Supplementary Section to

Novel and simple electrochemical biosensor monitoring attomolar concentrations of miRNA-155 in Breast Cancer

Ana R. Cardoso[a,b], Felismina T.C. Moreira[a], Rúben Fernandes[b,c], M. Goreti F. Sales[a]*

[a]BioMark-CINTESIS/ISEP, School of Engineering, Polytechnic Institute of Porto, Portugal

[b]Molecular Mechanisms of Disease Unit, Centre of Research in Health and Environment (CISA), ESTSP-IPP;

[c]Metabolism, Nutrition and Endocrinology Unit, Integrative Cancer Programe, Instituto de Investigação e Inovação em Saúde (i3S), University of Porto.

Section S.1 *- Morphological characterization of the biosensor*

The morphological features resulting from each modification stage were studied on planar gold surfaces, previously employed in regular SPR measurements and cleaned by oxidative solution. This was a fundamental strategy, as the Au layer on the SPEs was deposited by printing approaches and therefore its surface was too rough to allow detecting any morphological change promoted by a single monolayer modification of small oligonucleotides.

The morphological study was conducted on three different assemblies: clean Au-SPR, Au-SPR/Anti-miRNA155 and Au-SPR/Anti-miRNA-155/miRNA-155, prepared as described for the SPEs. The corresponding images are displayed as an inset in Figure 1. The first image shows the typical observation of the clean gold surface (Figure 1A). This surface was not as flat as expected, because the Au-SPR had been reused in previous experiments. Still, its roughness was low enough to allow detecting changes related to the subsequent chemical modification.

Indeed, the immobilization of the Anti-miRNA155 on the clean Au-SPE rendered significant changes (Figure 1B), both in surface roughness (2.84 nm) and deepness (127.50 nm). The observed surface showed more roughness and deepness compared with the clean gold (1.39 nm and 17.57 nm, respectively), which was consistent with the addition of a miRNA monolayer onto the Au-SPE surface.

Finally, the miRNA-155 hybridization on the Au-SPR/Anti-miRNA155 surface produced greater morphological changes (Figure 1C). Both roughness and deepness decreased due to the hybridization event, thereby suggesting that the complementary oligonucleotide sequences hybridized. The formation of an apparently flatter surface could be justified by the presence of stable double strand oligonucleotide formations of negative charge, evenly distributed all around the surface and all of the same length; the strong negative outer surface of each double strand would contribute to repel each other and create an image of a homogenous surface.

Section S.2 - *UV Characterization*

After making the morphological characterization of the surface, UV/Vis absorption spectroscopy studies were made to confirm that the reaction between Anti-miRNA-155 and miRNA-155 had occurred. This was done because, according to the literature, nucleic acids, nucleosides, and nucleotides absorb strongly UV light, and the sum of their individual nitrogenous bases generates the overall absorbance in the UV spectra of DNA and RNA (*RNA Nanotechnology*, 2014). The results obtained are shown in Figure S1.

