_3\text{CN} + \text{LiClO}_4$ 0.1 mol L^{-1} and transfer in $\text{CH}_3\text{CN} + \text{LiClO}_4$ 0.1 mol L^{-1} free of
 140 monomer.

141 A slight decrease in glucose sensitivity (0.85 instead of $1.32 \text{ mA mol L}^{-1} \text{ cm}^{-2}$), is observed
 142 and attributed to the underlying poly(pyrrole-adamantane) which generates, due to its
 143 hydrophobic character, steric constraints for the permeation of H_2O_2 and therefore for its
 144 electro-oxidation on the platinum surface. The limitation in H_2O_2 diffusion is corroborated by
 145 the marked increase in K_m^{app} value from 14 mmol L^{-1} in the absence of polymer to 47 mmol L^{-1}
 146 1 in its presence, this value being notably higher than that reported for the free enzyme.^[25,29]
 147 A positive corollary of this phenomenon is the extension of the linear domain of the
 148 calibration curve ($0.044 - 60.5 \text{ mM}$) (Table 1).

149 In order to reduce the steric limitations due to the hydrophobic character of poly(pyrrole-
150 adamantane), a lactobionic derivative has been introduced into the structure of the polymer by
151 electrochemical copolymerization of a pyrrole-adamantane and pyrrole-lactobioamide mixture
152 in the ratio 1/3 respectively. The copolymer formation was carried out in $\text{CH}_3\text{CN} + 0.1 \text{ M}$
153 LiClO_4 by controlled potential electrolysis at 0.74V, the charge passed 2.5 mC cm^{-2} being
154 identical to that used for the electrogeneration of poly(pyrrole-adamantane). The
155 electrochemical characterization of the resulting copolymer by cyclic voltammetry shows a
156 redox system at $E_{1/2} = 0.26 \text{ V}$ (data not shown) and the integration of the corresponding
157 charge leads to a surface coverage of $3.8 \cdot 10^{-7} \text{ mol m}^{-2}$ similar to that obtained for the
158 poly(pyrrole-adamantane) film. As previously demonstrated for a copolymer composed of
159 biotin and lactobioamide groups, the presence of polymerized lactobioamide groups improves
160 the hydrophilic character of the copolymer and enhances its permeability and the H_2O_2
161 permeation and hence the transduction step.^[30] The copolymer-based bioelectrode thus
162 exhibits a glucose sensitivity ($3.3 \text{ mA mol L}^{-1} \text{ cm}^{-2}$), 4 times higher than that obtained with
163 the polymer-based bioelectrode and a decrease in the K_m^{app} value to 22 mmol L^{-1} reflecting
164 both the best diffusion and thus detection of H_2O_2 (Table 1). In addition, the J_{max} value ($144 \pm$
165 $10 \mu\text{A cm}^{-2}$) increases markedly, reflecting an increase in the amount of immobilized enzyme.
166 This phenomenon can be attributed to a swelling process of the copolymer in water in contrast
167 to the hydrophobic film of poly(pyrrole-adamantane) thus increasing the specific anchoring
168 surface of the GNPs and therefore of the GOx-Ad (Figure 4).

