Label-free graphene oxide–based SPR genosensor for the quantification of microRNA21

Michael Michael Lopez Mujica, Yuanyuan Zhang, Fethi Bedioui, Fabiana Gutierrez, Gustavo A. Rivas

► To cite this version:

Michael Michael Lopez Mujica, Yuanyuan Zhang, Fethi Bedioui, Fabiana Gutierrez, Gustavo A. Rivas. Label-free graphene oxide–based SPR genosensor for the quantification of microRNA21. *Analytical and Bioanalytical Chemistry*, 2020, 412, pp.3539-3546. hal-03021298

HAL Id: hal-03021298

<https://hal.science/hal-03021298>

Submitted on 25 Nov 2020

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

2 **Label-free graphene oxide based-SPR genosensor for the quantification of**
3 **microRNA21**

4 Michael López Mujica¹, Yuanyuan Zhang², Féthi Bédioui^{2,*}, Fabiana Gutiérrez^{1,*},
5 Gustavo Rivas^{1,*}

6 ¹INFIQC-CONICET, Departamento de Fisicoquímica, Facultad de Ciencias
7 Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria. 5000 Córdoba,
8 Argentina.

9 ²Chimie ParisTech, PSL University, CNRS 2027, Institute of Chemistry for Life and
10 Health Sciences, SEISAD 75005 Paris, France.

11
12
13 ***Corresponding authors:**

14 Fabiana Gutierrez: fabgutierrez@gmail.com

15 Fethi Bedioui: fethi.bedioui@chimieparistech.psl.eu

16 Gustavo Rivas: grivas@fcq.unc.edu.ar

18 ABSTRACT

19 This work is focused on the development of a genosensor for microRNA-21
20 quantification using Surface Plasmon Resonance (SPR) to transduce the
21 hybridization event. The biosensing platform was built by self-assembling two
22 bilayers of poly(diallyldimethylammonium chloride) (PDDA) and graphene oxide
23 (GO) at a gold surface modified with 3-mercaptopropene sulfonate (MPS), followed
24 by the covalent attachment of the DNA probe. GO was used in two directions, to
25 allow the anchoring of the probe DNA and to increase the sensitivity of the
26 biosensing event due to its field enhancer effect. The new bioanalytical platform
27 represents an interesting alternative for the label-free biosensing of microRNA-21,
28 with a linear range between 1.0fM and 10nM, a sensitivity of $(5.1 \pm 0.1) \text{ m}^\circ\text{M}^{-1}$ and a
29 detection limit of 0.3fM. The proposed sensing strategy was successfully used for
30 the quantification of microRNA-21 in enriched urine samples.

32 KEYWORDS

33 microRNA-21; Genosensor; SPR; Graphene oxide; Cancer biomarker; Layer-by-
34 layer self-assembly.

36 1. INTRODUCTION

37 The microRNAs (miRNAs) are short endogenous and non-coding RNAs that
38 have an important effect on the expression of a large number of proteins and,
39 consequently on different molecular pathways [1]. They are present in several body
40 fluids, such as serum, blood, urine, saliva and tears, thereby enabling minimally

invasive detection [2]. Since the dysregulation in microRNAs-expression is an initial event in tumorigenesis, these microRNAs are very important biomarkers for early diagnosis of cancer and represent a good promise to reduce the worldwide health problem of this pathology [3, 4]. The advantages of miRNAs as biomarkers are connected with their high stability, their presence in several biological materials and the significant differences on their expression in healthy and pathological individuals [5].

microRNA-21 is an oncogenic microRNA that has been connected with different diseases, especially cancer. It has been found in patients with cervical cancer and has demonstrated to be useful for the prognosis and therapeutic application [6]. A critical study about the connection between dysregulated miRNAs and prostate cancer showed that miRNA-21 is one of the four microRNAs that give relevant information for the detection, prognosis and evaluation of the therapy [7]. microRNA-21 is also involved in the growth, metastasis and apoptosis of non-small cell lung cancer and is a promising biomarker for its diagnosis and prognosis [8]. Kura et al. [9] also showed that some biologically active nutrients like polyunsaturated fatty acids, vitamins E and D, dietary fiber, or selenium affect the expression of many miRNAs, connected with cardiovascular diseases, like miRNA-21, -30 family, -34, -155, or -199.

The determination of miRNAs is very complex due to their short sequence, low levels and high similarity between them. The current gold standard technique for miRNA detection is quantitative real-time polymerase chainreaction (qPCR) where the quantification of miRNAs extracted from body fluids is achieved through reverse

transcription of miRNA and labeling with fluorescent probes [10]. Other common methods used in miRNA detection are surface enhanced Raman scattering, microarrays and Northern blot [11-13]; however, they present important limitations like requirement of specialized personnel and need of centralized laboratories with expensive instrumentation [14]. Electrochemical biosensors have also demonstrated to be a very useful tool for the sensitive and selective quantification of microRNAs [15-17].

