

Supplementary Materials for

A Study on the Combination of Enzyme Stabilizers and Low Temperatures in the Long-Term Storage of Glutamate Biosensor

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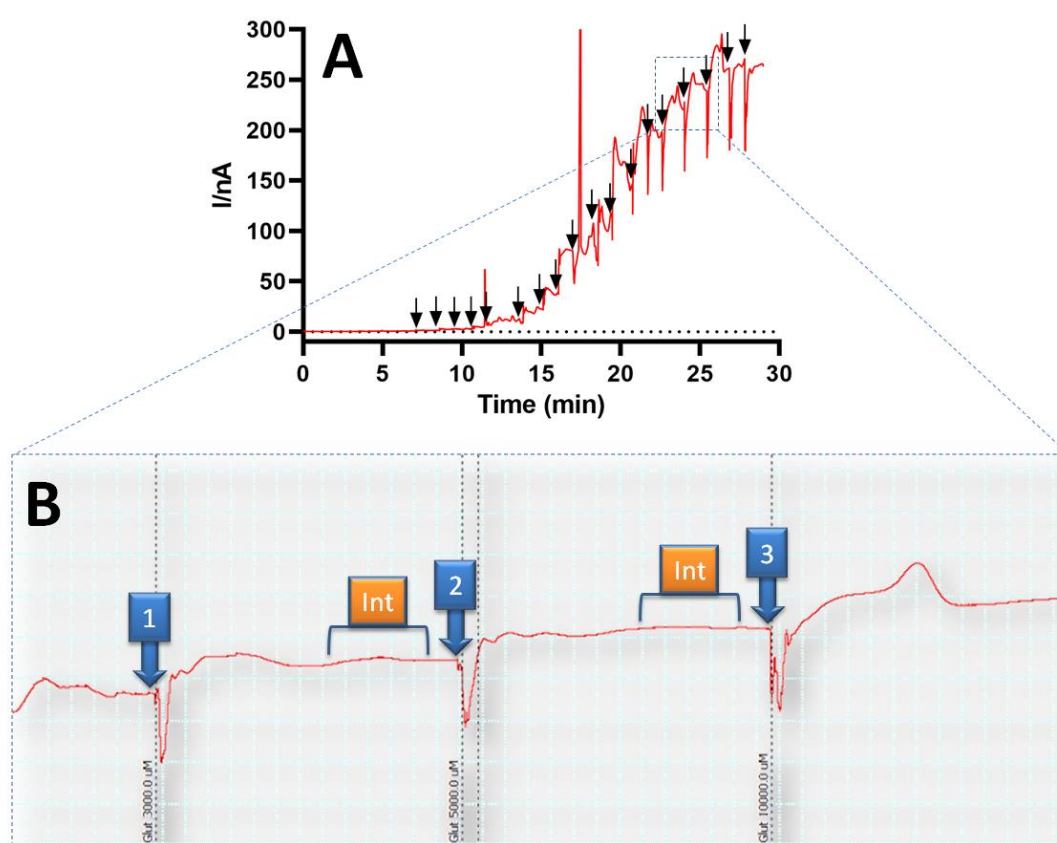


Figure S1. Illustrative plot that reports the raw currents derived from the calibration of a biosensor belonging to the control group (Ptc/PPD/PEI(1%)2/GlutOx5) on Day 56, from 0 to 50,000 μM concentration. Rows indicate the injections of the Glut stock solution corresponding to concentrations of 1, 2, 4, 10, 20, 50, 100, 200, 400, 600, 1000, 1500, 2000, 3000, 5000, 10,000, 20,000, and 50,000 μM .

