Supporting Information

Electrochemical immunosensing platform for the determination of the 20S proteasome using an aminophenylboronic/poly-indole-6-carboxylic acid -modified electrode

Francisco Martínez-Rojas[†], Victor C. Diculescu[‡], Francisco Armijo[†]**

† Pontificia Universidad Católica de Chile, Facultad de Quimica y de Farmacia, Departamento de Química Inorgánica, Laboratorio de Bioelectroquimica, Av. Vicuña Mackenna 4860, 7820436 Macul, Santiago, Chile.

‡ National Institute of Materials Physics, Atomistilor, No. 405A, Măgurele, Ilfov, Romania

Corresponding author Tel.: +56-2-26864389; fax: +56-2-26864744.

E-mail address: jarmijom@uc.cl (F. Armijo); victor.diculescu@infim.ro (V.C. Diculescu)

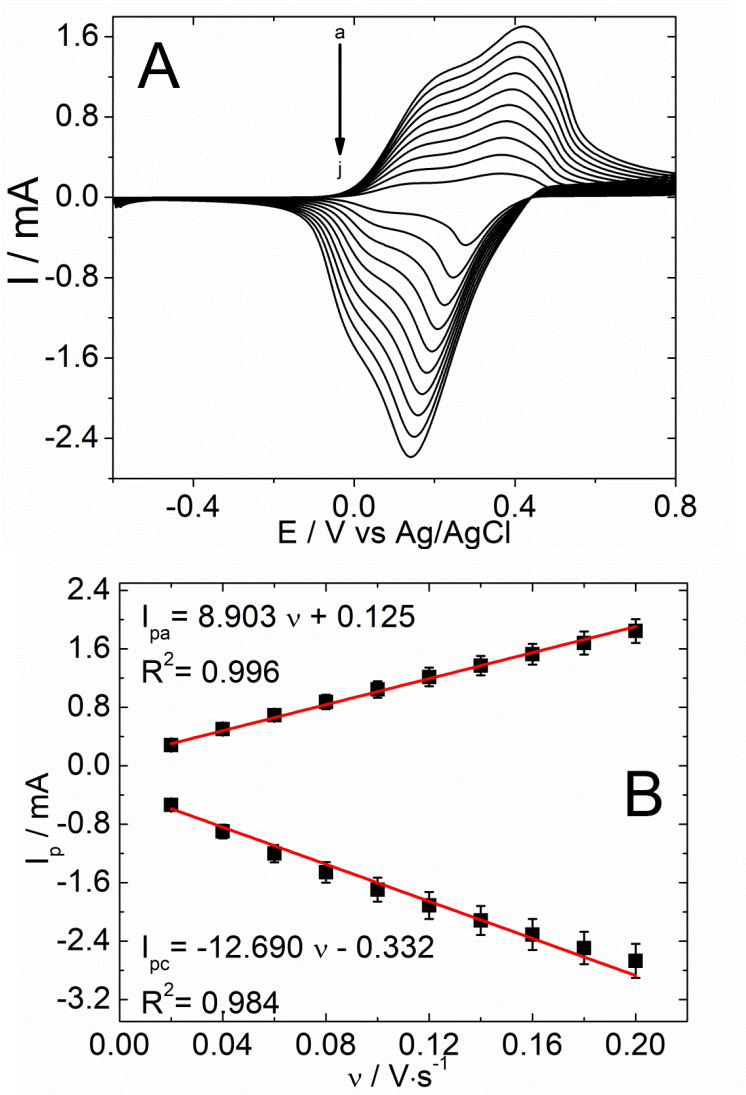


Figure S1. (A) Cyclic voltammograms of a 6-PICA film in NaAC–HAC buffer 0.1 mol·L^{-1} (pH 5.5) at different scan rates of 0.2 V·s^{-1} (a), 0.18 V·s^{-1} (b), 0.16 m·Vs^{-1} (c), 0.14 V·s^{-1} (d), 0.12 V·s^{-1} (e), 0.1 V·s^{-1} (f) 0.08 V·s^{-1} (g) 0.06 V·s^{-1} (h) 0.04 V·s^{-1} (i) and 0.02 V·s^{-1} (j). **(B)** The linear relationships of the anodic peak current (A) and cathodic peak current depended on different scan rates.