SPR biosensing strategies have made possible the real-time and label-free detection of different bioanalytes, providing additional useful information about binding kinetics and bioaffinity interactions. Q. Wang et al. [18] reported the selective femtomolar SPR detection of microRNA-21 by taking advantage of the electronic coupling between Au nanoparticles (AuNPs)-localized plasmon and Au surface plasmon, and the refractive index enhancement of the medium next to Au film caused by the hybrid generated between the DNA-linked AuNPs on the Au film surface and the terminus of capture DNA. A similar strategy was proposed by Liu et al. [19] for the sub-fM detection of microRNA-21 and cancer cells based on a multiple signal amplification strategy using a thiol-modified hairpin probe immobilized on a gold film with silver NPs deposited after the formation of the DNA supersandwich structure. Xue et al. [20] reported the use of antimonene for the specific label-free and aM detection of miRNA-21 and miRNA-155 due to the substantially stronger interaction of antimonene with ssDNA compared to DNA-RNA heteroduplex and the use of Au nanorods to amplify the SPR signal. Sipova et al. [21] presented a sensitive miRNA quantification strategy using a high-performance portable SPR sensor for

87 spectroscopy of surface plasmons based on a special diffraction grating
88 functionalized with the probe DNA and disperser (SPRCD) and the use of an
89 antibody able to recognize the DNA-RNA duplex. The sensor was used to quantify
90 miRNA from mouse liver tissues. Gayatri et al. [22] described a regenerative, solid-
91 state localized SPR (LSPR) sensor based on gold nanoprisms that circumvent the
92 need for labels or amplification, and enabled the detection of sub-fM microRNA-21
93 and microRNA-10b in human plasma of pancreatic cancer patients. SPR was also
94 used for the quantification of microRNA-10b at attomolar level [23] and microRNA-
95 93 at picomolar level [24]. Feng et al. [25] reported a surface plasmon-enhanced
96 electrochemiluminescence with Ag nanoclusters deposited at DNA as
97 electrochemiluminescence emitters and AuNPs deposited at glassy carbon electrode
98 (GCE) as localized surface plasmon resonance source in combination with a
99 catalytic hairpin assembly amplification that allowed to reach a detection limit of 0.96
100 aM and a successful determination of miRNA-21 in enriched diluted serum samples.

101 Here, we are proposing a bioanalytical platform for the detection of microRNA-
102 21 with SPR transduction based on the use of a gold disk modified with 3-
103 mercaptopropene-1-sulfonate (MPS) followed by the self-assembling of
104 poly(diallyldimethylammonium) chloride (PDDA) and graphene oxide (GO) and the
105 covalent attachment of capture DNA. In the following sections we discuss the
106 influence of the number of PDDA/GO bilayers on the plasmonic response of the
107 resulting platform; the optimization of the amount of DNA probe immobilized at the
108 surface and the hybridization time on the detection of the hybridization event; and
109 the analytical performance of the biosensor.

110

111 2. MATERIALS AND METHODS

112

113 2.1. Chemicals and reagents

114 Sodium 3-mercapto-1-propanesulfonate (MPS), N-hydroxysuccinimide
115 (NHS), Poly(diallyldimethylammonium chloride) (PDDA), N-(3-
116 dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), ethanolamine (Et),
117 benzoquinone, hydroquinone, and poly(acrylic acid) (PAA) were purchased from
118 Sigma-Aldrich. Ethanol, sulfuric acid (98%) and sodium hydroxide were provided by
119 J. T. Baker. Graphene oxide (GO) was obtained from Graphenea. Other chemicals
120 were reagent grade and used without further purification. The DNA and RNA
121 sequences (Invitrogen Life Technologies), are the following:

122 sequence DNA probe: 5'-NH₂-TCA-ACA-TCA-GTC-TGA-TAA-GCT-A-3'

123 miRNA-21: 5'-UAG-CUU-AUC-AGA-CUG-AUG-UUG-A-3'

124 Single base mismatch: 5'-UAG-CUU-AUC-ACA-CUG-AUG-UUG-A-3'

125 Non-complementary sequence: 5'-GGG-GGG-GGG-GGG-GGG-GGG-GGG-3'

126 Ultrapure water (UW, $\rho = 18.2 \text{ M}\Omega \text{ cm}$) from a Millipore-MilliQ system was
127 used for preparing all the solutions.

