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**CHRONOAMPEROMETRIC MAGNETOGENOSENSING FOR
SIMULTANEOUS DETECTION OF TWO ROUNDUP READY™ SOYBEAN
LINES: GTS 40-3-2 AND MON89788**

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Highlights

- Simultaneous electrochemical detection of two Roundup Ready™ soybean events is feasible
- Core@shell Fe₃O₄@Au MNPs proved to be a suitable nanosupport
- Detection limits at the subnM level were achieved
- Methodology specific for these events with no cross-reactivity

Abstract

Development of expeditious analytical methods for the detection of genetically modified organisms (GMOs) is increasingly necessary, not only to verify compliance with labelling, but also to help industry to efficiently control the reception of raw materials. On the basis of this, a disposable electrochemical magnetogenoassay is proposed for simultaneous detection of two Roundup Ready (RR) soybean lines GTS 40-3-2 and MON89788, using gold-coated magnetic nanoparticles ($\text{Fe}_3\text{O}_4@Au$) as nanosupport. To perform this magnetogenoassay, a sandwich-type hybridization assay was used with different enzymatic labelling systems (fluorescein isothiocyanate and digoxigenin) and dual screen-printed carbon electrodes (SPdCEs), which allowed the simultaneous readout of each target. A linear relationship ranging from 0.1 to 2.5 nM and from 0.1 to 1.0 nM was achieved for GTS-40-3-2 and MON89788 events, respectively, and both assays showed a similar detection limit of about 0.1 nM. Furthermore, a good performance in terms of precision and selectivity was achieved. The proposed approach is a step forward for event-specific multiplex detection.

Keywords: Core-shell $\text{Fe}_3\text{O}_4@Au$ magnetic nanoparticles, GTS 40-3-2, MON89788, Electrochemical genoassay, Simultaneous detection.

1. Introduction

Humans have long sought to select plants and animals with beneficial traits, such as an increased yield or resistance to diseases or environmental pressures, leading to a gradual modification of the genome. However, it takes hundreds of years before any detectable

improvement is obtained. Modern technology, and genetic engineering in particular, now makes it possible to alter genetic material by introducing a foreign gene or an altered gene from the same organism, to create novel traits in organisms like increased insect resistance and herbicide tolerance. Organisms with genetic material altered in this way are called genetically modified organisms (GMOs). Since 1996, there has been a consistent increase in the global area planted with biotech crops, having reached 185.1 million hectares in 2016, with 50% of this area corresponding to genetically modified (GM) soybean [1]. Currently, there are 37 GM soybean events, of which 19 have already been approved for food and feed, import / processing or cultivation within the European Union (EU) [2]. Glyphosate-tolerant GM soy, also known as Roundup Ready soybean, is the GM crop plant most commercialized in the world. Among these GM events, the GTS 40-3-2 and MON89788 lines from Monsanto Company are the most representative. The GTS 40-3-2 event, commercially known as Roundup Ready™ (RR1) soybean, corresponds to the first-generation glyphosate-tolerant GM-soy plant, having been granted approval for market launch in the EU in 1996. The principle underlying this strategy is based on the tolerance of these plants to glyphosate, the active component of the Monsanto herbicide Roundup, by incorporation of the CP4 EPSPS coding sequence. A similar approach was conducted in the development of the second-generation glyphosate-tolerant soybean, while maintaining weed control and crop safety benefits. However, soybean MON 89788 event, with the trade name of Genuity™ Roundup Ready 2 Yield® (RR2), has the added potential of enhancing yield, providing a benefit for farmers. This event was approved in the EU in 2008 [2].

In order to protect and provide information to the consumer, the EU decided that the labelling of food products containing more than 0.9% authorized GM material is mandatory [3]. For this reason, it became essential to develop new methodologies

capable of detecting these genetic modifications to guarantee the implementation of legislation.

