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1 õLabel-free electrochemical DNA sensor using õclickö-functionalized PEDOT electrodes.
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7 **Title:** Label-free electrochemical DNA sensor using õclickö-functionalized PEDOT

8 electrodes.

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Abstract: Here we describe a label-free electrochemical DNA sensor based on poly(3,4-ethylenedioxythiophene)-modified (PEDOT-modified) electrodes. An acetylene-terminated DNA probe, complementary to a specific õHepatitis Cö virus sequence, was immobilized onto azido-derivatized conducting PEDOT electrodes using õclickö chemistry. DNA hybridization was then detected by differential pulse voltammetry, evaluating the changes in the electrochemical properties of the polymer produced by the recognition event. A limit of detection of 0.13 nM was achieved using this highly selective PEDOT-based genosensor, without the need for labelling techniques or microelectrode fabrication processes. These results are promising for the

development of label-free and reagentless DNA hybridization sensors based on conducting polymeric substrates. Biosensors can be easily prepared using any DNA sequence containing an alkyne moiety. The data presented here reveal the potential of this DNA sensor for applications to point-of-care tests in the screening of diseases, such as õHepatitis Cö, and genetic mutations.

Keywords: Hepatitis C virus, azido-EDOT, click chemistry, DNA biosensor, differential pulse voltammetry, electrochemistry.

1. Introduction

Generally, DNA sensors are based on DNA hybridization. In this approach, a single-stranded DNA (ssDNA) probe is immobilized on a surface and exposed to a sample containing the specific complementary target sequence, which is captured by forming a double-stranded DNA (dsDNA) molecule. This recognition event (hybridization) is then transduced into a readable signal. A variety of transduction techniques can be used to monitor this process, including optical (Ma et al., 2013; Yan et al., 2014), mass-sensitive (García-Martinez et al., 2011), and electrochemical methods (Lazerges and Bedioui, 2013).

Electrochemical DNA sensors are reliable, fast, simple, and cost-effective devices that convert the hybridization occurring on an electrode surface into an electrical signal by means of direct or indirect methods. DNA sensors based on indirect methods require the use of labels or electroactive indicators, such as ferrocenyl derivatives (Nakayama, 2002), redox-active enzymes (Patolsky et al., 2001), nanoparticles (Ting et al., 2009), and redox intercalators (Ferapontova and Gothelf, 2009; Millan and Mikkelsen, 1993). Strategies involving labels are time- and labor-consuming and they do not allow real-time detection of target-probe coupling. In contrast, direct detection methods are based on the intrinsic electroactivity of DNA, mostly derived from the oxidation of guanine or

adenine bases (Karadeniz et al., 2003; Kerman et al., 2003), and they allow reagentless and simpler detection. However, this direct detection still has some drawbacks, such as its dependence on the number of guanine residues and the need for high oxidation potentials, which may generate side oxidation reactions. Electrochemical impedance spectroscopy (EIS) has also been used as direct technique for DNA detection (Park and Park, 2009). Even though this technique is highly sensitive, this advantage sometimes limits its application as a result of being liable to respond to interferences too. Nevertheless, it has been successfully replaced by other less sophisticated electrochemical techniques, such as differential pulse voltammetry or chronopotentiometry (Arora et al., 2007). Alternatively, electrochemical DNA sensors based on conducting polymers (CPs) have been used to directly detect DNA hybridization events in a label-free format. The electronic structure of CPs is highly sensitive to environmental changes occurring at the polymer surface, like those generated by a hybridization event (Garnier, 1989; Peng et al., 2009; Prabhakar et al., 2008).

Functionalized CPs are synthesized using pre- and post-functionalization strategies. The former consists of linking the desired functional biomolecule to the corresponding monomer, followed by its polymerization. However, instability or possible damage to oligonucleotides under electropolymerization conditions makes the post-functionalization strategy more suitable for the immobilization of DNA sequences onto CP surfaces. In the latter approach, the substrate is first electropolymerized from a solution containing precursor monomers modified with reactive groups and then subjected to a coupling reaction with a modified DNA probe at the polymer surface.