128

129 2.2. Apparatus

130 A platinum wire and Ag/AgCl, 3 MNaCl were used as counter and reference
131 electrodes, respectively. All potentials are referred to the latter. Electrochemical
132 Impedance Spectroscopy (EIS) measurements were performed with an Autolab

PGSTAT30 potentiostat (Methrom). The experiments were carried out in a 0.050 M phosphate buffer solution pH 7.40, using 2.0×10^{-3} M benzoquinone/hydroquinone as redox indicators. The EIS parameters were the following, amplitude: 0.010 V, frequency range: 1.0×10^{-2} to 1.0×10^6 Hz and working potential: 0.200 V. The impedance spectra were analyzed and fitted by using the Z-view program.

Surface Plasmon Resonance (SPR) measurements were performed using a single channel Autolab E-SPR SPRINGLE instrument (Eco Chemie). A standard gold disk (BK-7 Eco Chemie), mounted on a hemicylindrical lens through index-matching oil was used to build the platform. Sample solutions were injected manually and the measurements were carried out under batch conditions at 25 °C. The analytical signal was obtained from the change in the SPR minimum angle ($\Delta\theta_{\text{SPR}}$).

UV-Vis experiments were carried out with a Shimadzu UV1601 spectrophotometer and a quartz cuvette of 0.1 cm path length.

2.3. Construction of the biosensing platform

Figure 1 displays a scheme with the different steps during the construction of the biosensing platform. The gold disk was modified *in-situ* using a 2.00×10^{-2} M MPS solution (prepared in 1.60×10^{-3} M H_2SO_4) for 60 min. After washing the surface, it was allowed to interact with PDDA (1.00 mg mL^{-1} , prepared in 0.050 M phosphate buffer solution pH 7.40) for 15 min and after that, with GO (0.50 mg mL^{-1} GO solution prepared in 0.10 M NaOH) for 30 min. This procedure was repeated twice to obtain the required platform Au/MPS/(PDDA/GO)₂. The carboxylate residues of GO were activated with an EDC/NHS mixture (0.100 M prepared in 0.050 M phosphate buffer

1
2
3 156 solution pH 7.40) for 20 min and after that it was allowed to interact with a 50 ppm
4
5 157 DNA probe solution (prepared in 0.050 M phosphate buffer pH 7.40 + 0.500 M NaCl)
6
7 158 for 30 min. The unreacted GO carboxylate groups were quenched by adding 1.0 M
8
9
10 159 Et solution (pH 8.30) for 15 min. The hybridization event was performed at
11
12 160 Au/MPS/(PDDA/GO)₂/DNA by interacting with miRNA-21 (prepared in 0.050 M
13
14 161 phosphate buffer solution pH 7.40 + 0.500 M NaCl) for 120 min. The surface of the
15
16 162 modified gold-disk was carefully washed between the different steps. In fact, it was
17
18 163 washed with the medium used to prepare the solution that interacted with the surface
19
20 164 in the previous step, and then, for three times, with the medium used to prepare the
21
22 165 solution that will interact with the platform in the next step. To obtain additional
23
24 166 information, the platform Au/MPS/(PDDA/GO)₂ was also interrogated by EIS using
25
26 167 gold electrodes (CH Instruments, 3mm diameter) prepared under the same
27
28 168 experimental conditions as the SPR sensor.
29
30
31
32
33 169
34

35 170 3. RESULTS AND DISCUSSION

36
37 171 One interesting alternative to improve the sensitivity of SPR determinations is
38
39 172 the incorporation of plasmonic nanostructures to SPR substrates due to the coupled
40
41 173 field increments [26]. The high transmittance of the graphene-sheet and the high
42
43 174 confinement and propagation of the surface plasmonic polaritons make
44
45 175 graphenaceous materials very useful elements for the construction of SPR
46
47 176 biosensors [27-29]. Therefore, taking into account the importance of GO to improve
48
49 177 the sensitivity of SPR determinations, we evaluated the effect of the number of GO
50
51
52
53
54
55
56
57
58
59
60

layers immobilized at the gold disk by EIS, through the variations of the total charge transfer resistance (R_{ct}), and by SPR, through the changes in the plasmon angle.