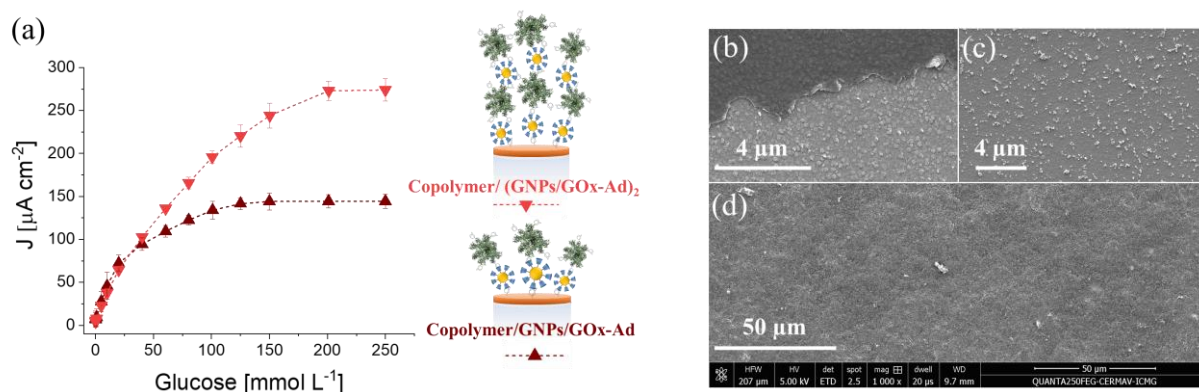
169 It should be noted that the control experiments performed by omitting the GNP deposition
170 step and directly adsorbing GOx-Ad onto the surface of the polymer or copolymer led to
171 bioelectrodes with an extremely low electrochemical response for the detection of glucose
172 (Figure 4). This confirms that the host-guest interactions approach is more efficient as
173 compared to non-specific interactions that are negligible and therefore highlights strongly the
174 key role of GNPs. With the aim to demonstrate that GOx-Ad immobilization was due to host-
175 guest interactions between adamantane and cyclodextrin at the shell of GNPs anchored on the
176 copolymer, the copolymer/GNPs electrode was first incubated with 1 mmol L^{-1} biotin aqueous
177 solution and then incubated in a second step with GOx-Ad solution. Due to the initial
178 formation of the biotin / cyclodextrin inclusion complex,^[28] a negligible quantity of enzyme
179 GOx-Ad was anchored to the electrode, as indicated by the low J_{max} value ($4.51 \mu\text{A cm}^{-2}$)
180 recorded (Figure 4c) for glucose oxidation.



181
 182 **Figure 4.** Calibration curves for glucose obtained at Pt electrode functionalized (a) by
 183 poly(pyrrole-adamantane) modified (light green) by adsorption of GOx-Ad and (dark green)
 184 by successive adsorption of GNPs and GOx-Ad, (b) by poly(pyrrole-
 185 adamantane)/poly(pyrrole-lactobionamide) modified (salmon) by anchoring of GOx-Ad and
 186 (burgundy) by successive anchoring of GNPs and GOx-Ad, (c) by poly(pyrrole-
 187 adamantane)/poly(pyrrole-lactobionamide) modified by successive anchoring of GNPs, biotin
 188 and GOx-Ad and their respective schematic representation. Experimental conditions as in
 189 Figure 2.

190 The possibility of generating a multilayer nanostructure was investigated by successively
 191 incubating the copolymer electrode with GNP and GOx-Ad and by repeating this procedure to
 192 theoretically make 2 layers of GNP and GOx-Ad. The resulting multilayer bioelectrode
 193 exhibits a value of J_{max} ($274 \mu\text{A cm}^{-2}$) almost twice that observed for the configuration based
 194 on a single enzyme incubation. This result illustrates the specific anchoring of an almost
 195 double amount of GOx-Ad resulting from the two successive incubations of the modified
 196 electrode in enzyme solution. In addition, the marked increase in $K_{\text{m}}^{\text{app}}$ value, namely 61
 197 mmol L^{-1} compared to 22mmol L^{-1} for the bioelectrode based on a single enzyme incubation,
 198 reflects the increase in steric hindrances for H_2O_2 diffusion generated by the increase in the
 199 number of layers (Table 1). Furthermore, it should be noted that the diffusion distance to the
 200 underlying platinum electrode for H_2O_2 which is preferentially produced at the interface

201 biomaterial / solution, must also increase with increasing layers and hence affect the K_m^{app}
 202 value. Although the sensitivity to glucose ($2.5 \text{ mA mol L}^{-1} \text{ cm}^{-2}$) is slightly reduced, a
 203 beneficial effect of multilayers approach is the linear range increase of the calibration curve
 204 ($0.068 - 100 \text{ mmol L}^{-1}$) while still maintaining a good detection limit, namely $68 \text{ } \mu\text{mol L}^{-1}$
 205 (Figure 5).