At present, nucleic acid biosensing technology has been demonstrated to be an interesting alternative to polymerase chain reaction (PCR), the reference method. The high specificity and sensitivity of genosensing platforms constitute the foundations for addressing some emerging issues in areas such as clinical diagnostics, environmental control and food safety [4-7]. In particular, electrochemical genosensing, which consists of converting nucleic acid hybridization, resulting from the biorecognition of the sequence-specific probes, into a detectable electrical signal, presents many advantages such as fast and simple protocol, low cost, and the possibility to be used for on-site monitoring, overcoming the limitations of the conventional method [8, 9]. In the field of GMO, these platforms have witnessed great advancements on all levels of specificity: screening, gene-specific, and event-specific methods [4, 10-12]. With the increase in the number of approved events, the development of multisensor approaches is essential.

However, to the best of our knowledge, no assay has yet been designed for the simultaneous identification of two transgenic events using the highest level of identification, that is, the event-specific detection. The most similar approach aimed to quantify the GTS40-3-2 event by detecting in parallel a specific sequence in the junction region and in a reference gene. The approach used magnetic microbeads in a sandwich format assay and two different enzymes, which implies different buffer conditions, so the detection is sequential rather than simultaneous [13]. The other known approach is only gene-specific [14]. In that work, Liao et al. developed a screening platform of GM events configured in an array chip, using a biomolecular computational assay, triggered by a multienzymatic cascade, having achieved a detection limit of 225 pM. However, a specific pretreatment of the extracted DNA to

convert it into single-stranded DNA is needed. Finally, a multiplex sensor for the simultaneous detection of promoter, terminator and gene-specific regions of the GMO genome has been reported [15]. Three different hairpin probes act as a capture probe for each target but the duplex is disrupted by an exonuclease, releasing the target for a new recognition event with an intact hairpin. The broken hairpin finally hybridizes with a complementary ssDNA-loaded AuNP labelled with electroactive compounds, which renders a double amplification scheme. This sensor is able to detect as low as 0.1% GMO but only for screening and qualitative purposes. Event identification is impossible with this design.

In recent years, the incorporation of nanotechnology in the field of electrochemical genosensing has been demonstrated as a good strategy to enhance the analytical features of these approaches [16]. It has been shown that core-shell gold-coated iron oxide magnetic nanoparticles ($\text{Fe}_x\text{O}_y@Au$ MNPs) are excellent platforms for electrochemical genosensing due to their high surface-to-volume ratio, superparamagnetic properties, high separation efficiency, high stability and biocompatibility [17, 18]. In both these works, efficiency and reproducibility were highlighted, probably due to the high monodispersity and stability of the MNPs used. Another characteristic that can contribute to this good performance are the magnetic properties that can be easily controlled by an external magnetic field, facilitating the washing steps in the genoassays and, consequently, avoiding nonspecific adsorptions.

Herein we report a step forward in electrochemical multiplexing for the rapid and simultaneous detection of two RR soybean lines GTS 40-3-2 and MON89788 using $\text{Fe}_3\text{O}_4@Au$ as an immobilization platform and two tracers (digoxigenin and fluorescein isothiocyanate). For that purpose, the magnetic cores (Fe_3O_4) were synthesized using the thermal decomposition method through the reduction of the organometallic

precursor iron(III) acetylacetonate [Fe(acac)₃] in 1-methyl-2-pyrrolidinone solvent, in the presence of oleic acid and oleylamine capping agents. This high-temperature synthesis approach method yielded high-quality Fe₃O₄ MNPs with well-controlled size and shape. The gold coating of the Fe₃O₄ cores was carried out by the chemical reduction of the Au(III) precursor in the presence of an organic capping agent (oleylamine) to prevent surface oxidation and agglomeration. A pure self-assembled monolayer (SAM) of mercaptohexanoic acid was formed on the Au shell as a linker between the MNPs and aminated DNA probes. Sandwich-type hybridization assays with enzyme-amplified electrochemical detection were performed for the determination of each target, using disposable dual screen-printed carbon electrodes (SPdCEs). This methodology allowed a sensitive, reproducible and selective approach to be developed for the simultaneous detection of RR1 and RR2 events.