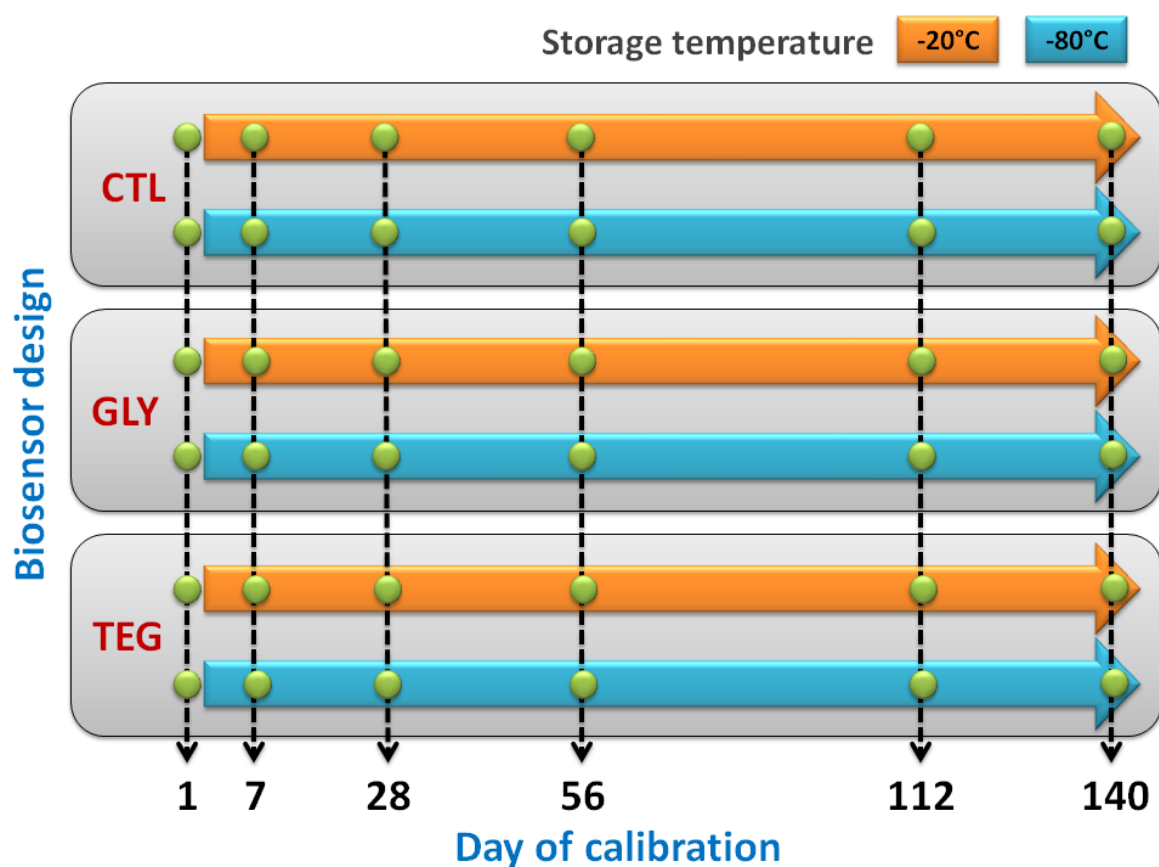


Figure S2. Schematic representation of the timeline of the experimental protocol. The biosensors were calibrated on Day 1 after RT over-night stabilization, with a range of concentration between 0 and 50,000 μM by means of injections of Glut stock solution (10 mM and 1 M). The same protocol was repeated on Day 7, Day 28, Day 56, Day 112, and Day 140 (for a total of 5 months) (green rings). The three different groups (Control, GLY, and TEG) were subjected to 6 calibrations each. After each calibration, each biosensor group was stored at two different temperatures, i.e., -20°C (orange rows) and -80°C (blue rows).