Figure 2A shows the changes in the total R_{ct} obtained after the assembling of several layers of PDDA and GO at Au/MPS using hydroquinone/benzoquinone as redox markers. The inset shows the Nyquist plots corresponding to the first two PDDA/GO bilayers, where the circles are the experimental points and the solid lines correspond to the fitting of these results with the equivalent circuit. The adsorption of GO at Au/MPS/PDDA produces an increment in the R_{ct} due to the poor conductivity of GO. The assembling of the next layer of PDDA produced a new increment in R_{ct} due to the blockage effect, to increase even more after the immobilization of the second layer of GO. Further immobilization of GO or PDDA does not produce significant changes, indicating that after the second PDDA/GO bilayer there is no substantial increment in the thickness of the multilayers system due to a poor accumulation either of GO or PDDA. The system was fitted with a double time constant equivalent circuit (also shown in the inset) based on the capillary membrane model, which has been used to evaluate the charge transport through multilayers of polyelectrolytes, to demonstrate the existence of possible defects in these multilayers systems [30, 31]. This model assumes that the electrode is not fully covered during the construction of the supramolecular architecture defining areas more accessible for the charge transfer of the redox mediator. The Nyquist plots present wide semicircles, product of the combination of two time constants, indicating the existence of areas with two different heterogeneous rate constants for hydroquinone/benzoquinone charge transfer [32]. Therefore, the

201 results presented in Figure 2A suggest that after the assembling of GO there is not
202 full coverage of the PDDA layer, with the consequent existence of regions with
203 different GO coverage and incomplete charge reversion of PDDA.

204 The platform was also evaluated by SPR. Figure 2B depicts the changes in
205 $\Delta\theta_{\text{SPR}}$ during the assembling of the different PDDA and GO layers. $\Delta\theta_{\text{SPR}}$ increases
206 after each immobilization step up to the second (PDDA/GO) bilayer, in agreement
207 with the EIS results, indicating that there is an effective immobilization of PDDA and
208 GO up to the second bilayer. Further adsorption of GO or PDDA produces almost
209 no change in θ_{SPR} . Therefore, two (GO/PDDA) bilayers were selected as the
210 optimum platform for building the biosensor.

211 One of the main advantages of SPR-based biosensors is the capability to
212 follow in real time the different steps during the construction of the bioanalytical
213 platform and the detection of the biosensing event. Figure 3A shows the SPR profiles
214 obtained during the building of the whole bioanalytical platform
215 (Au/MPS/(PDDA/GO)₂/DNA and the target recognition. The analytical signal in the
216 presence of microRNA-21 was obtained from the change in θ_{SPR} before and after
217 the target addition and rinsing with Tween + buffer solution (inset of Figure 3A). To
218 evaluate more clearly these changes, Figure 3B displays the increment of $\Delta\theta_{\text{SPR}}$ after
219 the incorporation of each layer of the biosensing platform and after the hybridization
220 with microRNA-21.

221 The effect of the hybridization time was studied for 1.0×10^{-8} M microRNA-21.
222 The SPR angle increases with the hybridization time from 60 to 120 min (13.9, 20.9
223 and $42.5 \text{ m}^\circ\text{M}^{-1}$ for 60, 90 and 120 min, respectively) and we selected 120 min as

the best compromise between assay time and sensitivity. The effect of the amount of DNA probe covalently attached at Au/MPS/(PDDA/GO)₂ through the EDC/NHS chemistry, was also investigated by SPR. No significant changes in θ_{SPR} were observed for 50, 100 and 300 ppm DNA probe; therefore, we selected 50 ppm for further work. To demonstrate the advantages of GO on the SPR response, we built another supramolecular architecture by using PAA as negatively charged polymer instead of GO. $\Delta\theta_{\text{SPR}}$ increases during the assembling of the two (PDDA/PAA) bilayers although after the covalent attachment of the DNA probe and further hybridization with microRNA-21, the changes in the SPR angle were very small, indicating that the presence of GO is really necessary to obtain a sensitive SPR response (not shown).

3.3. Analytical performance of the genosensor

Figure 4A displays the plot of $\Delta\theta_{\text{SPR}}$ as a function of microRNA-21 concentration. There is a linear dependence between 1.0 fM and 10 nM, with a sensitivity of $(5.1 \pm 0.1) \text{ m}^\circ\text{M}^{-1}$ and a detection limit of 0.3 fM (calculated as $3 \times \text{SD}/s$ where SD is the standard deviation of the blank signal and s , the sensitivity). The reproducibility, obtained from the determination of 10^{-10} M with three different biosensors was 5.8%. Table 1 compares the analytical characteristics of our biosensor with the most relevant SPR-based microRNA-21 biosensors reported in the last years. The bioanalytical platform reported here presents detection limits lower or comparable to most of the biosensors reported in the table and higher than the one obtained using an amplification signal based on the use of antimonene and

AuNRs [20]. Therefore, the proposed strategy demonstrated to be competitive compared to the SPR-based microRNA21 biosensors already reported since it presents very good analytical performance using a simple scheme without the need of amplification steps. The selectivity of the biosensor was examined through the interaction of the biosensor with two non-complementary sequences, a fully non-complementary one and a one-base mismatch sequence. The response of the biosensor in both cases is considerably smaller than the one for microRNA-21. In fact, the analytical signal for the fully non-complementary was 0% while for a one-base mismatched sequence, it was only 40% of the one obtained in the presence of microRNA-21.

