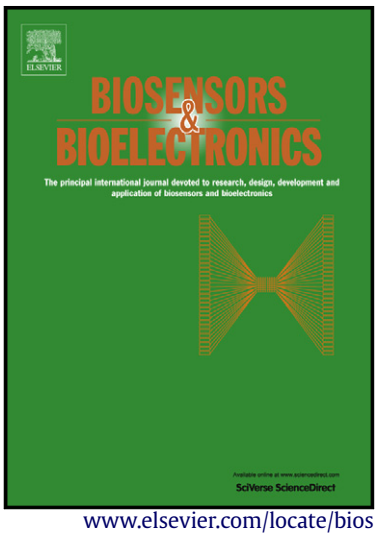

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Abstract:
Scientific knowledge of glutamate (GLU) neurobiology is severely hampered by the inadequacy of the available *in vivo* brain sampling techniques. Due to the crucial role of GLU in central nervous system function and pathology, the development of a reliable sampling device is mandatory. GLU biosensor holds potential to address many of the known issues of in vivo GLU measurement. We report here on the development and test of a labor- and cost-effective micro biosensor, suitable to be applied for measuring brain GLU. A glycerol-based cryopreservation method was also tested. Needle type Pt biosensors were coated with a permselective Nafion-Poly(o-phenylenediamine) layer and cross-linked to L-glutamate oxidase with poly(ethylene glycol) diglycidyl ether. Tested *in vitro*, the device shows high sensitivity and specificity for GLU, while being poorly influenced by common interfering substances such as ascorbate, dopamine and dihydroxyphenylacetic acid. Further, the cryopreservation procedure kept sensitivity unaltered for 30 days and possibly longer. We conclude that a highly efficient GLU biosensor of minimal dimensions can be consistently and affordably constructed with relative ease. Together with the possibility of cryopreservation this shall foster diffusion and exploitation of GLU biosensors technology.

Keywords:
Glutamate biosensor, Platinum, Permselective membrane, Amperometry, Cryopreservation.

1. Introduction

Glutamate (GLU) is the main excitatory neurotransmitter in the mammalian central nervous system (CNS) and is profoundly involved in most brain functions such as cognitive processes, plasticity, memory and movement (Niciu et al., 2012; Riedel et al., 2003; Platt SR, 2007). As a consequence, GLU is also implicated in pathological behaviors like stress, depression, and addiction (Enrico et al., 1996; Pittenger et al., 2007; Sun, 2011), as well as in many neurological disorders including epilepsy, schizophrenia, and Hungtington's chorea (Noetzel et al., 2012; Danbolt NC, 2001).

Despite such a prominent role in CNS physiology and pathology, GLU neurobiology remains obscure mainly because of the inadequacy of the available *in vivo* brain sampling techniques, in particular microdialysis (Westerink BHC, 1995). In fact, after many attempt to use microdialysis to study GLU neurotransmission, the reliability of this method has been severely questioned. Whilst microdialysis can sample brain GLU *in vivo*, dialysate GLU levels show insensitive to common tests used to ascertain its neuronal origin, such as local tetrodotoxin (TTX) administration or Ca deprivation (Timmerman and Westerink, 1997; van der Zeyden et al., 2008). Many physiological reasons may account for this fact, including the presence of a dual GLU pool (neuronal and metabolic), the complex anatomic structure of GLU synapse and the fast turnover of neuronal GLU (Rodriguez et al., 2013; Niciu et al., 2012; Kessler J-P, 2013). On the other hand, among the known disadvantages of microdialysis, its low spatial and temporal resolution, as well as the large tissue damage secondary to probe implantation (Borland et al., 2005), seems to be particularly adverse for monitoring neuronal GLU. However, many of these problems may be overcome by using specific biosensors (Kulagina et al., 1999; Cui et al., 2001), which are much smaller than a dialysis probe and provide excellent temporal and spatial resolution (Dale et al., 2005; Wilson and Gifford, 2005). Nowadays, biosensors have been successfully applied to measure GLU in brain slices (Ondenziel et al., 2007; Heinrich et al., 2012) and *in vivo* in anesthetized or awake rats (Day et al., 2006; Rutherford et al., 2007; Burmeister et al., 2002). However, GLU biosensors technology is still in its infancy, with a number of technical details needing improvement (Vasylieva et al., 2013; Kotanen et al., 2012).