2. Experimental section

2.1. Reagents and solvents

All commercially available reagents were used without further purification. 1-methyl-2-pyrrolidinone (NMP, ≥99.0%), anhydrous toluene (99.8%), 1-hexadecanol (95%), oleic acid (90%), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, ≥99.5%), concentrated saline sodium phosphate-EDTA (20× SSPE, pH 7.4), 6-mercaptohexanoic acid (90%), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), ethanolamine (≥99.0%), 3,3',5,5'-tetramethylbenzidine (TMB, Neogen K-blue enhanced activity substrate containing H₂O₂) were obtained from Sigma-Aldrich. Iron(III) acetylacetonate ([Fe(acac)₃], >99%), and oleylamine (80-90%) were purchased from Acros Organics. Alfa Aesar provided hydrogen tetrachloroaurate(III) trihydrate (HAuCl₄·3H₂O, 99.99%, ≥49.0% Au basis).

Antifluorescein-peroxidase (antiFITC-POD) and antidigoxigenin-peroxidase (antiDig-POD) Fab fragments were obtained from Roche Diagnostics GmbH (Mannheim, Germany). Casein 1% (w/v) blocking solution in 1× phosphate buffer saline (PBS) and absolute ethanol (analytical grade) were purchased from Thermo Scientific. Sodium chloride (NaCl, 99.5%) was supplied by Panreac, and the Tween 20 was received from Merck.

The following buffer solutions, prepared with Milli-Q water (specific resistivity 18.2 MΩ cm), were used: (i) immobilization buffer: HEPES (0.1 M, pH 7.4); (ii) hybridization buffer: SSPE (2× SSPE, pH 7.4); (iii) washing buffers: HEPES-T (0.1 M HEPES, 0.01% Tween 20); SSPE-T (2× SSPE, 0.01% Tween 20); and (iv) conjugate buffer: PBS-C (1× PBS solution containing 1% casein).

The DNA oligonucleotides, whose sequences are described in Table S1, were obtained as lyophilised desalted salts from Sigma-Aldrich. All stock solutions were prepared in Milli-Q water and stored at −20 °C.

2.2. *Synthesis and functionalization of magnetic Fe₃O₄@Au nanomaterial*

The Fe₃O₄@Au nanosupports were prepared by thermal decomposition and subsequently coated with a gold shell through reduction of Au(III) precursor on the surface of the MNPs in the presence of an organic capping agent (oleylamine) as used in our previous work [17] (Scheme S1).

Before SAM formation, a solvent-exchange step from toluene to ethanol is needed, in which the Fe₃O₄@Au MNPs concentration was determined. Afterwards, the Fe₃O₄@Au MNPs surface was functionalized with 6-mercaptopentanoic acid (MCHac) in ethanol (10 mL), under stirring for 16 h at room temperature, magnetically separated, washed

and dispersed in 0.1 M HEPES buffer (pH 7.4). A final $\text{Fe}_3\text{O}_4@Au$ MNPs concentration of 1 mg mL^{-1} was obtained. The nanomaterial will be denoted as $\text{Fe}_3\text{O}_4@Au\text{-SAM}$.

2.3. Dual magnetogenoassay procedure

The experimental procedure was carried out at room temperature and protected from light using aluminium foil.

2.3.1 Immobilization of DNA capture probes on $\text{Fe}_3\text{O}_4@Au\text{-SAM}$ MNPs

To biofunctionalize the $\text{Fe}_3\text{O}_4@Au\text{-SAM}$ with the ssDNA from both soybean events, the EDC/NHS reaction was used by the addition of a solution of 200 mM EDC and 50 mM NHS prepared in 500 μL of HEPES buffer to 0.0625 mg of $\text{Fe}_3\text{O}_4@Au\text{-SAM}$ for 15 min under stirring. After the activation of the carboxylic groups, the supernatant was removed, and 1 μM solution of both aminated capture probes (CP) in 1000 μL HEPES buffer was incubated with $\text{Fe}_3\text{O}_4@Au\text{-SAM}$ at room temperature for 1 h (Scheme 1, Step 1). Afterwards, two washing steps with HEPES-T were performed before addition of 1000 μL of 1 M ethanolamine in 0.1 M HEPES for 10 min to block the unreacted carboxylic groups. The CP-MNPs were then magnetically separated, and washed twice with HEPES-T and SSPE-T, each for 5 min. Finally, the CP-MNPs were resuspended in SSPE buffer (100 μL for each assay).