DNA probes are commonly functionalized with amino or carboxyl groups and then covalently attached to the polymer surface through peptide bonds using carbodiimide

coupling chemistry (Peng et al., 2007, 2005). However, although widely used, these reactions are not fully chemoselective in aqueous solvents, and hydrolysis occurs along with the desired coupling reaction, thereby lowering the efficiency of the immobilization. Thus, a more chemoselective coupling reaction would be more advantageous. In this respect, the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides with terminal alkynes, frequently referred to as the õclickö reaction, shows high reliability, specificity, and biocompatibility and has been successfully used in post-functionalization reactions.

A few years ago *Bäuerle at al.* reported the first example of post-functionalization of a novel conducting poly-(azidomethyl-EDOT) polymer (azido-PEDOT) by õclickö chemistry with various types of terminal alkynes (Bu et al., 2008). Since then, several examples of derivatization of azido-PEDOT with alkyne-containing small molecules such as fluorophores (Daugaard et al., 2008), ferrocene, glycosides, and fullerenes (Bu et al., 2011) have been described. However, to the best of our knowledge, no study has yet applied õclickö chemistry to develop label-free DNA hybridization sensors based on conducting PEDOT polymers.

Here we report on the first voltammetric genosensor based on azido-derivatized PEDOT electrodes for the label-free detection of a sequence correlating with the õHepatitis Cö virus (HCV). PEDOT was selected as CP due to the simplicity of EDOT monomer functionalization and to its high electrochemical stability (Bu et al., 2008; Kros et al., 2005). An acetylene-terminated oligonucleotide probe, complementary to a HCV target sequence, was immobilized onto an azido-PEDOT polymer by covalent binding using õclickö chemistry. DNA hybridization was detected by differential pulse voltammetry (DPV), directly measuring changes in the electrochemical properties of the polymer

1 triggered by the recognition event. We characterized the selectivity of the sensor and the

2 limit of detection (LOD) was determined to fall in the nanomolar range.

3 **2. Materials and methods**

4 2.1. Materials

1 Azidomethyl-substituted 3,4-ethylenedioxythiophene (azido-EDOT) was
2 synthesized following the protocol developed by *Bu et al.* (Bu et al., 2008). 99.8%
3 anhydrous dichloromethane (DCM) and tetrabutylammonium hexafluorophosphate
4 ($TBAPF_6$) were supplied by Sigma-Aldrich Co (USA). Gold working electrodes
5 (disk diameter 1.6 mm) were purchased from BASi (Indiana, USA). Dimetyl
6 sulfoxide (DMSO), tris [(1-benzyl-1H-1,2,3-triazol-4-yl)methyl)]amine (TBTA),
7 N,N-diisopropylethylamine (DIPEA), and copper iodide (CuI) were supplied by
8 Sigma-Aldrich Co (USA). Phosphate-buffered saline (PBS) and
9 tris(hydroxymethyl)aminomethane were purchased from Sigma Aldrich Co (USA)
10 and Panreac Química S. L. U. (Spain), respectively. A 21-mer oligonucleotide
11 related to the õHepatitis Cö virus (HCV-probe) bearing an acetylene group was
12 synthesized by solid-phase methodology, using the phosphoramidite derivative of
13 hex-5-yn-1-ol, according to the protocol reported by the authors of this work *Alvira*

and Eritja (Alvira and Eritja, 2007). This DNA sequence is complementary to a specific õHepatitis Cö DNA sequence (base location: 8245-8265). The

Table 1: List of oligonucleotides. *X* represents the acetylene group and the five next *T* bases were introduced as spacers.

DNA sequences	
Probe (HCV-probe)	5¢-*XTT TTT* TGG GGA TCC CGT ATG ATA CCC-3ø
Complementary target (HCV-target)	5ø-GGG TAT CAT ACG GGA TCC CCA-3ø
Non-complementary sequence 1 (Nc1-target)	5ø-CTC GAT GAC TCA ATG ACT CG-3ø
Non-complementary sequence 2 (Nc2-target)	5¢-CCC GCA CTT CAC CAC TCC TCA CCA CTT CAC GCC C-3ø

complementary and non-complementary sequences, used to study the selectivity of the sensor through their hybridization with the probe, were provided by Sigma-Aldrich Co (USA). All DNA sequences listed in **Table 1** were supplied as lyophilized powder. All solutions were prepared using Milli-Q water.