In this paper we report the characterization of a GLU amperometric biosensor, derived from Wahono et al (2012), that might be applied to measure GLU release *in vivo*. Our specific aim was to develop a cost- and labor-advantageous, efficient device with high sensitivity and specificity for GLU, while still being easy enough to assemble and manipulate. To this end we projected and tested *in vitro* several I-shaped sensor prototypes based on the device described by Wahono et al (2012); the most important differences among prototypes were: permselective membranes and enzyme immobilization procedures. Coating with permselective membranes is a key strategy to reduce the magnitude of electroactive interference from endogenous molecules in the extracellular fluid (Dale et al., 2005; Kirwan et al., 2007). Among the various non-conductive polymers

referenced in the literature we focused on nafion and poly-o-phenylenediamine (alone and in combination), because of their recognized efficacy and relative ease of use (Hascup et al., 2008; Ekingi et al., 2001). Among the potential interfering species in the brain extracellular fluid, ascorbic acid, dopamine, and dihydroxyphenylacetic acid were chosen to test the shielding efficacy of permselective membranes, owing to their high concentrations and ubiquity. The efficiency of any biosensor depends on the nature and the state of its biological recognition element (Chambers et al., 2008; Wilson and Gifford, 2005). Covalent immobilization of L-glutamate oxydase on the electrode surface is a critical step in the production of GLU biosensors, since this procedure can profoundly impact enzyme substrate specificity and efficiency (Vasylieva et al., 2013). In a recent paper a new poly(ethylene glycol) diglycidyl ether-based procedure for L-glutamate oxydase cross-linking has been proposed (Vasylieva et al., 2011). Apart from being effective in covalently linking the enzyme while preserving its biological properties, this technique offers the important advantage of making no use of toxic components. In this paper we compared the performances of GLU biosensors made with the poly(ethylene glycol) diglycidyl ether-based immobilization procedure against the well-known glutaraldheyde-based method (Burmeister et al., 2000) Lastly, in order to improve biosensors duration, a specific cryopreservation method was tested. Of the many prototypes made in our laboratory, three of them (closely related) are reported herein with an in-depth description of the best performing model.

2. Materials and methods

2.1. Chemicals and biosensors components

L-glutamate oxidase (*Streptomyces sp*; GLU-ox) was purchased from US Biological. Bovine serum albumin (BSA) fraction V 99%; glutaraldehyde (grade I, 25%, acqueous solution); poly(ethylene glycol) diglycidyl ether (PEDGE); Nafion; o-phenylendiamine; L-glutamic acid; dopamine hydrochloride (DA); dihydroxyphenylacetic acid (DOPAC); l-ascorbic acid (AA); H_2O_2 20% wt %; glycerol; were obtained from Sigma-Aldrich. Fused silica capillary (40 μm ID, 105 μm OD) was purchased from Polymicro technologies, LLC; Pt wire (25 μm), Pt/Ir wire (20 μm), and Ag wire (150 μm) were purchased from Advent RM, USA; epoxy glue (UHU plus, Bostik); silver epoxy glue (Epoxy technology, Inc, USA).

2.2. Biosensors manufacturing

All prototypes share the same basic structure: a metal wire (Pt or Pt/Ir) inserted into a fused silica capillary and sealed with epoxy glue. One end of the wire allowed for connection to the potentiostat, while the other end was trimmed to a length of 500 μm and enzyme-coated. The main differences among prototypes reside in the nature of the metal wire and in the coupling method between the wire and the potentiostat. In prototype 1, the wire (Pt or Pt/Ir) was coupled with Ag epoxy glue to an Ag wire (Fig. 1a). In prototype 2, the wire (Pt) was coupled with epoxy glue to a gold-coated pin (Fig. 1b). In prototype 3, the wire (Pt) was coupled to a gold-coated pin filled with silver epoxy glue (Fig. 1c).

2.3. Permselective membrane

Before enzyme coating Pt or Pt/Ir wires where coated with protective (permselective) membranes; Nafion, poly-o-phenylenediamine (Poly(oPD)) or a combination of both were used. Nafion was applied manually by dipping the tip of the wire in a Nafion solution for 10 s twice, with 20 s interval. Nafion-coated wires were allowed to dry at room temperature for 5 min and then cured in oven at 80° C for 4 min. Poly(oPD) was deposited over bare or Nafion-coated wire by electro-polymerization, by applying a constant potential of +700 mV for 30 minutes. O-phenylenediamine monomer solution (300 mM) were made in 0.01 M phosphate-buffered saline solution, pH 7.4.

2.4. Enzyme immobilization procedure

Biosensors were coated with GLU-ox using two different immobilization methods. In the first case, enzyme solution was prepared adding 0.5 unit of GLU-ox (0.5 unit/μl) to a solution containing 0.8% BSA and 0.1% glutaraldehyde. In the second case, PEDGE (100 mg/ml) was used instead of glutaraldehyde. Biosensor were coated by hand, dipping the tip of the wire into the enzyme solution while working under a microscope. After coating, glutaraldehyde-treated sensors were left

for at least 72 h at room temperature prior to use. Instead, PEDGE-treated sensors were cured in oven at 48° C for 90 min and then stored at 4° C or cryopreserved at -20° C.