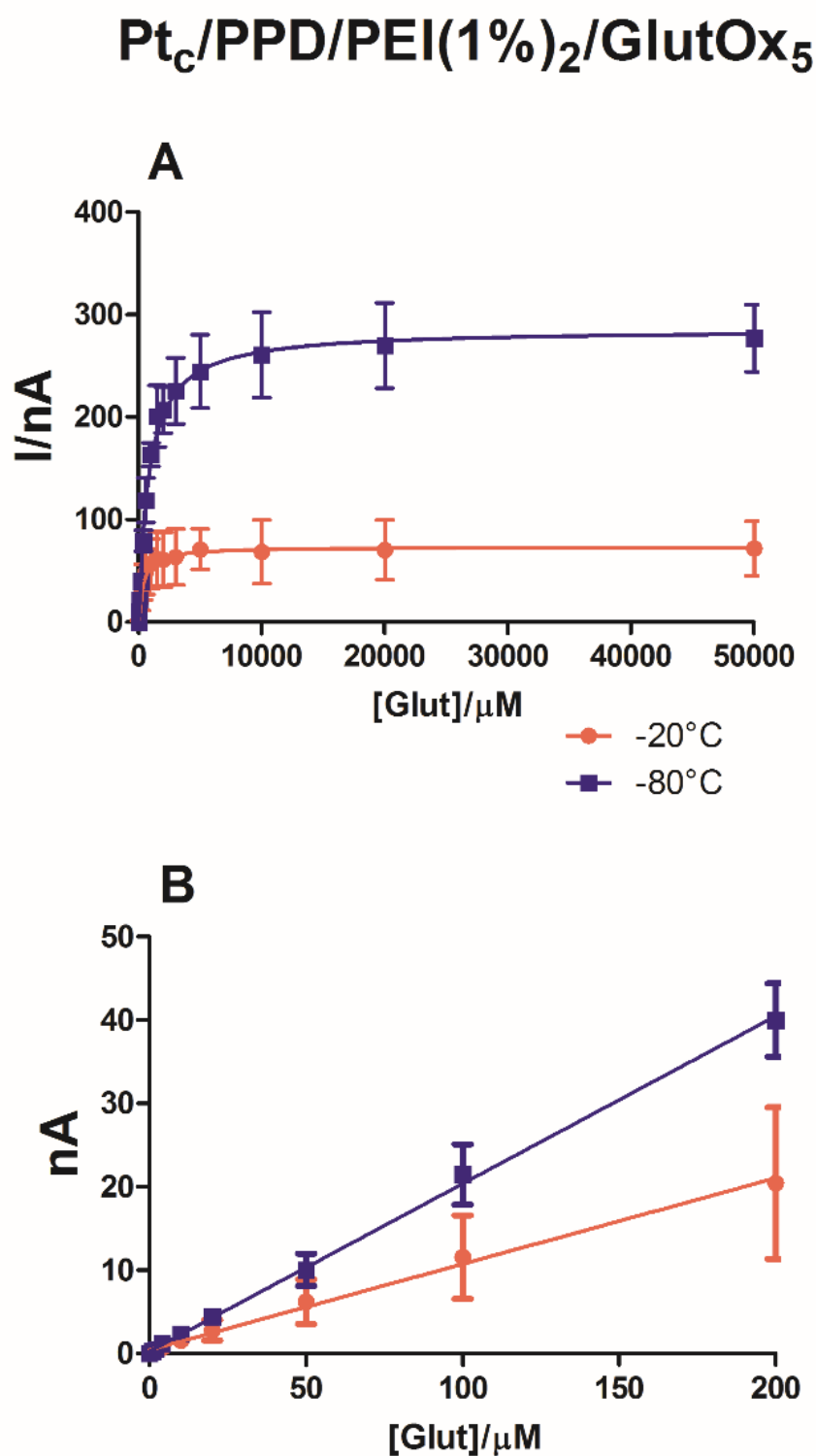


Figure S3. Representative calibration plots of the control group (Pt_c/PPD/PEI(1%)₂/GlutOx₅) on Day 56 for biosensors (n = 4) stored at -20 °C (blue plot) and -80 °C (red plot). Panel A: Michaelis–Menten graph for a concentration range of 0–50,000 μM. Panel B: Linear regression graph in a concentration range of 0–200 μM. Concentration steps for calibrations were 1, 2, 4, 10, 20, 50, 100, 200, 400, 600, 1000, 1500, 2000, 3000, 5000, 10,000, 20,000, and 50,000 μM.