2.2. Instrumentation

Electrochemical measurements were performed on an AUTOLAB PGSTAT 30 electrochemical analysis system (Eco Chemie, The Netherlands). Cyclic voltammetry (CV) and DPV experiments were conducted in a three-electrode electrochemical cell, which consisted of a gold working electrode, a platinum wire as counter electrode, and a Ag/AgCl-NaCl (3M) reference electrode. For electrochemical polymerization in DCM,

a Ag/AgCl pseudoreference electrode was used and referenced after each use against ferrocene-ferricenium (Fc/Fc^+).

Polymer oxidation signals (between 0.5 V and 1 V vs. Ag/AgCl) were measured by DPV. The oxidation current intensity after background current correction was used as analytical signal. Raw DPV data were treated with the GPES 4.7 software package, using the Savitzky and Golay filter, followed by the moving average baseline correction (peak width of 0.01) provided by the software.

Synthesis of HCV-probe bearing an acetylene group was performed on an Applied Biosystems model 3400 DNA synthesizer.

2.3. Preparation of azido-PEDOT electrodes

Gold electrodes were carefully polished with diamond paste and alumina powder of different grain sizes (from 1 µm to 0.05 µm) prior to use. The electrodes were then washed in ultrasonic baths of acetone and ethanol. Electropolymerization of azido-EDOT was performed on the electrodes by CV, using 1.5 mM azido-EDOT monomer and 100 mM $TBAPF_6$ in DCM under argon atmosphere. CV between -1.5 V and +1.2 V (vs. Fc/Fc^+) at 100 mV/s was applied on the electrodes, resulting in the azido-PEDOT coverage.

2.4. Immobilization of DNA probes on azido-PEDOT electrodes

Immobilization of acetylene-terminated DNA probes (HCV-probe) on azido-PEDOT electrodes was performed by means of the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition (õclickö reaction), as depicted in **Figure 1**.

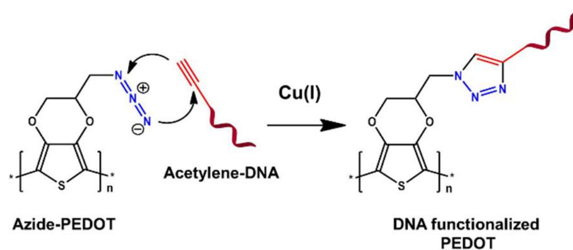

Figure 1: Construction of PEDOT-based DNA sensor: DNA immobilization process by õclickö reaction, using Cu(I) source.

The õclickö reaction was performed in a 1:1 mixture of water and DMSO, requiring a large excess of Cu(I) source to proceed (Alvira and Eritja, 2007). HCV-probe concentrations between 10 µM and 10 nM were evaluated. The azido-PEDOT electrodes were immersed in a solution (õclickö mixture) consisting of the desired concentration of HCV-probe in 50 mM Tris-HCl (pH 7.4) and 100 equivalents CuI, 100 equivalents TBTA, and 120 equivalents DIPEA in DMSO. The electrodes were incubated in the õclickö mixture for 20ó24 h at 45ºC. They were then washed with 0.05% Tween 20 in PBS (100 mM, pH 7.4) in order to remove any HCV-probe non-covalently bound to the surface. Electrodes were finally rinsed with Milli-Q water. õClickö reactions with a solution free of DNA and with a solution containing an acetylene-free DNA sequence (Nc1) were performed as controls. DPV measurements were conducted between +0.5 V and +1 V (vs. Ag/AgCl reference electrode) at 100 mV/s in 20 mM Tris-HCl buffer at room temperature. The efficiency of the immobilization was then evaluated by comparing DPV results obtained using the HCV-probe and controls.

2.5. DNA hybridization

After immobilization of the HCV-probe, electrodes were incubated for 30 min at room temperature using several concentrations of different DNA sequences, prepared in 20 mM Tris-HCl (pH 7.4) with 20 mM NaCl. The HCV-target sequence in a concentration

1 range from 20 nM to 1 nM was used to evaluate the analytical performance of the

2 sensor. 50-nM solutions of the non-complementary sequences listed in **Table 1** were

3 used to test the selectivity of the sensor. After hybridization, the sensors were washed

4 with 0.05% Tween 20 in PBS (100 mM, pH 7.4) in order to remove any non-hybridized

5 DNA target. DPV measurements were then conducted between +0.5 V and +1 V (vs.

6 Ag/AgCl reference electrode) at 100 mV/s in 20 mM Tris-HCl buffer at room

7 temperature. Three independent electrodes per concentration were used, evaluating the

8 analytical performance of the PEDOT-based sensor.