To evaluate the usefulness of Au/MPS/(PDDA/GO)₂/DNA biosensing platform for further analytical applications, we quantify microRNA-21 in a 1:10 diluted urine sample enriched with 1.0×10^{-10} M microRNA-21, being the recovery 91.3 and 87.0 %. These results indicate that the proposed biosensor could be used for practical applications.

4. CONCLUSIONS

In summary, we reported a SPR biosensor for the femtomolar quantification of microRNA-21 using a simple scheme based on a supramolecular architecture built at a thiolated gold platform containing GO with successful application for the quantification of microRNA-21 in enriched urine samples. The incorporation of GO in the multistructure facilitates the covalent attachment of the NH₂-DNA probe and makes possible the enhancement of the SPR analytical signal, eliminating, in this

way, the need for additional amplification scheme. The proposed strategy opens the door to the development of other biosensors by anchoring the corresponding biorecognition molecule at Au/MPS/(PDDA/GO)₂.

ACKNOWLEDGEMENTS

Financial support from CONICET, ANPCyT, SECyT-UNC (Argentina) and ECOS-SUD program (A16E02). M. L.-M. thankfully acknowledges CONICET for the PhD fellowship. Y. Zhang thanks Chinese Scholarship Council for providing a three-year scholarship for her stay in France and LabEx MICHEM for supporting her living expenses in UNC Argentina.

DECLARATION OF CONFLICT OF INTEREST

No authors have any conflicts of interest to disclose.

REFERENCES

1. Mazunder S, Datta S, Ray JG, Chaudhuri K, Chateerjee R. Liquid biopsy: miRNA as a potential biomarker in oral cancer. *Cancer Epidemiol.* 2019;58:137-145.
2. C.-S. Zhu CS, Zhu L, Tan DA, Qiu XY, Liu CY, Xie S, Zhu LT. Avenues Toward microRNA Detection In Vitro: A Review of Technical Advances and Challenges *Comput. Struct. Biotechnol. J.* 2019;17:904-916.
3. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. *Trends in Molecular Medicine.* 2014;20:460-469.

- 293 4. Shajari E, Mollasalehi H. Ribonucleic-acid-biomarker candidates for early-
294 phase group detection of common cancers. *Genomics*. 2020;112:163-168.
- 295 5. Peng Y, Croce CM. The role of MicroRNAs in human cancer. *Signal*
296 *Transduct. Target. Ther.* 2016;1:15004.
- 297 6. Hasanzadeh M, Movahedi M, Rejali M, Maleki F, Ahmadi MM, Seifi S,
298 Hosseini Z, Khazaei M, Amerizadeh F, Ferns GA, Rezayi M, Avan A. The
299 potential prognostic and therapeutic application of tissue and circulating
300 microRNAs in cervical cancer. *J Cell Physiol*. 2019;234:1289-1294.
- 301 7. Sharma N, Baruah MM. The microRNA signatures: aberrantly expressed
302 miRNAs in prostate cancer. *Clin Transl Oncol*. 2019;21:126-144.
- 303 8. Zheng W, Zhao J, Tao Y, Guo M, Ya Z, Chen C, Qin N, Zheng J, Luo J, L, Xu
304 L. MicroRNA-21: A promising biomarker for the prognosis and diagnosis of
305 non-small cell lung cancer. *Oncology Letters*. 2018;16:2777-2782.
- 306 9. Kura B, Parikh M, Slezak J, Pierce GN. The Influence of Diet on MicroRNAs
307 that Impact Cardiovascular Disease. *Molecules* 2019;24:1509.
- 308 10. Varkonyi-Gasic E, Wu R, Wood M, Walton EF, Hellens RP. Protocol: a highly
309 sensitive RT-PCR method for detection and quantification of microRNAs.
310 *Plant Methods* 2007;3:12.
- 311 11. Várallyay E, Burgyán J, Havelda Z. MicroRNA detection by northern blotting
312 using locked nucleic acid probes. *Nat. Protoc.* 2008;3:190.
- 313 12. Lee I, Ajay SS, Chen H, Maruyama A, Wang N, McInnis MG, Athey BD.
314 Discriminating single-base difference miRNA expressions using microarray
315 Probe Design Guru (ProDeG). *Nucleic Acids Res.* 2008;36:e27.