Pt ₂ /PPD/PEI(1%) ₂ /GlutOx _s								
DAY	−20 °C				−80 °C			
	V _{MAX(app)} (nA)	K _{M(app)} (μM)	LRS (nA μM ^{−1})	LOD (μM ^{−1})	V _{MAX(app)} (nA)	K _{M(app)} (μM)	LRS	LOD (μM ^{−1})
1	149.2 ± 111.1	585.5 ± 189.6	0.144 ± 0.001	0.116 ± 0.009	139.1 ± 15.4	373.1 ± 70.2	0.165 ± 0.001	0.097 ± 0.016
7	128.0 ± 3.4*	390.5 ± 49.6	0.135 ± 0.001*	0.205 ± 0.017*	172.8 ± 4.4 [§]	698.8 ± 74.9 [§]	0.110 ± 0.003 [§]	0.178 ± 0.025 [§]
28	103.7 ± 5.2*	297.6 ± 76.6	0.163 ± 0.003*	0.324 ± 0.022*	199.4 ± 9.8 [§]	1253.0 ± 223.8 [§]	0.067 ± 0.003 [§]	0.214 ± 0.036 [§]
56	73.4 ± 7.7*	373.6 ± 189.9	0.103 ± 0.003*	0.777 ± 0.033*	218.9 ± 8.7 [§]	833.7 ± 130.2 [§]	0.157 ± 0.002 [§]	0.358 ± 0.029 [§]
112	62.5 ± 4.4*	599.7 ± 60.8	0.059 ± 0.001*	0.674 ± 0.028*	172.1 ± 3.2 [§]	734.4 ± 55.5	0.155 ± 0.002 [§]	1.613 ± 0.078 [§]
140	50.2 ± 5.0*	541.3 ± 239.9	0.063 ± 0.001*	1.215 ± 0.054*	152.4 ± 6.8 [§]	732.8 ± 133.8	0.122 ± 0.003 [§]	1.204 ± 0.066 [§]

Table S1. Data obtained by calibrations of the control design from Day 1 to Day 140 for biosensors (n = 4) stored at −20 and −80 °C. V_{MAX(app)} data are given as nA, K_{M(app)} data as μM, LRS data as nA μM^{−1}, and LOD data as μM. Data are expressed as the mean ± SEM. * *p* < 0.05 −20 °C data vs. corresponding Day 1 data; § *p* < 0.05 −80 °C data vs. corresponding Day 1 data; # *p* < 0.05 vs. −20 °C.

Pt ₂ /PPD/PEI(1%) ₂ /GlutOx _s /GLY(0.1%)								
DAY	−20 °C				−80 °C			
	V _{MAX(app)} (nA)	K _{M(app)} (μM)	LRS (nA μM ^{−1})	LOD (μM ^{−1})	V _{MAX(app)} (nA)	K _{M(app)} (μM)	LRS (nA μM ^{−1})	LOD (μM ^{−1})
1	256.3 ± 12.5	467.1 ± 104.7	0.141 ± 0.002	0.063 ± 0.014	264.7 ± 8.7	474.4 ± 71.0	0.147 ± 0.001	0.074 ± 0.022
7	211.4 ± 10.7*	333.4 ± 83.7	0.304 ± 0.003*	0.182 ± 0.026*	258.8 ± 6.1	422.3 ± 46.5 [§]	0.338 ± 0.007 [§]	0.098 ± 0.031 [§]
28	275.9 ± 6.5	360.2 ± 41.3	0.339 ± 0.004*	0.382 ± 0.034*	254.2 ± 8.0	483.9 ± 69.5 [§]	0.292 ± 0.005 [§]	0.222 ± 0.043 [§]
56	310.7 ± 8.2*	366.5 ± 46.8	0.318 ± 0.004 [§]	0.235 ± 0.029*	285.9 ± 14.3	605.5 ± 130.9 [§]	0.220 ± 0.003 [§]	0.173 ± 0.029 [§]
112	266.8 ± 9.0	483.8 ± 74.3	0.276 ± 0.015 [§]	0.689 ± 0.057*	239.7 ± 6.9	623.7 ± 77.2 [§]	0.183 ± 0.003 [§]	0.803 ± 0.051 [§]
140	254.8 ± 7.5	508.4 ± 67.2	0.216 ± 0.001 [§]	1.007 ± 0.041*	204.0 ± 8.7 [§]	648.5 ± 117.6	0.165 ± 0.002 [§]	0.619 ± 0.092 [§]