9 **3. Results and discussion**

10 3.1. Preparation and characterization of azido-PEDOT electrodes

11 **Figure 2** shows the polymerization of the monomer during cyclic voltammetry, as

12 described in previous sections. Compared to these measurements, the current intensity

13 for bare gold electrodes tested in electrolyte solution free of monomer (100 mM

14 $TBAPF_6$ in DCM) was very low. After addition of the azido-EDOT monomer to the

15 electrolyte, a large current increase was observed at around 1 V, corresponding to the

16 polymerization of the monomer, as reported by *Bu et al.* (Bu et al., 2008). In the second

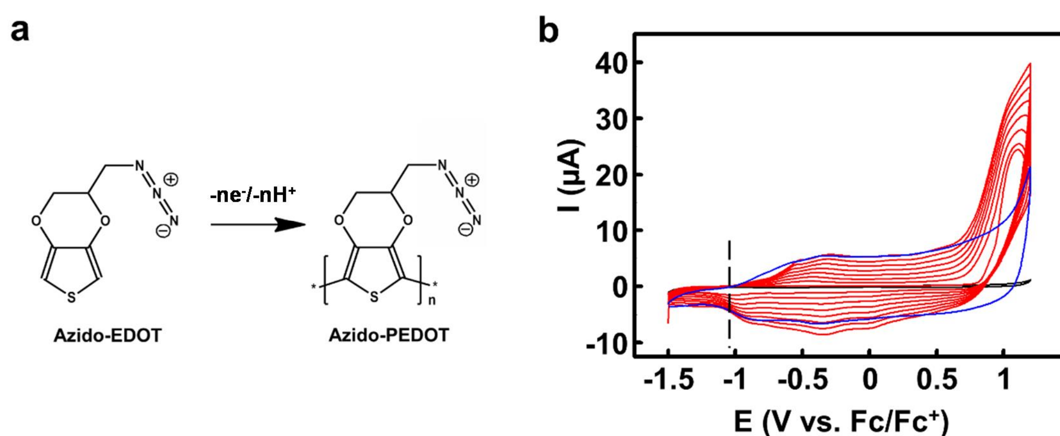

Figure 2: Preparation of azido-PEDOT electrodes. a) Electropolymerization reaction of azido-EDOT. b) Electrochemical characterizations in DCM and 100mM $TBAPF_6$ using cyclic voltammetry between -1.5 V and +1.2 V (vs. Fc/Fc^+), at 100 mV/s. Black line: bare gold electrode in electrolyte solution free of monomer. Red lines: polymer formation of azido-EDOT (using 1.5 mM of monomer). Blue line: electroactivity of an azido-PEDOT film in an electrolyte solution free of monomer; dotted line highlights the transition of the polymer from a semiconducting to a conducting state.

polymerization scan, a new irreversible redox wave was detected in the low-potential region, indicating the deposition of azido-PEDOT on the gold electrode. This redox wave gradually increased with the number of scans, thereby revealing the increasing amount of azido-PEDOT polymerized during each anodic sweep (red voltammograms). After electropolymerization, voltammograms of azido-PEDOT electrodes were recorded in an electrolyte solution free of modified monomer (blue voltammogram, for detailed CV characterization of azido-PEDOT electrodes see Supplementary information **A3**). These electrodes presented the typical CP shape of p-type semiconductors (Bu et al., 2011). At -1.03 V (vs. Fc/Fc^{+}) the electrografted polymer shifted from a semiconducting to a conducting regime.

3.2. DNA probe immobilization and characterization

Immobilization of HCV-probes on azido-PEDOT electrodes was performed as described before by means of the õclickö reaction. Immobilization was confirmed by DPV, X-ray photoelectron spectroscopy (XPS), and time-of-flight secondary ion mass spectrometry (ToF-SIMS), as described below.