Fig. S1A shows the cyclic voltammograms of 6-PICA examined with different scan rate in pH 5.5 acetate buffer. According to the obvious redox couples, it represents that the 6-PICA can be well modified on GCE and it can show stable current response in the scan rate of 20–200 mV·s^{-1}. In the potential range of 0 V to 0.8 V, the 6-PICA film exhibits two well-defined redox couples, indicating reversible and fast electron transfer processes. Both anodic and cathodic peak currents are directly proportional to scan rate Fig. S1B, suggesting that it is a surface controlled process in the system. The observation of well-defined and

persistent cyclic voltammetric peaks indicates that the 6-PICA/GCE exhibits electrochemical response characteristics of redox species confined on the electrode. The apparent surface coverage (Γ) was estimated by equation [1, 2]:

$$Ip = n^2 F^2 \nu A \Gamma /4RT \qquad (1)$$

where, Ip is the peak current of the 6-PICA; n is the number of electron transfer; F is Faraday constant (96 485 $C \cdot mol^{-1}$); ν is the scan rate ($mV \cdot s^{-1}$); A is the area of the electrode surface (0.0125 cm^2); R is gas constant (8.314 $J \cdot mol^{-1} K^{-1}$); and T is the room temperature (298.15 K). Assuming a three-electron process in the present case, the Γ as calculated in $7.59 \cdot 10^{-8}$ mol cm^{-2} for 6-PICA.

[1] Compton, R. G., Banks, C. E., 2010. Understanding Voltammetry, 2nd Ed. Word Scientific Publishing Co. Pte. Ltd., Singapore

[2] Laviron, E., 1974, J. Electroanal. Chem. 52, 355–393.

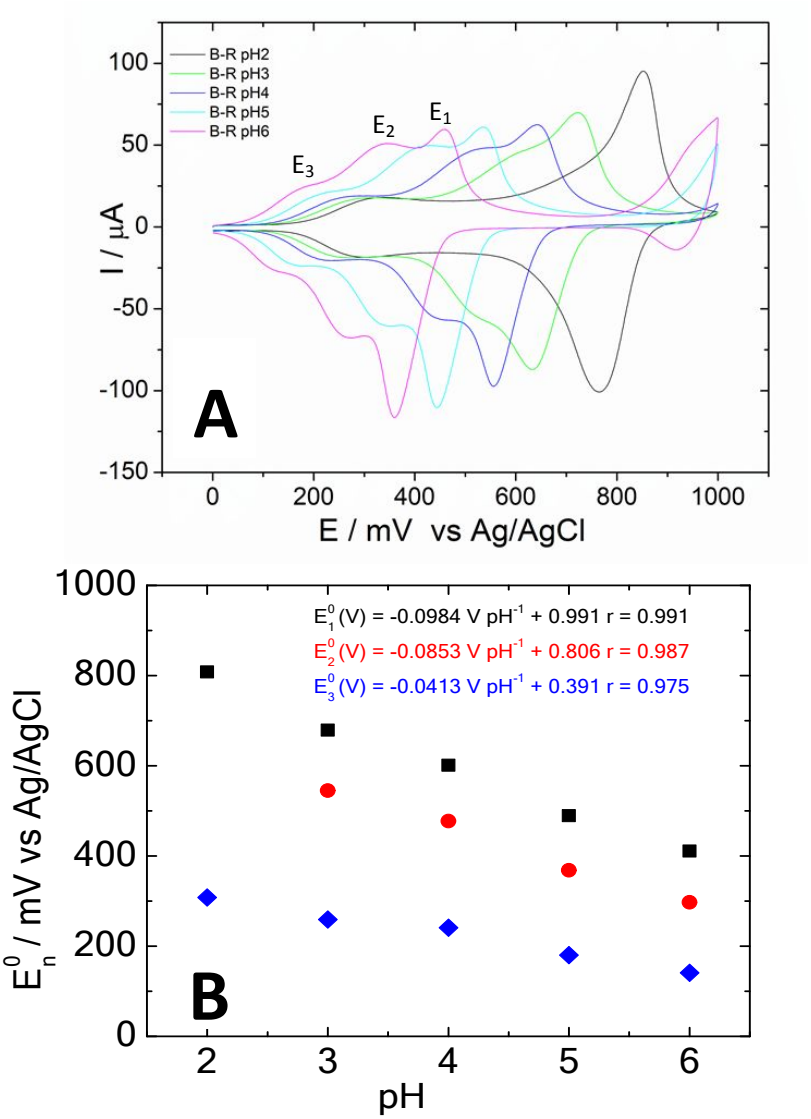
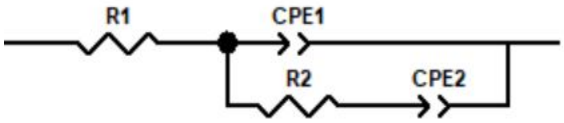


Figure S2. (A) Cyclic voltammograms at pH between 2.0 and 6.0 of 6-PICA film in Britton-Robinson buffer 0.1 mol·L^{-1} (B-R) at 0.1 V·s^{-1}. **(B)** Plots of formal potential (E_1^0 E_2^0 and E_3^0) as a function of pH.