2.5. Electrochemical measurements

All recordings were performed using a four channel potentiostat (BioStat, ESA, Inc USA), connected to a personal computer. *In vitro* biosensors calibration was carried out in PBS (pH 7.4, 37° C), at constant +700 mV potential versus Ag/AgCl reference electrode; a stable baseline current was normally achieved within 30-45 min. Bare and permselective-membrane coated wires were calibrated with H_2O_2 (20-200 μM), DA (0-100 μM), DOPAC (0-10 μM) and AA (0-20 μM). The current was recorded after every addition, and changes in baseline values were used to evaluate device performances. Biosensor sensitivity was obtained by calculating the slope of the analyte calibration curve by linear regression analysis. Selectivity is expressed as the ratio of sensitivity values of AA (250 μM), DA (5 μM), DOPAC (20 μM) versus H_2O_2.(100 μM) (Hascup et al., 2008).

2.6. Biosensor cryopreservation

After a first calibration with H_2O_2 and GLU (time 0), biosensors were stored at -20° C with the enzyme-coated tip immersed in a H_2O-glycerol (1:1) solution. At 3, 7 and 30 days cryopreserved biosensors were allowed to thaw, tested for H_2O_2 and GLU, and returned to -20° C in H_2O-glycerol.

2.7. Statistical analysis

All data are expressed as mean ± standard deviation (SD). Comparisons between data groups were performed using Bonferroni test or Umpaired t test were appropriate; significance level $p<0.05$.

3. Results

3.1. Sensitivity for H_2O_2 and GLU in different biosensor prototypes.

H_2O_2 and GLU sensitivity values for prototypes 1 (Pt and Pt/Ir), 2 and 3 are reported on Table 1. Sensitivity for H_2O_2 was determined on bare electrodes while sensitivity for GLU was determined on Nafion-Poly(oPD)-coated sensors. H_2O_2 sensitivity: among Pt-based biosensors, prototype 3 (242.7 ± 12.50 pA/μM, R^2 = 0.997, n = 15) significantly ($p<0.001$) outperform both prototype 2 (179.5 ± 21.67 pA/μM, R^2 = 0.986, n = 20) and prototype 1 (134.5 ± 11.35 pA/μM, R^2 = 0.9791, n = 20). Fig. 2 shows linearity of response for H_2O_2 and GLU of prototype 3 biosensors. In prototype 1, Pt/Ir-based devices (152.4 ± 18.35 pA/μM, R^2 = 0.958, n = 20) are significantly (two-tailed p = 0.007, t = 3.710) more sensitive with respect to Pt-based biosensors (134.5 ± 11.35 pA/μM, R^2 = 0,9791, n = 20). GLU sensitivity: among Pt-based biosensors, prototype 3 (11.42± 0.94 pA/μM, R^2 = 0.9867, n = 15) significantly ($p<0.01$) outperform both prototype 2 (5.900 ± 0.53 pA/μM, R^2 = 0.991, n = 18) and prototype 1 (3.71± 0.55 pA/μM, R^2 = 0.958, n = 18). In prototype 1, Pt/Ir-based devices (3.876 ± 0.56 pA/μM, R^2 = 0.985, n = 18) do not show significant difference in sensitivity ($p=0.465$) with respect to Pt-based biosensors (3.71 ± 0.55 pA/μM, R^2=0.9577, n=18).

3.2 Permselective membranes

The effect of permselective membranes application on prototype 3 sensitivity for H_2O_2, AA, DOPAC and DA is reported on table 2. Bare Pt electrodes exhibit high sensitivity for H_2O_2 (242.7 ± 12.50 pA/μM, R^2 = 0.989, n = 15), AA (176.2 ± 4.840 pA/μM, R^2=0.997, n=15), DA (69.74 ± 4.26 pA/μM, R^2=0.968, n=15) and DOPAC (122.38 ± 1.20 pA/μM, R^2=0.984, n=15). All permselective coatings partly reduced H_2O_2 sensitivity of bare Pt electrodes (27.4% Nafion, 0.1% Poly(oPD); 29.4% Nafion-Poly(oPD)), while effectively shielding all other compounds tested. Nafion-Poly(oPD) coating yielded best all-round results reducing AA 85.4% ($p<0.01$ vs bare), DOPAC 95.9% ($p<0.01$ vs bare), DA 86.7% ($p<0.01$ vs bare), while modestly affecting H_2O_2 signal (29.4% vs bare). Selectivity values (expressed as the ratio of sensitivity values of AA (250 μM), DA (5 μM), DOPAC (20 μM) versus H_2O_2 (100 μM)), are shown in Fig. 3.