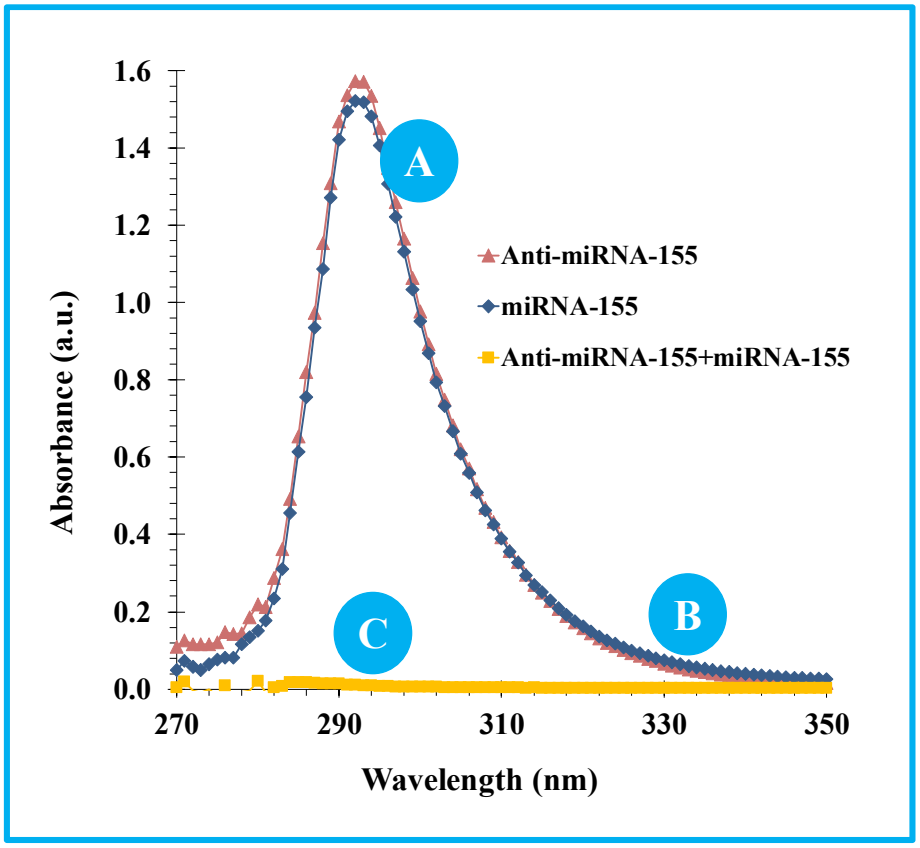

Figure S1 UV/Vis absorption spectra of single solutions of Anti-miRNA155 (A) and miRNA-155 (B), and a mixture of these compounds, in the same concentration as in the individual solutions (C).

The UV spectra of individual solutions of anti-miRNA-155 and mirRNA155 had a single peak, around ~290 nm (Figure S1-A and B). This confirmed the existence of oligonucleotide species in single-strand formation. According to literature, the molecules responsible for absorbing radiation at this wavelength are aromatic nitrogenous bases (adenine and uracil; cytosine and guanine) (*RNA Nanotechnology*, 2014).

After mixing both solutions (Figure S1-C), the spectra showed no peaks. During this stage, hybridization led to nitrogenous base pairing, resulting in the absence of absorbance by the solution. This lack of the absorption upon hybridization was led by a hypsochromic shift, as stacking of nitrogenous bases in native miRNA-155 interfered with UV absorption. Thus, these results have

proven that the hybridization process had successfully occurred between anti-miRNA-155 and miRNA-155 single strands in the test solution.

Moreover, when the hybridized strands were separated by heat, the peak of absorbance of light was recovered. This phenomenon corresponded to subsequent bathochromic shift (*RNA Nanotechnology*, 2014) and confirmed the possibility of reversing the hybridization stage.

Section S.3 – *Complementary data for the regeneration of the electrode*

The biosensor was regenerated by physical and chemical treatment, described in the main paper. Three consecutive calibrations on the same device were made for this purpose, reaching stable calibration features, as displayed in Figure S2. Additional calibration assays should be done however, if one aims to identify how many calibrations may be performed consecutively on the same device.