DPV was used to test the efficiency of capture probe immobilization. **Figure 3** shows the DPV signals for the immobilization performed using 50 nM HCV-probe, as described in the experimental section. õClickö reactions with a solution free of DNA and with a solution containing 50 nM acetylene-free DNA sequence (Nc1) were used as controls. The electroactivity of the azido-PEDOT electrodes incubated with the HCV-probe showed a dramatic decrease compared to controls. This is likely due to the bond formation of the HCV-probe, which acts as insulating layer on the polymeric film, impeding ion exchange and hence reducing the electrochemical activity of the polymer (Pham et al., 2003; Thompson et al., 2003). In contrast, electrodes in which DNA was not anchored to the surface did not show such a large electrochemical change, thereby

1 indicating that covalent immobilization (the õclickö reaction) was accomplished only

2 when the acetylene-terminated HCV-probe was used.

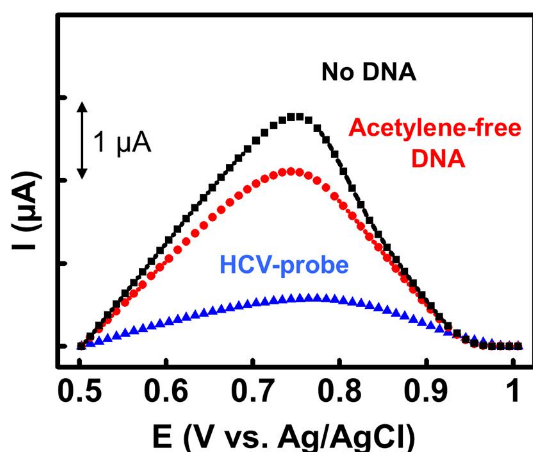

Figure 3: DPV of õclickö reaction. Azido-PEDOT electrodes incubated with the HCV-probe (blue triangles) showed a decreased current intensity in comparison with azido-PEDOT electrodes incubated with acetylene-free DNA, Nc1 (red dots), and a DNA-free solution (black squares). DPV measurements were conducted between +0.5 V and +1 V (vs. Ag/AgCl reference electrode) at 100 mV/s in 20 mM Tris-HCl buffer at room temperature.

3 The ToF-SIMS and XPS analyses of azido-PEDOT surfaces and HCV-modified

4 PEDOT surfaces were compared (see Supplementary information **A1** and **A2**). The

5 ToF-SIMS results revealed ion fragments representative of DNA in electrodes

6 incubated with the HCV-probe, thus confirming its presence on the azido-PEDOT

7 electrodes. Alternatively, XPS high-resolution spectra of the N 1s binding energies

8 revealed a decrease of the peak at 404.3 eV in favor of the lower energy peak (at 400.6

9 eV). This observation provides additional evidence of a reaction between surface azides

10 and acetylenes (Collman et al., 2006).

11 ### 3.3. DNA hybridization

12 Hybridization experiments were performed on HCV-modified PEDOT electrodes (using

13 50 nM HCV-probe for immobilization), incubated with distinct DNA sequences at a

concentration of 50 nM. DPV measurements were performed on electrodes incubated with the following: HCV-target; non-complementary Nc1-sequence; a mixture of non-complementary sequences (Nc1+Nc2) and HCV-target; and a DNA-free solution (**Figure 4a**). The highest current intensity was observed for electrodes incubated in a DNA-free solution, remaining almost unchanged upon exposure to the Nc1-sequence. In contrast, when electrodes were incubated with the HCV-target, the current intensity decreased sharply, revealing a change in the electrochemical behavior of the system. These results suggested that only DNA strains complementary to the immobilized capture probe underwent hybridization, thus affecting the electroactivity of the polymer. The decrease in current intensity observed for electrodes exposed to the HCV-target was attributed to changes in the polymer environment caused by DNA hybridization. It has been reported that the formation of hydrogen bonds after hybridization creates potential barriers that slow down the diffusion of ions into the polymer (Bäuerle and Emge, 1998; Cha et al., 2003; Korri-Youssoufi and Makrouf, 2002; Navarro et al., 2005). These barriers thus reduce the electroactivity and conductivity of the polymer backbone, which is in good agreement with the electrochemical behavior observed.