3.3. Enzyme immobilization procedures

The effects of GLU-ox immobilization procedures (glutaraldehyde or PEDGE) on prototype 3, Nafion-Poly(mPD)-coated GLU biosensor performances are reported on table 3. Both devices show the same detection limit (LOD) but a different response time: 2910 ms for glutaraldehyde- and 3429 ms for PEDGE-treated biosensors. Further, GLU sensitivity for PEDGE-treated biosensors (14.59 ± 0.2594 pA/μM, R^2=0.9994, n=18) is significantly different (two-tailed $p<0.0001$, t = 13.775) to glutaraldehyde-treated biosensors (11.42 ± 0.9379 pA/μM, R^2 = 0.9867, n = 15).

3.4. Biosensor cryopreservation

Prototype 3 Nafion-Poly(oPD)-coated PEDGE-treated GLU biosensor, were cryopreserved at -20° C for 3, 7 and 30 days. A time-corse of H_2O_2 and GLU sensitivity recorded in cryopreserved biosensors vs room temperature-preserved devices is shown in table 4. Biosensors cryopreservation with glycerol does not influence H_2O_2 nor GLU sensitivity, with only negligible fluctuations in performances.

4. Discussion

Our work stem from a recent paper from Wahono et al (2012), describing a newly designed GLU amperometric biosensor. Aiming to build a feasible and robust device to be used in the lab routine, we derived several prototypes using different materials and technical solutions (Fig. 1). As shown in Table 1, our findings indicate that different constructive details do influence final biosensor performances. The electrical contiguity between the electrode wire and the potentiostat appeared particularly critical, both in terms of total resistance and device reliability. In our hands, best all-round performances were obtained by joining the Pt electrode to a gold pin using silver-epoxy glue (prototype 3).

Biosensor sensitivity was also affected by electrode's chemical nature. In prototype 1, bare Pt/Ir electrodes showed higher sensitivity for H_2O_2 than Pt electrodes, but enzyme-coated Pt/Ir did not perform significantly better in detecting GLU (less than 5% difference). Pt/Ir wire was also found fragile and of difficult manipulation during manufacturing, compared to Pt wire. Such a mechanical boon for the pure metal compared with the alloy is in contrast with a previous study (Kirwan et al., 2007); however, this discrepancy can be ascribed to different wire proportions (125 µm diameter and 1 mm in lengths versus 20 µm diameter and 0.5 mm in lengths). Since tissue damage secondary to device implantation severely hampers the efficiency of any brain monitoring technique (Borland et al., 2005; Clapp-Lilly et al., 1999), 25 µm Pt wire was chosen for production biosensors.

The use of permselective membranes in biosensors is an efficient strategy to reduce the magnitude of non-specific signals from electroactive endogenous molecules (Dale et al., 2005; Kirwan et al., 2007). In this study we applied two well-known permselective membranes (Nafion and Poly(oPD)) in order to achieve high H_2O_2 sensitivity and selectivity against interfering species (Ekinci et al., 2001; Hascup et al., 2008; Killoran and O'Neill, 2008). Bare and coated devices were tested against H_2O_2 and AA, DOPAC and DA which are among the major electroactive endogenous molecules susceptible to oxidation at a Pt surface at +700 mV. Results in Table 2 show that the association of Nafion and Poly(oPD) yields best results, highly reducing all non-specific signals (up to 95.9% for DOPAC) with a 30% decrease of H_2O_2 signal. These *in vitro* results are in agreement with previous evidence (Wahono et al., 2012; Kirwan et al., 2007) and indicate that Nafion-Poly(oPD) coating may help to maximize biosensor selectivity in order detect GLU in a complex physiologic media, rich in electroactive endogenous molecules.

Biosensors performances largely rely on the activity and specificity of the enzyme linked at the electrode surface. Several immobilization methods are currently available, including glutaraldehyde-based cross-linking (Burmeister et al., 2000; Netchiporouk et al., 1996) and enzyme entrapment in different matrices (Tian et al., 2009; Liu et al., 2005; Oldenziel et al., 2006). While glutaraldehyde-based immobilization remains the most used enzyme cross-linking method, recent evidence suggest that PEGDE may be an advantageous alternative to glutaraldehyde (Vasylieva et al., 2013 and 2011; Zigah et al., 2009). Since enzyme immobilization procedure is a

crucial step in biosensors construction, we tested how glutaraldehyde- and PEDGE-based enzyme cross-linking affected our prototype 3 performances. The results in Table 3 show that our PEDGE-treated devices are significantly more sensitive to GLU than glutaraldehyde-treated ones (14.59 ± 0.2594 pA/μM vs 11.42 ± 0.9379 pA/μM respectively, $p<0.01$). This is in line with previous studies and may be attributed to a sustained catalytic activity of immobilized GLU-ox, due to PEDGE-based mild cross-linking mechanism (Vasylieva et al., 2013 and 2011). However, our data are in contrast with a recent preliminary report of no significant advantages of PEDGE over glutaraldehyde (Burmeister et al., 2013). Notwithstanding, it is important to consider that PEDGE offers a non-toxic alternative to glutaraldehyde which, together with its low cost and ease of use, makes PEDGE ideal as a production technique.