206
 207 **Figure 5.** (a) Calibration curves for glucose obtained at a Pt electrode functionalized by
 208 copolymer (poly(pyrrole-adamantane)/poly(pyrrole-lactobionamide)) modified (burgundy) by
 209 successive adsorption of GNPs and GOx-Ad and (red) by anchoring 2 layers of GNPs
 210 alternated by two layers of GOx-Ad and their schematic representation (experimental
 211 conditions as in Figure 2); SEM images of the (poly(pyrrole-adamantane)/poly(pyrrole-
 212 lactobionamide) film (b) before, (c) after 30 min of GNPs incubation and (d) after the final
 213 formation of the GNP/GOx-Ad multilayer nanostructure onto the copolymer.

214 3. Conclusion

215 The examples of enzyme electrodes developed here show clearly the ability of
 216 glyconanoparticles to be efficiently used for the elaboration of electrode biomaterials using
 217 host-guest interactions like adamantane / β -cyclodextrin. The versatility of this approach
 218 relies on the diversity of hydrophobic compounds that fit into the cyclodextrin cavity, such as
 219 biotin or pyrene. Furthermore, the use of electrogenerated functionalized poly(pyrrole) films
 220 for the firm anchoring of these nanoparticles paves the way for the spatially controlled
 221 immobilization of these GNPs by electrochemical addressing. This innovative and versatile
 222 concept will be extended in the near future to the anchoring of other biological
 223 macromolecules such as antibodies, DNAs and aptamers or even to enzyme / antibody
 224 associations as markers for immunosensors.

225 4. Experimental Section

226 *Materials and reagents:* all reagents (analytical grade) and glucose oxidase (GOx, Type VII,
227 from *Aspergillus niger*, lyophilized min 60 % protein (Lot #: 086K2076; 179000 units g⁻¹
228 solid), ITO electrodes were purchased from Sigma-Aldrich. Amicon[®] Ultra-15 Centrifugal
229 Filter Unit-30 KDa were purchased from Merck. The TEM images were registered with
230 TEM-CAM 216 (TVIPS) camera at 200 kV using a JEOL 2100 Plus microscope equipped
231 with a RIO16 (GATAN) camera, and the SEM images were acquired using an ULTRA 55
232 FESEM based on the GEMINI FESEM column with beam booster (Nanotechnology Systems
233 Division, Carl Zeiss NTS GmbH) with tungsten gun.

234 *Glyconanoparticles (GNPs) suspension preparation:* The polystyrene-block- β -cyclodextrin
235 (PS-*b*- β CD) synthesis has been realized by click chemistry as previously reported ^[23] and
236 used for self-assembly of GNPs by direct nanoprecipitation.^[23] Briefly, to obtain stable GNPs
237 suspension in water, 15 mg of PS-*b*- β CD was solubilized in 2 mL THF / H₂O solution (80:20)
238 v/v stirred at 1000 rpm for 24 h. The resulting solution was added using a syringe pump at a
239 speed of 10.2 mL h⁻¹ in 80 mL of Milli-Q water under stirring (500 rpm) and then stirred for 2
240 h at room temperature. Finally, organic solvent was removed under reduce pressure and the
241 final volume was adjusted to 80 mL, in order to get a GNPs concentration of 0.187 mg L⁻¹.

242 *Pyrrole derivatives:* adamantyl-11-pyrrolyl-1-undecyl carboxylic acid amide (pyrrole-
243 adamantane)^[31] and 8-pyrrol-1-lactobionamide (pyrrole-lactobionamide) monomers were
244 synthesized as previously described in the literature.^[32] Pyrrole-adamantane was synthesized
245 by formation of succinimide (NHS) ester from the pyrrole-carboxylic acid using standard
246 Steglich reaction conditions. Then the pyrrole NHS (199.7 mg, 0.57 mmol) ester was
247 dissolved in 5 mL of DMF and 1-adamantylamine (86.1 mg, stoichiometric amount) and
248 tetraethylamine (300 mg, an excess) were added. The resulting solution was stirred for 3 days
249 at 60 °C, evaporated to dryness and then purified by column chromatography on silica gel
250 (Et₂O–hexane (1/1)) in order to obtain the pure product. For the preparation of pyrrole-
251 lactobionamide, 8-pyrrol-1-aminooctane (0.500 g, 2.6 mmol) was solubilized in 100 mL
252 methanol containing 26 mmol L⁻¹ lactobionic acid. Then the solution was stirred under reflux
253 for 24 h and methanol was evaporated under vacuum by freeze-drying.