In addition, the selectivity of the sensor with respect to the HVC-target in a mixture with non-complementary DNA sequences, Nc1 and Nc2 (see **Table 1**), was tested. The three DNA sequences were mixed at the same concentration (50 nM), resulting in a total DNA concentration of 150 nM. DPV measurements revealed that exposing the polymer to the mixture of non-complementary and complementary sequences resulted in a similar electrochemical behavior, as observed when using the HCV-target alone. This finding indicates that the DNA sensor developed here is capable of detecting the complementary target within a more complex mixture and that it does not show

1 remarkable interferences caused by the presence of other non-complementary

2 sequences, an important feature required for the analysis of real samples.

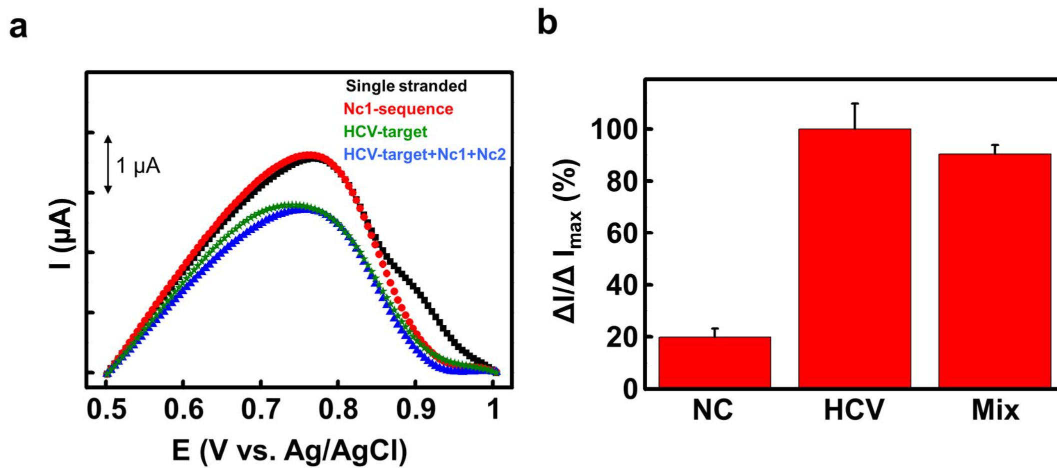

Figure 4: Selectivity of the DNA sensor. a) DPV measurements of HCV-modified PEDOT electrodes incubated in hybridization solutions with HCV-target, Nc1-sequence, a mixture of non-complementary sequences (Nc1+Nc2) and HCV-target, and a DNA-free solution. All DNA sequences were used at a concentration of 50 nM. DPV measurements were conducted between +0.5 V and +1 V (vs. Ag/AgCl reference electrode) at 100 mV/s in 20 mM Tris-HCl buffer at room temperature. b) Relative current changes of the sensor to different DNA sequences. HCV shows the response of the sensor to the complementary sequence (taken as reference, 100%). Nc1 shows the response of the sensor to the non-complementary sequence. Mix shows the response of the sensor to a mixture of non-complementary (Nc1, Nc2) and complementary sequences. Error bars are the standard deviation of three independent measurements.

3 These hybridization results are summarized in **Figure 4b**, which shows the relative

4 current changes against the blank experiment (hybridization experiment without DNA).

5 Three electrodes were used per experiment. Thus, taking as reference the response of

6 the sensor to a complementary target (100 ± 9.7%, HCV in **Figure 4**), the response to

7 the non-complementary sequence (Nc1 in **Figure 4**) did not exceed 20% (19.9 ± 3.3%),

8 which is most likely explained by non-specific adsorption of non-complementary DNA

9 onto the electrode. In contrast, the response of the sensor to the cocktail of non-

10 complementary and complementary sequences (õMixö in **Figure 4**) remained above

1 90% (90.33 ± 3.5%), which suggests that the sensor has the capacity to detect the

2 complementary target over several non-complementary targets with good resolution.

3 On the basis of our results, we conclude that the DNA sensor developed here allows the

4 effective discrimination of all the sequences tested, at a fixed concentration of 50 nM,

5 and thus shows high selectivity.