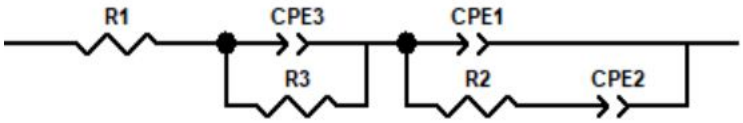
To ascertain the effect of pH, 6-PICA/GCE was examined in different pH solutions (pH 2–6). Fig. S2A presents the redox couples of 6-PICA which are shifted to more negative potential as increasing pH value of NaAC–HAC buffer solution. It shows stable redox peaks with various pH conditions. Even though repeatedly examine 6-PICA and change testing order of pH conditions, the results are the same. This result indicates that the 6-PICA conducting polymer is active and stable in a wide pH condition. The 6-PICA redox couples (with formal potential of E_1^0 and E_2^0) exhibit the significant slopes of -0.097 mV·pH^{-1} and 0.0895 mV pH^{-1} for redox couple E_1 and redox couple E_2, respectively.

Table S3. Fitting results of EIS data. The quality of the fitting to the equivalent circuit was evaluated by an acceptable error value of $\chi^2 < 0.001$. The changes in the electrochemical properties of the system were simulated using the Randles equivalent circuit model.



Electrode	R_1 (Ω)	CPE_1-T (mF)	CPE_1-P	R_2 (Ω)	CPE_2-T (mF)	CPE_2-P	χ^2
6-PICA/GCE	32	1.46	0.88	35	3.45	0.96	$9.45 \cdot 10^{-3}$

Table S4. Fitting results of EIS data. The quality of the fitting to the equivalent circuit was evaluated by an acceptable error value of $\chi^2 < 0.001$. The changes in the electrochemical properties of the system were simulated using the modified Randles equivalent circuit model.



electrode	APBA-6-PICA/GCE	mAb-APBA-6-PICA/GCE	P20S-mAb-APBA-6-PICA/GCE
R_1 (Ω)	**23**	**25**	**31**
CPE_3-T (mF)	10.42	5.45	3.18
CPE_3-P	0.38	0.41	0.37
R_3 (Ω)	**225**	**$1.61 \cdot 10^{12}$**	**$1.0 \cdot 10^{20}$**
CPE_1-T (μF)	19.1	7.51	4.14
CPE_1-P	0.69	0.77	0.84
R_2 (Ω)	**23.23**	**59.17**	**166.8**
CPE_2-T (mF)	3.54	3.02	2.26
CPE_2-P	0.98	0.98	0.96
χ^2	$0.43 \cdot 10^{-3}$	$0.29 \cdot 10^{-3}$	$7.32 \cdot 10^{-5}$