254 *Adamantane-tagged glucose oxidase:* preparation of adamantane-tagged glucose oxidase was
255 adapted from the synthesis previously described.^[31] 2.5 mg of 1-adamantylamine (0.016
256 mmol) and 3 mg 1-ethyl-3-(3-dimethylamino-propyl) carbodiimid hydrochloride (EDC, 0.016
257 mmol) were dispersed in phosphate buffer (3 mL, 0.1 mol L⁻¹, pH 6.0) by sonication. Then

258 GOx (1.6 mg) was mixed in this solution for 22 h kept under stirring. In order to purify the
 259 resulting solution, it was centrifuged using different volumes of phosphate buffer (0.1 mol L⁻¹,
 260 pH 7) using an Amicon[®] Ultra-15 Centrifugal Filter Unit-30 kDa to get a 0.5 mg mL⁻¹ final
 261 concentration.

262 *Preparation of the bioelectrode configurations:*

263 ○ *Pt//Gox-Ad, Pt/GNPs/GOx-Ad and Pt/GNPs/GOx*

264 Pt/GOx-Ad was obtained by spreading and drying 25 μL of GOx-Ad onto Pt electrode surface.
 265 For Pt/GNPs/GOx-Ad and *Pt/GNPs/GOx*, a drop of 50 μL of GNP_s (0.187 mg L⁻¹) were
 266 spread onto Pt electrode surface and dried at room temperature. The resulting electrodes are
 267 then incubated respectively with 25 μL of GOx-Ad and 25 μL of GOx for 7h at 4 °C and
 268 rinsed in phosphate buffer (0.1 mol L⁻¹, pH 7) for 5 minutes under weak stirring at room
 269 temperature.

270 ○ *Pt/polymer/ GNPs/GOx-Ad*

271 The poly(pyrrole-adamantane) was prepared by controlled potential oxidation at a fixed
 272 potential (0.79 V vs. Ag⁺ 10⁻² mol L⁻¹/ Ag) in pyrrole-adamantane 3 mmol L⁻¹ in CH₃CN +
 273 LiClO₄ 0.1 mol L⁻¹ until to get a charge of 2.5 mC cm⁻². Then 50 μL of GNPs (0.187 mg mL⁻¹)
 274 were deposited onto the electrogenerated film for incubation. After rinsing the electrode, 25
 275 μL of GOx-Ad was then incubated for 7 H at 4°C and then rinsed in phosphate buffer as
 276 previously described.

277 ○ *Pt/copolymer/GNPs/GOx-Ad*

278 The electropolymerization of a solution of pyrrole-adamantane (0.75 mM) and pyrrole-
 279 lactobionamide (2.25 mM) in CH₃CN + 0.1 mol L⁻¹ LiClO₄ was achieved by controlled
 280 potential electrolysis (2.5 mC cm⁻²) at 0.74V vs. Ag⁺ 10⁻² mol L⁻¹/ Ag. The steps to anchor
 281 successively GNPs and GOx-Ad were achieved as described previously for Pt/polymer/
 282 GNPs/GOx-Ad.

283 ○ *Pt/copolymer/GNPs/biotin/GOx-Ad*

284 A Pt/copolymer/GNPs as previously described was incubated in saturated biotin solution (1
 285 mM) for 2 h at 25°C. After rinsing the electrode, 25 μL of GOx-Ad was then incubated for 7
 286 h at 4°C and then rinsed in phosphate buffer for obtaining the
 287 Pt/copolymer/GNPs/biotin/GOx-Ad.