Table S2. Data obtained by calibrations of glycerol-loading design from Day 1 to Day 140 for biosensors (n = 4) stored at −20 and −80 °C. V_{MAX(app)} data are given as nA, K_{M(app)} data as μM, LRS data as nA μM^{−1}, and LOD data as μM. Data are expressed as the mean ± SEM. * *p* < 0.05 −20 °C data vs. corresponding data on Day 1; § *p* < 0.05 −80 °C data vs. corresponding Day 1 data; # *p* < 0.05 vs. −20 °C.

Pt ₂ /PPD/PEI(1%) ₂ /GlutOx _s /TEG(0.1%)								
DAY	−20 °C				−80 °C			
	V _{MAX(app)} (nA)	K _{M(app)} (μM)	LRS (nA μM ^{−1})	LOD (μM ^{−1})	V _{MAX(app)} (nA)	K _{M(app)} (μM)	LRS (nA μM ^{−1})	LOD (μM ^{−1})
1	240.9 ± 7.3	414.9 ± 59.4	0.232 ± 0.003	0.289 ± 0.024	207.6 ± 7.3	471.3 ± 75.5	0.185 ± 0.001	0.029 ± 0.012
7	299.8 ± 10.7*	499.5 ± 89.2	0.375 ± 0.009	0.410 ± 0.016*	207.3 ± 2.6	412.7 ± 24.6	0.292 ± 0.005 [§]	0.035 ± 0.009 [§]
28	228.6 ± 6.7	485.8 ± 64.6	0.211 ± 0.007	0.506 ± 0.019*	252.7 ± 3.4 [§]	534.8 ± 31.9	0.280 ± 0.003 [§]	0.101 ± 0.017 [§]
56	179.2 ± 2.5*	510.7 ± 32.2	0.199 ± 0.010	0.620 ± 0.031*	207.3 ± 2.6 [§]	380.3 ± 52.8	0.267 ± 0.007 [§]	0.204 ± 0.015 [§]
112	165.8 ± 2.9*	884.1 ± 60.2*	0.141 ± 0.004	0.497 ± 0.043*	194.4 ± 3.7 [§]	522.4 ± 44.1	0.194 ± 0.003 [§]	0.165 ± 0.021 [§]
140	149.8 ± 5.6*	1295 ± 175.6*	0.127 ± 0.004	1.016 ± 0.087*	204.0 ± 8.7 [§]	554.2 ± 81.0	0.186 ± 0.005 [§]	0.536 ± 0.043 [§]

Table S3. Data obtained by calibrations of the triethylene glycol-loading design from Day 1 to Day 140 for biosensors (n = 4) stored at −20 and −80 °C. V_{MAX(app)} data are given as nA, K_{M(app)} data as μM, LRS data as nA μM^{−1}, and LOD data as μM. Data are expressed as the mean ± SEM. * *p* < 0.05 −20 °C data vs. corresponding Day 1 data; § *p* < 0.05 −80 °C data vs. corresponding Day 1 data; # *p* < 0.05 vs. −20 °C.