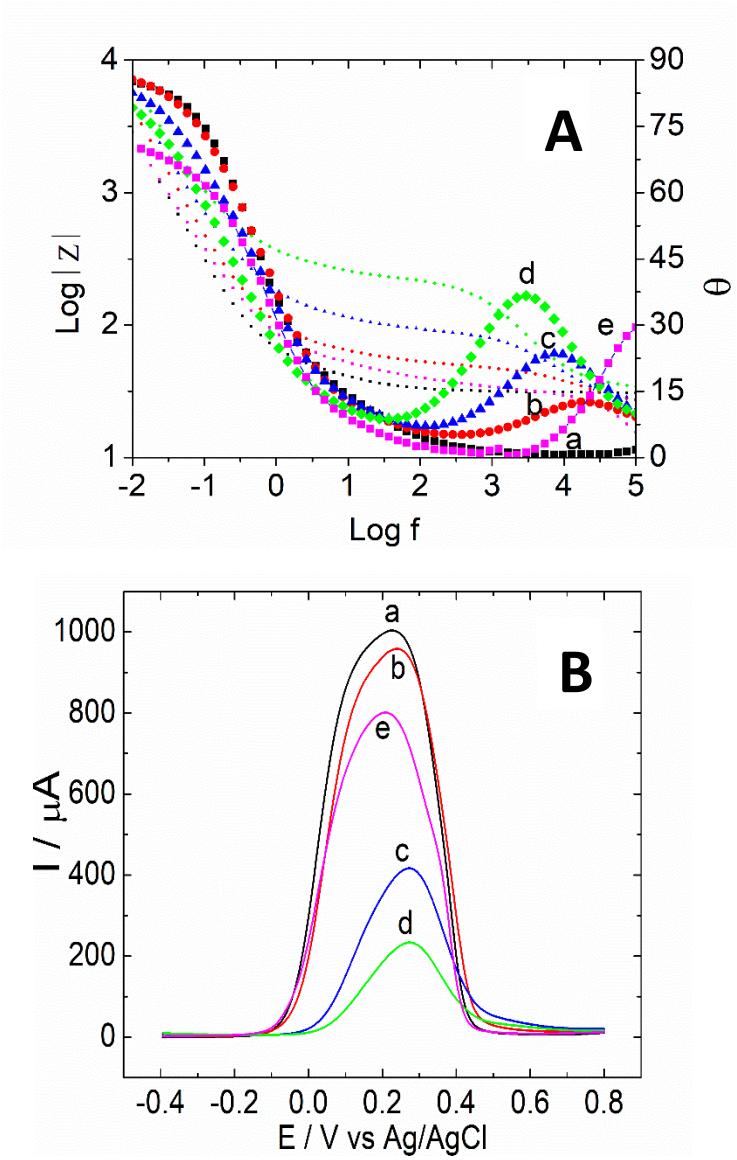


Figure S5. (A) Bode plots and **(B)** SWV of (**a**) 6-PICA/GCE (black line), (**b**) APBA-6-PICA/GCE (red line), (**c**) mAb-APBA-6-PICA/GCE (blue line), (**d**) P20S-mAb-APBA-6-PICA/GCE (green line) and (**e**) mAb-6-PICA-GCE (magenta line) (The concentration of P20S is 100 ng·mL^{-1}). EIS: 0.01 Hz to 100 KHz, 10 mV of amplitude in NaAC–HAC buffer 1.0 mol·L^{-1} (pH 5.5).

Fig S5 control experiment was carried out to evaluate the non-specific interaction of mAb with the surface 6-PICA/GCE, the results de SWV and EIS shows that only exist a specific interaction when the APBA was present.