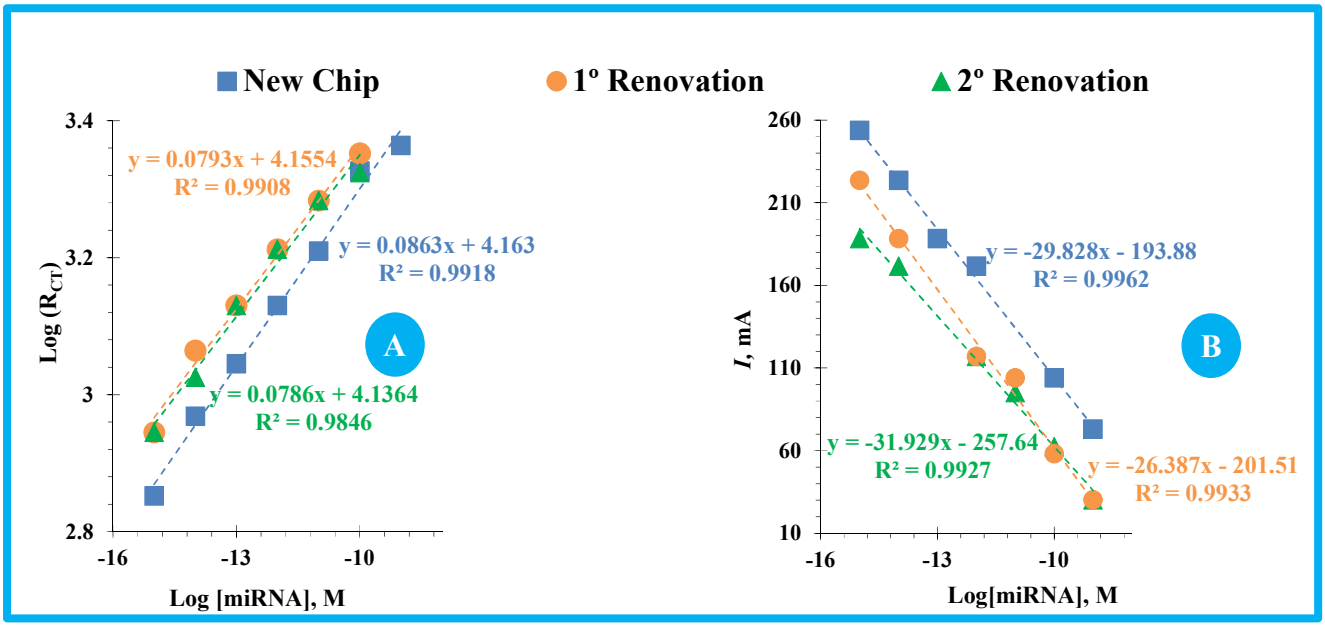

Figure S2 Consecutive calibrations in the same chip, first and after regeneration, (A) in EIS and (B) in SWV assays, performed in 5.0×10^{-3} M $[Fe(CN)_6]^{3-}$ and 5.0×10^{-3} M $[Fe(CN)6]^{4-}$, in buffer (TRIS and EDTA with NaCl), pH 7.4, in the same concentration range of miRNA-155 as before.

Section S.4 – Complementary data for Selectivity

The selectivity study was made by evaluating the combined effect of miRNA-155 with other biomolecule that may be present in serum. This was done by incubating a solution with miRNA-155 and another one with miRNA-155 and possible interfering species. The resulting electrical readings are plotted in Figure S3, where it a negligible effect is evidenced.

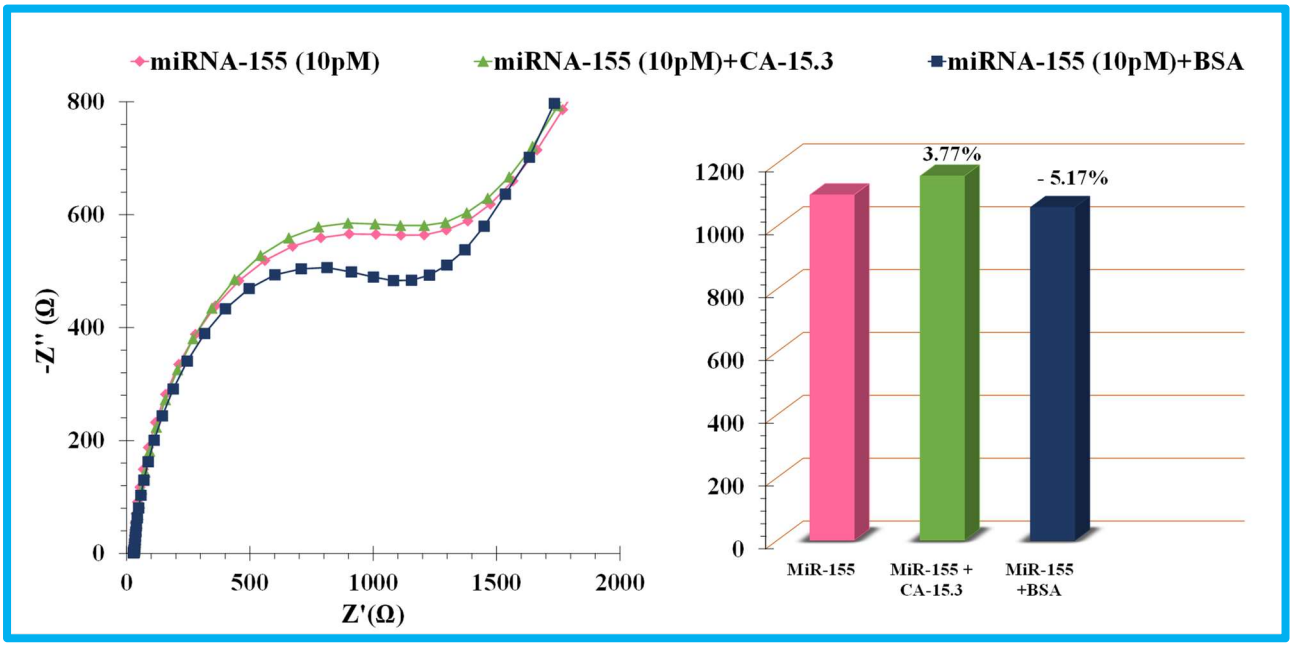

Figure S3 Selectivity behaviour of the biosensor for miRNA-155 (10 pM) against CA-15.3 (30 U/mL) and BSA (0.30 µg/mL), after 30 minutes incubation and for the same redox probe tested before.