Short half life represent another limitation in biosensors utilization. Biosensors degrade mainly because isolated enzymes, away from their physiological conditions, are easily denatured or inactivated by environmental stresses (Yancey et al., 1982). To extend their lifetime, we stored our devices at -20° C with the enzyme-coated tip immersed in a H_2O-glycerol (1:1) solution. The ability of glycerol to act as protein-stabilizing agent is long documented, yet its mechanism of action is still not clear (Bradbury and Jakoby, 1976; Gekko and Timasheff, 1981; Yancey PH, 2005). Recent evidence show that glycerol shift proteins to more compact conformations and prevents protein aggregation by inhibiting unfolding, thus preserving the active structure of the enzymes (Meng et al., 2004; Vagenende et al., 2009). Cryopreservation in H_2O-glycerol maintained GLU biosensors sensitivity unaltered after 30 days, suggesting that this methodology may actually be effective for longer times.

5. Conclusions

In this paper we report the construction and *in vitro* characterization of 3 amperometric micro biosensor for GLU detection, derived from a newly described device (Wahono et al., 2012). Our best prototype shows high sensitivity and specificity for GLU, while being poorly influenced by common interfering substances such as AA, DOPAC and DA. Further, the structure is easily constructed, very small and streamlined and may represent a labor- and cost-effective tool to be applied for measuring brain GLU release *in vivo* (Kulagina et al., 1999, van der Zeyden et al., 2008).

We have developed and *in vitro* tested several I-shaped GLU biosensors (Wahono et al., 2012) built with different constructive details. We found that the choice of enzyme immobilization procedures and permselective membrane as well as the electrical contiguity between the enzyme-coated Pt wire and the potentiostat, profoundly influence final biosensor performances. The overall results show that a specific and sensitive GLU biosensor can be reliably constructed and criopreserved for a long time before use. With high performances and minimal dimensions, this biosensor type holds potential to address many of the known issues in the field of *in vivo* GLU study. However, a more definite answer shall only arise from a routine laboratory use of this device. An affordable and consistent construction and criopreservation method will help diffusion and full exploitation of biosensors technology.

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Highlights

- The construction and *in vitro* characterization of an efficient and cost-effective amperometric glutamate biosensor is presented.
- Permselective coating with Nafion-Poly(o-phenylenediamine) enhanced biosensor selectivity against common interferents.
- Glutamate-oxydase cross-linking with PEGDE is an advantageous alternative to glutaraldehyde-based immobilization method.
- A glycerol-based cryopreservation method maintained biosensors sensitivity unaltered after 30 days.

	Sensitivity (pA/µM) - H_2O_2	Sensitivity (pA/µM) - GLU
Prototype 1 (Pt)	134.5 ± 11.35 (n=20)	3.711 ± 0.5523 (n=18)
Prototype 1 (Pt/Ir)	152.4 ± 18.35 (n=20)	3.876 ± 0.5652 (n=18)
Prototype 2	179.5 ± 21.67 (n=20)	5.9 ± 0.5264 (n=18)
Prototype 3	242.7 ± 12.5 (n=15)	11.42 ± 0.9379 (n=15)

Table1. Sensitivity for H_2O_2 and glutamate for different sensors type. Data are expressed as mean ± standard deviation (SD)

Prototype3 sensors				
Permselective membrane	H_2O_2 sensitivity (pA/μM)	AA sensitivity (pA/μM)	DA sensitivity (pA/μM)	DOPAC sensitivity (pA/μM)
Bare (n=15)	242.7 ± 12.50	176.2 ± 4.840	69.74 ± 4.26	122.38 ± 1.20
Nafion (n=15)	176.0 ± 12.71	67.38 ± 5.180 *	45.43 ± 2.80 *	20.79 ± 1.03 *
Poly(oPD) (n=15)	243.1 ± 32.56	42.50 ± 1.398 **	19.74 ± 1.20 **	5.20 ± 0.34 **
Nafion-Poly(oPD) (n=15)	171.3 ± 6.080	25.67 ± 1.707 **	9.28 ± 0.73 **	4.98 ± 0.34 **

Table 2. Sensitivity values for prototype 3 sensor with different permselective coatings. Data are expressed as mean ± standard deviation (SD). (* $p<0.05$ vs. bare; ** $p<0.01$ vs. bare). H_2O_2 (100 μM); AA: ascorbic acid (250 μM); DA: dopamine (5 μM); DOPAC: dihydroxyphenylacetic acid (20 μM).