- 1
2
3 316 13. Li P, Long F, Chen W, Chen J, Chu PK, Wang H. Raman scattering,
4
5 317 Fundamentals and applications of surface-enhanced Raman spectroscopy–
6
7 318 based biosensors. *Current Opinion in Biomedical Engineering*. 2020;13:51–
8
9 319 59.
10
11
12 320 14. Liu L, Song C, Zhang Z, Yang J, Zhou L, Zhang X, Xie GG. Ultrasensitive
13
14 321 electrochemical detection of microRNA-21 combining layered nanostructure
15
16 322 of oxidized single-walled carbon nanotubes and nanodiamonds by
17
18 323 hybridization chain reaction. *Biosens. Bioelectron*. 2015;70:351-357.
19
20
21 324 15. Kutluk H, Bruch R, Urban GA, Dincer C. Impact of assay format on miRNA
22
23 325 sensing electrochemical microfluidic biosensors for miRNA-197 detection.
24
25 326 *Biosensors and Bioelectronics*. 2020;148:111824.
26
27
28 327 16. Zouari M, Campuzano S, Pingarron JM, Raouafi N. Femtomolar direct
29
30 328 voltammetric determination of circulating miRNAs in sera of cancer patients
31
32 329 using an enzymeless biosensor. *Analytica Chimica Acta*. 2020 (in press).
33
34
35 330 17. Jirakova L, Hrstka R, Campuzano S, Pingarron J, Bartosik M. Multiplexed
36
37 331 immunosensing platform coupled to hybridization chain reaction for
38
39 332 electrochemical determination of microRNAs in clinical samples.
40
41 333 *Electroanalysis*. 2019;31:293-302.
42
43
44 334 18. Wang Q, Liu R, Yang X, Wang K, Zhu J, He L, Li Q. Surface plasmon
45
46 335 resonance biosensor for enzyme-free amplified microRNA detection based
47
48 336 on gold nanoparticles and DNA supersandwich. *Sensors and Actuators B*.
49
50
51 337 2016;223:613-620.
52
53
54
55
56
57
58
59
60

19. Liu R, Wang Q, Li Q, Yang X, Wang K, Nie W. Surface plasmon resonance biosensor for sensitive detection of microRNA and cancer cell using multiple signal amplification strategy. *Biosensors and Bioelectronics*. 2017;87:433–438.
20. Xue T, Liang W, Li Y, Sun Y, Xiang Y, Zhang Y, Dai Z, Duo Y, Wu L, Qi K, Shivananju BN, Zhang L, Cui X, Zhang H, Bao Q. Ultrasensitive detection of miRNA with an antimonene-based surface plasmon resonance sensor. *Nature Communications*. (2019);10:1.
21. Sipova H, Zhang S, Dudley AM, Galas D, Wang K, Homola J. Surface Plasmon Resonance Biosensor for Rapid Label-Free Detection of Microribonucleic Acid at Subfemtomole Level. *Anal. Chem*. 2010;82:10110–10115.
22. Joshi GK, Deitz-McElyea S, Johnson M, Mali S, Korc M, Sardar R. Highly Specific Plasmonic Biosensors for Ultrasensitive MicroRNA Detection in Plasma from Pancreatic Cancer Patients. *Nano Lett*. 2014;14:6955-6963.
23. Joshi GK, Deitz-McElyea S, Liyanage T, Lawrence K, Mali S, Sardar R, Korc M. Label-Free Nanoplasmonic-Based Short Noncoding RNA Sensing at Attomolar Concentrations Allows for Quantitative and Highly Specific Assay of MicroRNA-10b in Biological Fluids and Circulating Exosomes. *ACS Nano*. 2015;9:11075-11089.
24. Schmieder S, Weißpflog J, Danz N, Klotzbach U, Sonntag F. Detection of miRNA using a surface plasmon resonance biosensor and antibody amplification. *Current Directions in Biomedical Engineering*. 2016;2:135–138.