288 ○ *Pt/copolymer /2 (GNPs/GOx-Ad)*

289 The copolymer film was electropolymerized as described previously for Pt/copolymer/
 290 GNPs/GOx-Ad. The multilayered enzyme and GNPs assembly was created onto the

291 copolymer surface by depositing a drop of GNPs (34 μL , 0.077 mg mL^{-1}) for 2 hours at room
292 temperature, rinsed in phosphate buffer (0.1 mol L^{-1} , pH 7) followed by an incubation of a
293 drop of 25 μL of GOx-Ad for 7 h at 4°C and then rinsed in phosphate buffer as previously
294 described. These two steps have been reproduced once more.

295 *Electrochemical measurements:*

296 electrochemical measurements were performed using a potentiostat a PGSTAT 100 operated
297 by Nova 2.1.4 software using a conventional three-electrode electrochemical cell. The
298 working electrode was a Pt electrode (diameter 5 mm) thoroughly cleaned using 20 μm
299 diamond paste, and rinsed several times successively with acetone, ethanol, and distilled
300 water. The ITO electrode was rinsed with ethanol and water before use. The counter electrode
301 was a platinum wire. The reference electrode used is a SCE saturated calomel electrode in
302 aqueous solutions whereas a $10^{-2} \text{ mol L}^{-1} \text{ Ag}^+/\text{Ag}$ electrode was used as a reference electrode
303 in organic media ($\text{CH}_3\text{CN} + 0.1 \text{ mol L}^{-1} \text{ LiClO}_4$). All experiments were performed at 25°C in
304 0.1 mol L^{-1} phosphate buffer (pH 7) for aqueous solution and in $\text{CH}_3\text{CN} + 0.1 \text{ mol L}^{-1} \text{ LiClO}_4$
305 to perform the electropolymerization of the different monomers.

306 **Acknowledgements**

307 The authors thank the French National Research Agency in the framework of the
308 “Investissement d’avenir” program Glyco@Alps (ANR-15-IDEX-02), ANR-18-CE09-0022
309 and CBH- EUR- GS (ANR- 17- EURE- 0003). The NanoBio ICMG (UAR 2607), is
310 acknowledged for providing facilities for characterization of block copolymers and
311 glyconanoparticles morphology and size. This work was also supported by the Institute
312 Carnot PolyNat (CARN 0007-01).

313 **Conflicts of Interest**

314 The authors declare no conflicts of interest.

315 **References**

- 316 [1] M. Holzinger, A. Le Goff, S. Cosnier, *Front. Chem.* **2014**, *2*, 1.
317 [2] A. Mishra, R. Bhatt, J. Bajpai, A.K. Bajpai, *Int. J. Hydrog. Energy* **2021**, *46*, 19085.
318 [3] X. Xiao, H.-Q. Xia, R. Wu, L. Bai, L. Yan, E. Magner, S. Cosnier, E. Lojou, Z. Zhu, A.
319 Liu, *Chem. Rev.* **2019**, *16*, 9509.
320 [4] G. De Crozals, R. Bonnet, C. Farre, C. Chaix, *Nano Today* **2016**, *11*, 435.
321 [5] Q. Palomar, C. Gondran, M. Holzinger, R. Marks, S. Cosnier, *Biosens. Bioelectron.* **2017**,
322 *97*, 177.