	Glutaraldehyde	PEDGE
Slope	11.42 ± 0.9379	14.59 ± 0.2594
R^2	0,99	1
Linearity range **Linear Regression Equation**	0-100 µM Y = 11.42*X + 31.31	0-100 µM Y = 14.59*X + 1.426
LOD	≤1 µM	≤1 µM
Response time	2.91 s	3.4 s

Table 3. Comparison of prototype 3 biosensors performances using glutaraldehyde versus PEDGE

Sensitivity (pA/μM)	Time 0	3 days	7 days	30 days
Cryo - H_2O_2	244.5 ± 11.54 (n=8)	244.3 ± 9.30 (n=8)	236.4 ± 11.35 (n=8)	231.5 ± 14.31 (n=8)
RT - H_2O_2	239.6 ± 13.21 (n=6)	241.4 ± 10.41 (n=6)	232.5 ± 12.13 (n=6)	228.5 ± 9.17 (n=6)
Cryo - GLU	11.86 ± 0.9379 (n=8)	12.40 ± 0.7336 (n=8)	10.96 ± 0.8509 (n=8)	11.53 ± 0.3304 (n=8)
RT - GLU	12.14 ± 1.434 (n=6)	11.81 ± 1.712 (n=6)	10.01 ± 1.961 (n=6)	5.57 ± 2.704 (n=6)

Table 4. Glutamate and H2O2 sensitivity for sensors cryopreserved in H2O-glycerol solution and kept at room temperature after 3, 7, 30 days. Data are expressed as mean ± standard deviation (SD).

Fig.1 Schematic representation of microsensors construction. Ag: silver wire; Pt: platinum wire; Pt/Ir: platinum/iridium wire; ST: silica capillary; EG: epoxy glue; Ag-EG: silver-epoxy glue; GP: gold-coated pin.

Fig. 2 Linear response to H_2O_2 (20, 40, 60 100 and 200 µM; R^2 = 0.897, n=9) and glutamate (GLU, 20, 40, 60 and 100 µM; R^2 = 0.987, n=9) of PEDGE-based Nafion-Poly(oPD)-coated biosensors.

Fig. 3 Selectivity values of bare Pt wire, Nafion, Poly(oPD) and Nafion-Poly(oPD)-coated Pt wire. Values are expressed as the ratio of sensitivity values of AA (250 µM), DA (5 µM), DOPAC (20 µM) versus H_2O_2 (100 µM).