- 1
2
3 361 25. Feng X, Han T, Xiong Y, Wang S, Dai T. J. Chen, X. Zhang, G. Wang.
4
5 362 Plasmon-Enhanced Electrochemiluminescence of Silver Nanoclusters for
6
7 363 microRNA Detection. ACS Sens. 2019;4:1633–1640.
8
9
10 364 26. Zeng S, Baillargeat D, Ho HP, Yong KT. Nanomaterials enhanced surface
11
12 365 plasmon resonance for biological and chemical sensing applications Chem.
13
14 366 Soc. Rev. 2014;43:3426–3452.
15
16
17 367 27. Grigorenko AN, Polini M, Novoselov KS. Graphene plasmonics. Nat.
18
19 368 Photonics 6 (2012) 749–758.
20
21 369 28. Jung I, Rhyee JS, Son JY, Ruoff RS, Rhee KY. Nanotechnology.
22
23 370 2012;23:25708–25716.
24
25
26 371 29. Primo E, Kogan M, Verdejo H, Bollo S, Rubianes MD, Rivas G. Label-Free
27
28 372 Graphene Oxide-Based Surface Plasmon Resonance Immunosensor for the
29
30 373 Quantification of Galectin-3, a Novel Cardiac Biomarker. ACS Applied
31
32 374 Materials and Interfaces. 2018;10:23501-23508.
33
34
35 375 30. Barreira SVP, Garcia-Morales V, Pereira CM, Manzanares JA, Silva F.
36
37 376 Electrochemical Impedance Spectroscopy of Polyelectrolyte Multilayer
38
39 377 Modified Electrodes. J. Phys. Chem. B. 2004;108:17973–17982.
40
41
42 378 31. Fernández-Sánchez C, McNeil CJ, Rawson K. Electrochemical impedance
43
44 379 spectroscopy studies of polymer degradation: application to biosensor
45
46 380 development. Trends Anal. Chem. 2005;24:37–48.
47
48
49 381 32. Chirea M, Garcia-Morales V, Manzanares JA, Pereira C, Gulaboski R, Silva
50
51 382 F. Electrochemical Characterization of Polyelectrolyte/Gold Nanoparticle
52
53
54
55
56
57
58
59
60

383 Multilayers Self-Assembled on Gold Electrodes. J. Phys. Chem. B.
384 2005;109:21808–21817.

LEGENDS OF THE FIGURES

388 **Figure 1.** Scheme for the different steps during the building of microRNA-21
389 genosensor at Au/MPS/(PDDA/GO)₂ platform.

391 **Figure 2. A)** Bars plot for the variation of total R_{ct} during the self-assembling of 1.00
392 mg mL⁻¹ PDDA (red) and 0.50 mg mL⁻¹ GO (blue) at Au/MPS platform. The inset
393 shows the Nyquist plots for the two layers of PDDA and two layers of GO as well as
394 the corresponding equivalent circuit. Redox marker: 2.0×10^{-3} M
395 hydroquinone/benzoquinone; EIS parameters: amplitude: 0.010 V, frequency range:
396 1.0×10^{-2} to 1.0×10^6 Hz; Working potential: 0.200 V. B) $\Delta\theta_{SPR}$ calculated from the
397 sensorgram obtained during the consecutive self-assembling of 4 bilayers of 1.00
398 mg mL⁻¹ PDDA (red) and 0.50 mg mL⁻¹ GO (blue). Rinsing solutions: 0.050 M
399 phosphate buffer solution pH 7.40 before and after self-assembling of PDDA; and
400 0.100 M NaOH before and after the immobilization of GO.

402 **Figure 3.A)** SPR sensorgram obtained during the building of microRNA-21
403 genosensor: immobilization of MPS at Au (black arrow), PDDA at Au/MPS (red
404 arrow), GO at Au/MPS/PDDA (blue arrow), activation of Au/MPS/(PDDA/GO)₂ with
405 EDC/NHS (green arrow), covalent attachment of DNA probe (pink arrow), quenching

with Et (orange arrow), and interaction with microRNA-21 (violet arrow). The inset shows the $\Delta\theta_{\text{SPR}}$ obtained after addition of microRNA-21, used as the analytical signal. **B)** $\Delta\theta_{\text{SPR}}$ calculated from the sensorgram obtained in A).

409

Figure 4. Calibration plot for microRNA-21 quantification. Each point is an average of three different experiments.

412

413

LEGENDS OF THE FIGURES

Figure 1. Scheme for the different steps during the building of microRNA-21 genosensor at Au/MPS/(PDDA/GO)₂ platform.

Figure 2. A) Bars plot for the variation of total R_{ct} during the self-assembling of 1.00 mg mL⁻¹ PDDA (red) and 0.50 mg mL⁻¹ GO (blue) at Au/MPS platform. The inset shows the Nyquist plots for the two layers of PDDA and two layers of GO as well as the corresponding equivalent circuit. Redox marker: 2.0×10^{-3} M hydroquinone/benzoquinone; EIS parameters: amplitude: 0.010 V, frequency range: 1.0×10^{-2} to 1.0×10^6 Hz; Working potential: 0.200 V. **B)** $\Delta\theta_{SPR}$ calculated from the sensorgram obtained during the consecutive self-assembling of 4 bilayers of 1.00 mg mL⁻¹ PDDA (red) and 0.50 mg mL⁻¹ GO (blue). Rinsing solutions: 0.050 M phosphate buffer solution pH 7.40 before and after self-assembling of PDDA; and 0.100 M NaOH before and after the immobilization of GO.