- 323 [6] A. Balasubramanian, M. Burghard, *Anal. Bioanal. Chem.* **2006**, 385, 452.
- 324 [7] A. Le Goff, M. Holzinger, S. Cosnier, *Analyst* **2011**, 136, 1279–1287.
- 325 [8] V. Vamvakaki, N. A Chaniotakis, *Sens. Actuators B. Chem.* **2007**, 126, 193.
- 326 [9] J. Wang, *Electroanalysis* **2005**, 17, 7.
- 327 [10] V. Papper, K. Gorgy, K. Elouarzaki, A. Sukharaharja, S. Cosnier, R S. Marks, *Chem.*
328 *Eur. J.* **2013**, 19, 9639.
- 329 [11] O. Hosu, K. Elouarzaki, K. Gorgy, C. Cristea, R. Sandulescu, R. S.Marks, S. Cosnier ,
330 *Electrochem. Com.* **2017**, 80, 5.
- 331 [12] M. Singh, M. Holzinger, M. Tabrizian, S. Winters, N. C. Berner, S. Cosnier, G. S.
332 Duesberg, *J. Am. Chem. Soc.* **2015**, 137, 2800.
- 333 [13] M. Pumera, *Materials Today.* **2011**, 14, 308.
- 334 [14] Y. Zang, J. Fan, Y. Ju, H. Xue, and H. Pang, *Chem. Eur. J.* **2018**, 24,14010.
- 335 [15] S.-N. Ding, B.-H. Gao, D. Shan, Y.-M. Sun, S. Cosnier, *Biosens. Bioelectron.* **2013**, 39,
336 342.
- 337 [16] J. M. Pingarrón, P. Yáñez-Sedeño, A. González-Cortés, *Electrochim. Acta* **2008**, 53,
338 5848.
- 339 [17] Q. Palomar, X. Xing Xu, C. Gondran, M. Holzinger, S. Cosnier, Z. Zhang, *Microchim.*
340 *Acta* **2020**, 187, 1.
- 341 [18] G. Doria, J. Conde, B. Veigas, L. Giestas, C. Almeida, M. Assunção, J. Rosa, P. V.
342 Baptista, *Sensors* **2012**, 12, 1657.
- 343 [19] A. Sperling, W. J. Parak, *Phil. Trans. R. Soc. A.* **2010**, 368, 1333.
- 344 [20] A. J. Gross, R.Haddad, C.Travelet, E.Reynaud, P.Audebert, R.Borsali, S. Cosnier,
345 *Langmuir* **2016**, 32, 11939.
- 346 [21] A. J. Gross, X. Chen, F. Giroud, C. Travelet, R .Borsali, S. Cosnier, *J. Am. Chem. Soc.*
347 **2017**, 139, 16076.
- 348 [22] J. L. Hammond, A. J. Gross, F. Giroud, C. Travelet, R. Borsali, S. Cosnier. *Energy Lett.*
349 **2019**, 4, 142.
- 350 [23] M. Carrière, P. H. M. Buzzetti, K. Gorgy, M. Mumtaz, C. Travelet, R. Borsali, S. Cosnier,
351 *Nanomaterials* **2021**, 11, 1162.
- 352 [24] G. Chen, M. Jiang, *Chem. Soc. Rev.* **2011**, 40, 2254.
- 353 [25] B.E. Swoboda, V. Massey, *J. Biol. Chem.* **1965**, 240, 2209.
- 354 [26] I. Kazane, K. Gorgy, C. Gondran, N. Spinelli, A. Zazoua, E. Defrancq, S. Cosnier, *Anal.*
355 *Chem.* **2016**, 88, 7268.
- 356 [27] N. Haddour, S. Cosnier, C. Gondran, *J. Am. Chem. Soc.* **2005**, 127, 5752.

- 357 [28] M. Holzinger, M. Singh, S. Cosnier, *Langmuir* **2012**, 28, 12569.
- 358 [29] A. Ramanavičius, A. Kaušaitė, A. Ramanavičienė, *Sensors and Actuators B. Chem.* **2005**,
359 111,532.
- 360 [30] R.E. Ionescu, C. Gondran, L.A. Gheber, S. Cosnier, R.S. Marks. *Anal. Chem.* **2004**, 76,
361 6808.
- 362 [31] M. Holzinger, L. Bouffier, R. Villalonga, S. Cosnier, *Biosens. Bioelectron.* **2009**, 24,
363 1128.
- 364 [32] S. Cosnier, A. Lepellec, B. Guidetti, I. Rico-Lattes. *J. Electroanal. Chem.* **1998**, 449, 165.