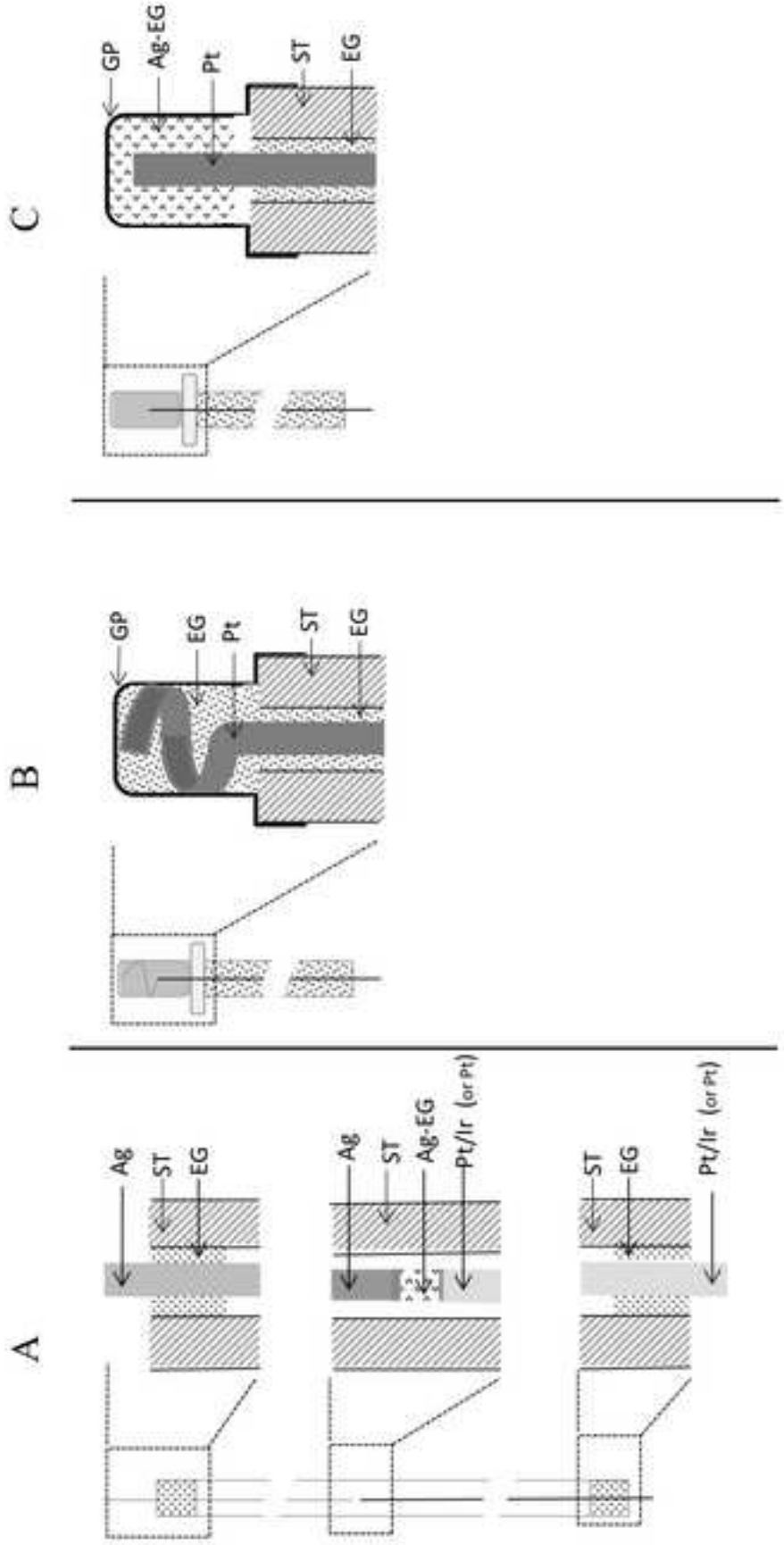
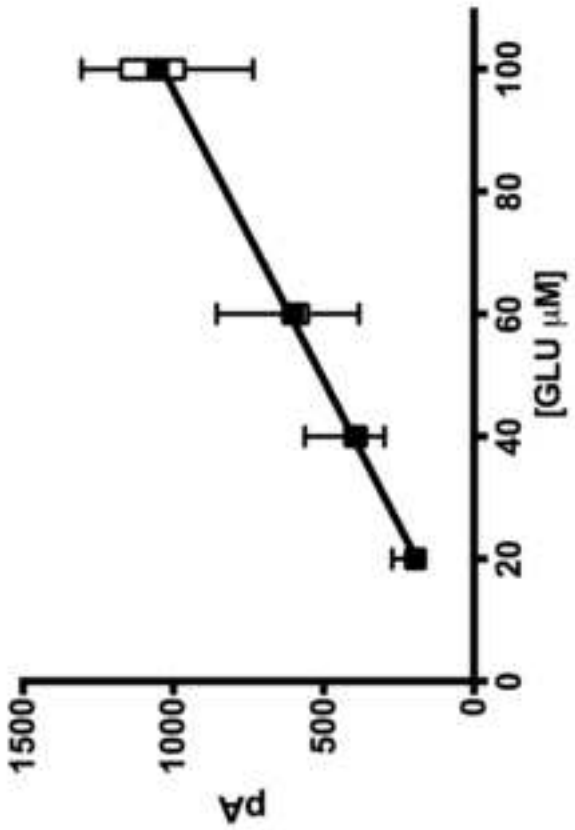

A
Ag
ST
EG
Ag
ST
Ag-EG
Pt/Ir (or Pt)
ST
EG
Pt/Ir (or Pt)
B
GP
EG
Pt
ST
EG
C
GP
Ag-EG
Pt
ST
EG

Figure_1


pA
1500
1000
500
0
0
20
40
60
80
100
[GLU μM]

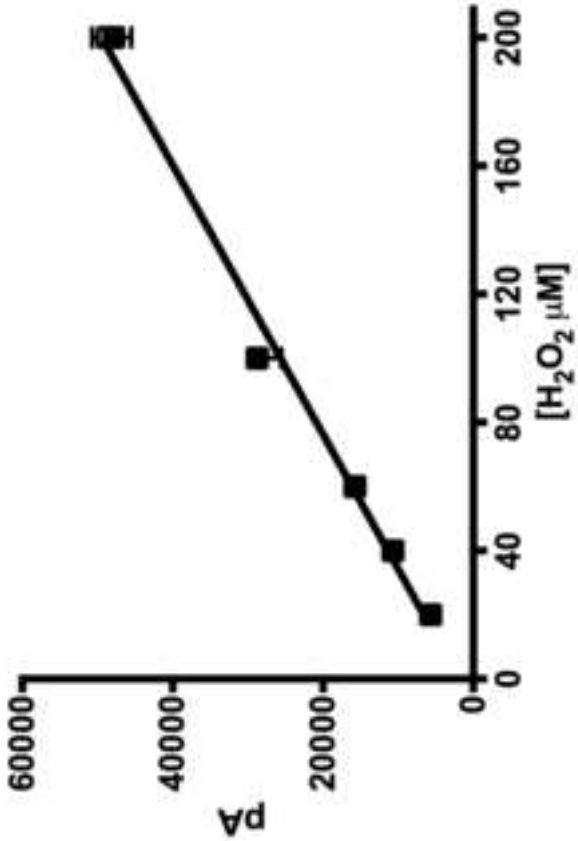

pA
60000
40000
20000
0
0
40
80
120
160
200
[H2O2 μM]