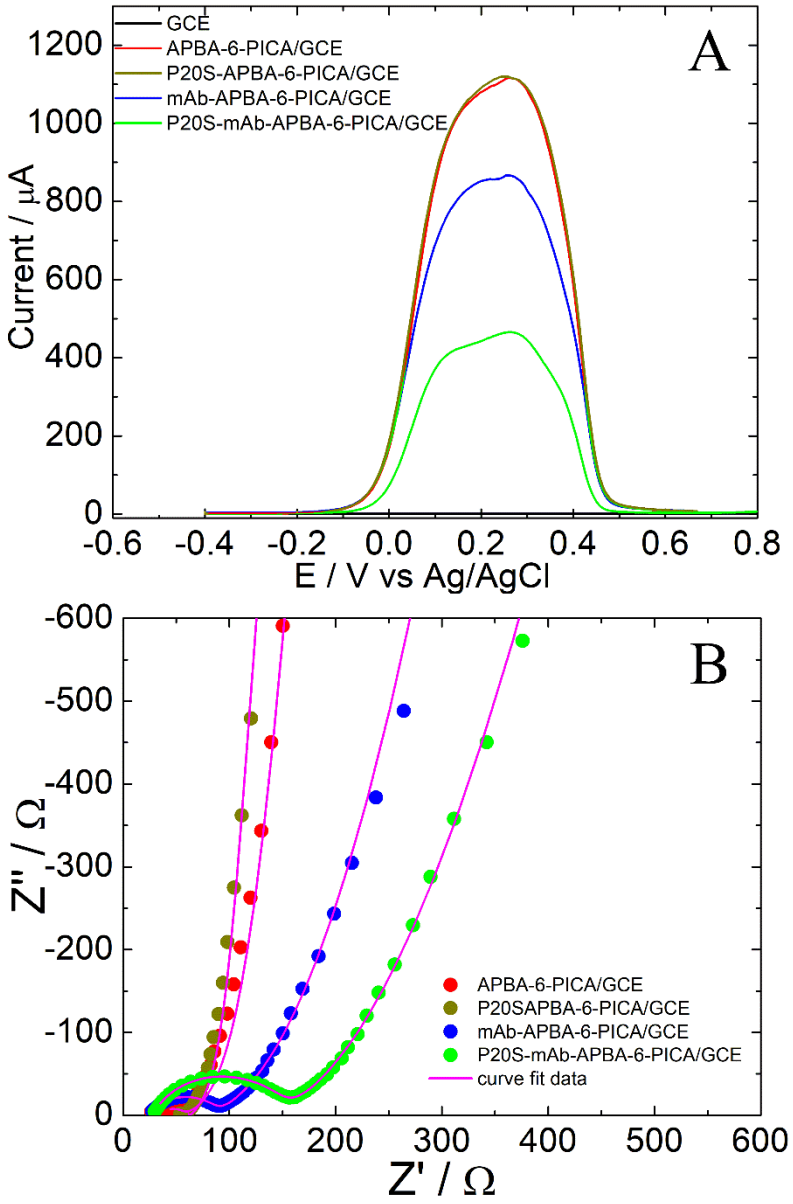


Figure S6. (A) SWV and **(B)** Nyquist plots (•) APBA-6-PICA/GCE, (•) P20S-APBA-6-PICA/GCE, (•) mAb-APBA-6-PICA/GCE and (•) P20S-mAb-APBA-6-PICA/GCE (The concentration of P20S is 60 ng·mL^{-1}). EIS: 0.01 Hz to 100 KHz, 10 mV of amplitude in NaAC–HAC buffer 1.0 mol·L^{-1} (pH 5.5).

Fig S6 control experiment was carried out to evaluate the non-specific interaction of PS20 with the surface ABPA-6PICA/GCE without and with monoclonal antibody, the results de SWV and EIS shows that only exist a specific interaction when the mAb was present.

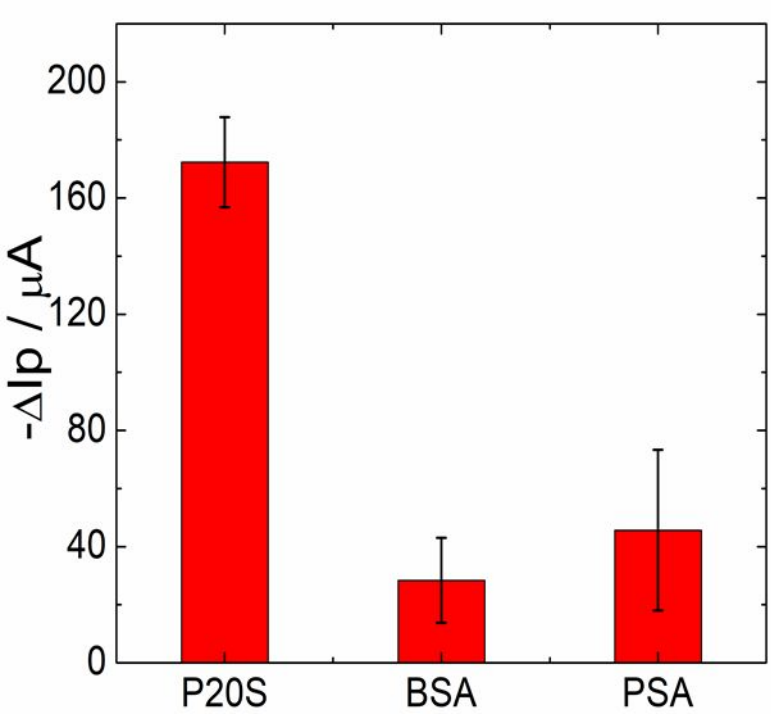


Figure S7. The selectivity test of the assay for P20S on the fabricated immunosensor after incubation in 120 ng/mL of P20S, BSA and PSA, respectively. Standard deviations of the measurements performed with three independent tests are indicated by error bars.

Selectivity

Specificity of an electrochemical immunosensor is an important parameter in clinical analysis, which consists of the ability to measure accurately a biomarker in the presence of other proteins and ions that may be expected to be present in the sample biologic. Selectivity was evaluated by comparing the responses obtained by SWV, after binding to other proteins, such as BSA, which constitutes approximately 50-60% in plasma / serum (120 ng·mL^{-1}) and PSA (120 ng·mL^{-1}) which were incubated for 2 h at 20 ° C in NaAC-HAC buffer (pH 5.5). The results shown in Figure S7, indicated lower response of current could be due to the minimal interaction between the mAb and the non-specific proteins and an adequate selectivity for P20S.