Figure 3.A) SPR sensorgram obtained during the building of microRNA-21 genosensor: immobilization of MPS at Au (black arrow), PDDA at Au/MPS (red arrow), GO at Au/MPS/PDDA (blue arrow), activation of Au/MPS/(PDDA/GO)₂ with EDC/NHS (green arrow), covalent attachment of DNA probe (pink arrow), quenching with Et (orange arrow), and interaction with microRNA-21 (violet arrow). The inset shows the $\Delta\theta_{SPR}$ obtained after addition of microRNA-21, used as the analytical signal. **B)** $\Delta\theta_{SPR}$ calculated from the sensorgram obtained in A).

Figure 4. Calibration plot for microRNA-21 quantification. Each point is an average of three different experiments.

For Peer Review

FIGURE 2-RIVAS ET AL.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47

FIGURE 3A-RIVAS ET AL.

FIGURE 3B-RIVAS ET AL.

FIGURE 4-RIVAS ET AL

Target	Technique used for detection	Signal amplification strategy	DL	Linear range [μM]	Real sample	Ref.
miRNA-21	SPR	gold nanoparticles (AuNPs) coupled with DNA supersandwich	8 fM	Up to 150 pM	human serum	[18]
miRNA-21	SPR	DNA-linked AuNPs, DNA supersandwich structure and positively charged silver nanoparticles (AgNPs)	0.6 fM	Up to 10pM	-	[19]
miRNA-21 miRNA-55	SPR	Au nanorods connected to amplify SPR signal and antimonene for discriminating ssDNA and DNA-RNA heteroduplex	10 aM	10 ⁻¹⁷ to 10 ⁻¹¹ M	-	[20]
miRNA-21 miR-10b	LSPR	-	23–35 fM	0.01-100 nM	plasma	[21]
miRNA-21	SPR	[PDDA-GO] ₂	0.3 fM	1.0 x 10 ⁻¹⁵ to 1.0 x 10 ⁻⁶ M	urine	This work

solid-state localized surface plasmon resonance (LSPR)

TABLE 1-RIVAS ET AL.

Michael LOPEZ MUJICA

obtained the Bachelor in Chemistry from Orient University. Currently, he is PhD student in the group of Biosensors at Physical Chemistry Department, Chemical Sciences Faculty, Cordoba National University (Argentina). His work is focused on the development of new biosensing strategies for the quantification of clinical biomarkers based on the use of nanomaterials.

Fabiana GUTIERREZ

obtained her PhD from the Cordoba National University (Argentina). Currently, she is Researcher at Argentine Research Council (CONICET), Laboratory of Analytical Development and Chemometrics (LADAQ) Faculty of Biochemistry and Biological Sciences, Litoral National University (Santa Fe, Argentina). Her research interests focus on the development of impedimetric and plasmonics genosensors and immunosensors.

Yuanyuan ZHANG

is a PhD of the Equipe "Synthèse, Electrochimie, Imagerie et Systèmes Analytiques pour le Diagnostic" of Chimie ParisTech (Université Paris sciences et lettres - PSL) in Paris, France. She works on the detection method of the clinical biomarker microRNA.

Fethi BEDIUI

is Senior Researcher at CNRS working at ChimieParisTech-PSL (Institute of Chemistry for Life and Health Sciences). His current research interests revolve around the development and the implementation of advanced molecular materials for electrocatalysis and electrochemical sensing devices for biologically relevant molecules (thiols, nitric oxide, peroxynitrite etc.). His scientific work is reflected in over 260 publications including books and book chapters (H index = 51).

Gustavo RIVAS

is Plenary Full Professor at Chemical Sciences Faculty (FCQ), Cordoba National University (Argentina) and Superior Researcher at Argentine Research Council (INFIQC-CONICET). He is member of the National Academy of Sciences of Argentina and Co-Editor-in-Chief of Sensors and Actuators B: Chemical. He is author of more than 170 publications including book chapters, with more than 7500 citations and H index = 48. He was President of the Argentinean Association of Analytical Chemists and Head of the Physical Chemistry Department (FCQ). His current research activities are focused on the development of enzymatic and affinity nano(bio)sensors devoted to the quantification of clinical and environmental relevant (bio)markers.