Figure_2

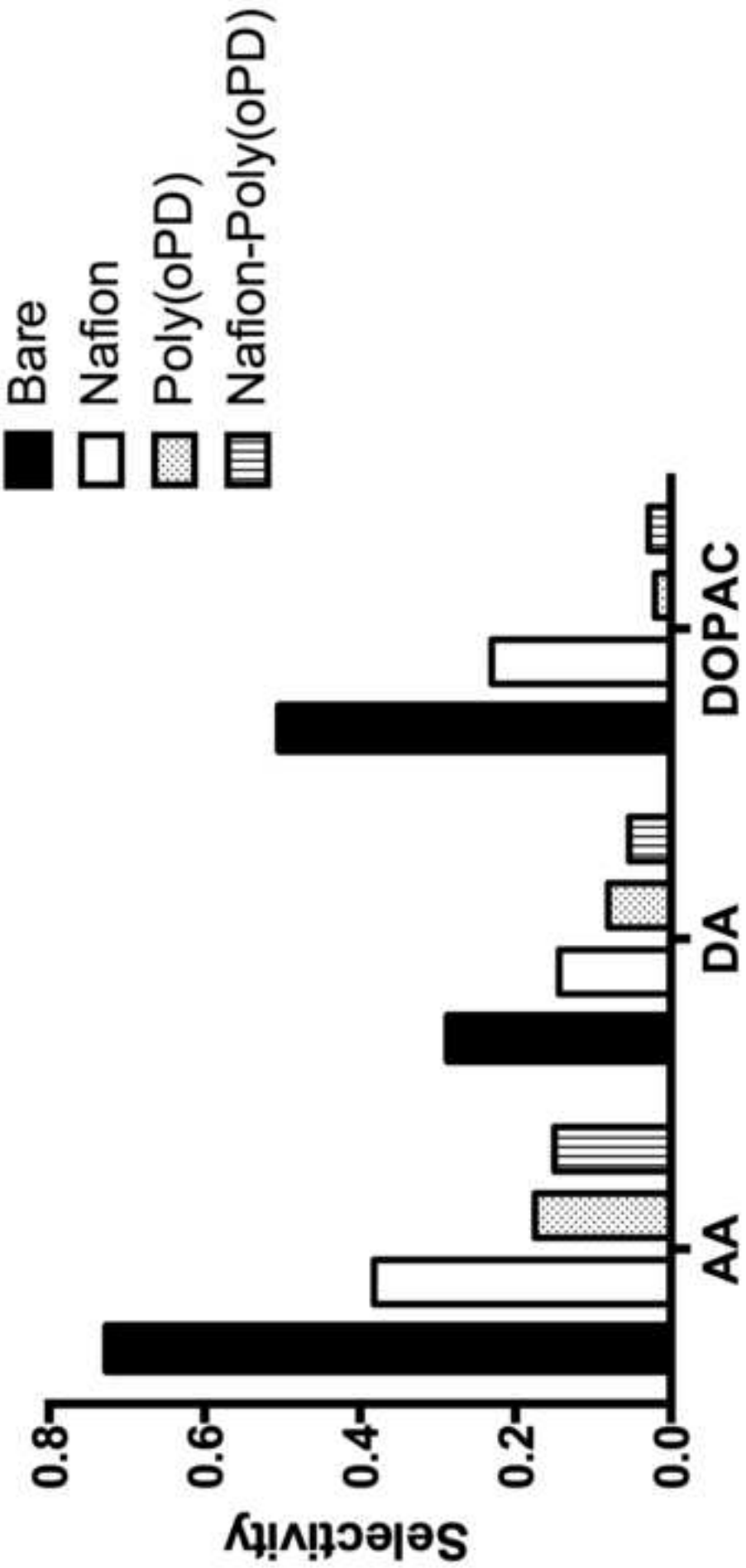

Bare
Nafion
Poly(oPD)
Nafion-Poly(oPD)
Selectivity
0.0
0.2
0.4
0.6
0.8
AA
DA
DOPAC

Figure_3

- A highly efficient yet simple and cost-effective glutamate biosensor is proposed
- The needle-type structure easily allow *in vivo* use
- The cryopreservation methodology highly increases sensors half life