6 ### 3.4. Analytical performance of the sensor

7 20 nM HCV-probe was immobilized on azido-PEDOT electrodes following the

8 protocol described in the experimental section. Hybridization experiments with HCV-

9 target at concentrations ranging from 20 nM to 1 nM were then conducted and evaluated

10 by DPV. **Figure 5a** shows the DPV measurements for the different electrodes, revealing

11 a gradual decrease in the oxidation current of the polymer as the concentration of the

12 HCV-target increased. This observation points to a change in the polymer behavior

13 upon hybridization. Moreover, the potential corresponding to the oxidation peak of the

14 polymer shifted towards more positive potentials for increasing concentrations, which is

15 in agreement with the formation of hydrogen bonds (potential barriers) that slow down

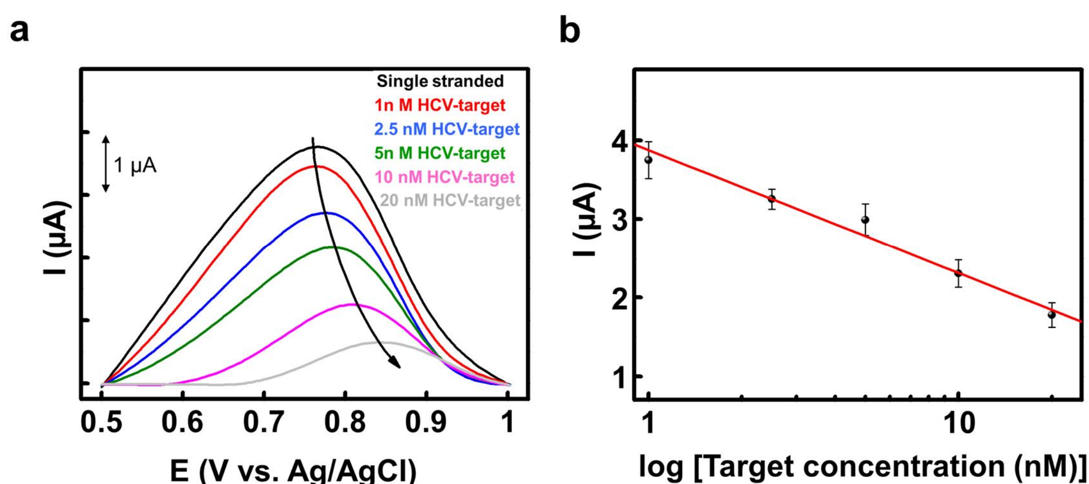

Figure 5: Sensor response to a range of concentrations of HCV-target. a) DPV measurements, conducted between +0.5 V and +1 V (vs. Ag/AgCl reference electrode) at 100 mV/s in 20 mM Tris-HCl buffer at room temperature and b) corresponding calibration curve, where current intensity is plotted against the logarithm of HCV-target concentration. Error bars are the standard deviation of three independent experiments.

the diffusion of ions through the polymer. These results demonstrate the dependence of the electrochemical behavior of the polymer on the changes in target concentration, thereby showing its suitability for the quantification experiments required for clinical analyses.

Figure 5b shows the calibration curve obtained from the quantitative electrochemical measurements, taken from at least three independent electrodes per concentration. When the current intensity is plotted against the logarithm of the target concentration, the graph is linear with a correlation coefficient of 0.990. The limit of detection (LOD) was obtained by applying the equation $Y_{LOD} = Y_B + 3\ _B$ and the regression equation $Y(X) = 3.878 - 1.563X$ of the plot. Where Y_B is the mean current for the blank experiment (hybridization experiment without DNA) and $_B$ is the standard deviation of the same blank experiment. In this way, a LOD of 0.13 nM was calculated. This result is comparable to values achieved by other electrochemical DNA sensors with enhanced sensitivity, provided by the use of redox indicators, microelectrodes, nanostructured surfaces, etc. (Kannan et al., 2011). In the particular case of the HCV detection, the existing label-based DNA electrochemical sensors report LODs between tens of nM (Liu et al., 2009) to pM (Zhang et al., 2013). Regarding the few reports related to label-free electrochemical DNA sensors for the detection of HCV (Uliana et al., 2014), they are based on either the oxidation signal of guanine or on the electrochemical behavior of CPs. On the one hand, *Pournaghi-Azar et al.* developed a label-free sensor for the HCV, based on the detection of guanine oxidation, with a LOD of 6.5 nM (Pournaghi-Azar et al., 2009). That sensor proved to be as sensitive as other DNA sensors based on the same principle of detection, but 50 times less sensitive than the PEDOT-based sensor presented here. On the other hand, as far as we know, only one study has addressed the

electrochemical label-free detection of HCV using CPs. In that case, the sensor was based on the electropolymerization of a probe-modified pyrrole monomer (dos Santos Riccardi et al., 2008). Although it showed a very low LOD (10^{-21} M), it required microelectrode fabrication technology. Instead, the system we report herein is based on a straightforward fabrication strategy that does not involve the use of complex equipment or processes. Moreover, this fabrication strategy provides a readily azido-functionalized platform for further probe immobilization via õclickö reaction under mild chemical conditions, thus protecting the integrity of HCV-probe from eventual damage during the electrochemical synthesis. In addition, this novel PEDOT sensor offers excellent LODs, taking into account that the concentration analyzed in clinical tests for the HCV in serum or plasma is usually between 1.5 and 2.0 µM (Liu et al., 2009).

4. Conclusions

Here we have presented a new approach for a highly selective and sensitive label-free electrochemical genosensor. In this regard, we used azido-PEDOT electrodes as platforms for the direct immobilization of acetylene-DNA probes by means of covalent binding using õclickö chemistry. This immobilization strategy protects DNA integrity from electropolymerization and can be easily applied to any DNA sequence containing an alkyne moiety. Hybridization events are detected directly by DPV, evaluating changes in the electrochemical properties of the polymer after the DNA recognition process. The sensor described here allowed effective discrimination between all the target sequences tested at a fixed concentration, thereby revealing its potential for applications in the screening of genetic mutations and diseases, such as the HCV. This novel genosensor achieved LODs below the nanomolar range for complementary target sequences related to the HCV, which were comparable to those of genosensors that use extra labelling steps and microfabrication.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at

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Captions:

Table 1: List of oligonucleotides. *X* represents the acetylene group and the five next *T* bases were introduced as spacers.

Figure 1: Construction of PEDOT-based DNA sensor: DNA immobilization process by õclickö reaction, using Cu(I) source.

Figure 2: Preparation of azido-PEDOT electrodes. a) Electropolymerization reaction of azido-EDOT. b) Electrochemical characterizations in DCM and 100mM $TBAPF_6$ using cyclic voltammetry between -1.5 V and +1.2 V (vs. Fc/Fc^+), at 100 mV/s. Black line: bare gold electrode in electrolyte solution free of monomer. Red lines: polymer formation of azido-EDOT (using 1.5 mM of monomer). Blue line: electroactivity of an azido-PEDOT film in an electrolyte solution free of monomer, dotted line highlights the transition of the polymer from a semiconducting to a conducting state.

Figure 3: DPV of õclickö reaction. Azido-PEDOT electrodes incubated with the HCV-probe (blue triangles) showed a decreased current intensity in comparison with azido-PEDOT electrodes incubated with acetylene-free DNA, Nc1 (red dots), and a DNA-free solution (black squares). DPV measurements were conducted between +0.5 V and +1 V (vs. Ag/AgCl reference electrode) at 100 mV/s in 20 mM Tris-HCl buffer at room temperature.

Figure 4: Selectivity of the DNA sensor. a) DPV measurements of HCV-modified PEDOT electrodes incubated in hybridization solutions with HCV-target, Nc1-sequence, a mixture of non-complementary sequences (Nc1+Nc2) and HCV-target, and a DNA-free solution. All DNA sequences were used at a concentration of 50 nM. DPV measurements were conducted between +0.5 V and +1 V (vs. Ag/AgCl reference electrode) at 100 mV/s in 20 mM Tris-HCl buffer at room temperature. b) Relative current changes of the sensor to different DNA sequences. HCV shows the response of the sensor to the complementary sequence (taken as reference, 100%). Nc1 shows the response of the sensor to the non-complementary sequence. Mix shows the response of the sensor to a mixture of non-complementary (Nc1, Nc2) and complementary sequences. Error bars are the standard deviation of three independent measurements.

Figure 5: Sensor response to a range of concentrations of HCV-target. a) DPV measurements, conducted between +0.5 V and +1 V (vs. Ag/AgCl reference electrode) at 100 mV/s in 20 mM Tris-HCl buffer at room temperature and b) corresponding calibration curve, where current intensity is plotted against the logarithm of HCV-target concentration. Error bars are the standard deviation of three independent experiments.