2.3.2 Sandwich-type hybridization assay and dual electrochemical detection

A sandwich-type hybridization assay was carried out with the desired concentrations of target DNA and 0.25 μM of both signalling probes (SP) labelled with fluorescein isothiocyanate (FITC) and digoxigenin (Dig) for RR1 and RR2, respectively, and mixed in SSPE buffer to a final volume of 1000 μL . To facilitate the homogeneous hybridization, a thermal shock consisting of 5 min at 98 $^\circ\text{C}$ and 5 min in an ice bath was

performed, and then the mixtures were left for 25 min at room temperature.

Subsequently, 100 μL of the CP-MNPs were added to each vial tube and the heterogeneous hybridization reaction occurred for 1 h under rotation. After that, the hybrid-conjugated MNPs were washed with SSPE-T and PBS-C buffers (Scheme 1, Step 2).

The solution containing hybrid-conjugated MNPs was split into two vials in order to perform the enzymatic labelling of each soybean event separately by adding 0.25 U mL⁻¹ of antiFITC-POD (RR1) or antiDig-POD (RR2) conjugate, prepared in PBS-C buffer, and incubated at room temperature (Scheme 1, Step 3). After 30 min, the resulting MNPs were magnetically separated, washed twice with PBS-C buffer, and then washed with SSPE-T buffer. Finally, these modified MNPs were resuspended in 14 μL of SSPE.

The dual electrochemical measurements were performed on the surface of a disposable SPdCE (geometric area of each working electrode is 4.7 mm²) after entrapping with two magnets placed under the working electrodes (WE), adding 4 μL of the modified RR1-MNPs on one of the WE and 4 μL of the modified RR2-MNPs on the second WE for 2 min (Scheme 1, Step 4). Subsequently, the liquid was carefully removed, and 60 μL of the TMB, in a ready-to-use reagent format, was added, covering the four electrodes for 1 min. The chronoamperometric measurements of the oxidized TMB were performed using a potential pulse at 0 V for 60 s (Scheme 1, Step 5). A new SPdCE was used for each measurement, and three replicates were carried out for all measurements.

2.4. Single RR2 magnetogenoassay procedure

The experimental procedure was based on our previous work [17]. Briefly, magnetic entrapment of RR2 DNA sequences through sandwich-type hybridization between aminated CP anchored to Fe₃O₄@Au MNPs and partial duplex labelled with Dig,

followed by enzymatic labelling (antiDig-POD), and subsequent detection of electroactive enzymatic product (TMB_{ox}) on the surface of SPCE, with a geometric working electrode area of 0.063 cm². For the sake of comparison with dual assays, all analytical signals are given in current densities.

3. Results and discussion

3.1. Selection of probes and target sequences

To achieve a genoassay able to unambiguously identify and quantify the GMO construct, an event-specific approach was chosen. These methods detect the junction between the recipient genome and the DNA inserted in the integration *locus*, since this site is characteristic of each transformation event. The sequences used in this work are presented in Table S1.

For the event-specific detection of RR2 soybean, the target sequence was specifically designed targeting the plant genome and the promoter junction zone. The resulting 87-nt target, containing 36-nt from the soybean genome and 51-nt from the promoter, presents a relatively stable secondary structure ($\Delta G = -9.42 \text{ kcal.mol}^{-1}$) as predicted using mfold Web Server [19] under the hybridization conditions used for the analysis (25 °C and 0.3 M Na⁺). In accordance with the guidelines for performing a sandwich-type hybridization assay on SAM [20], both probes (capture and signalling) were designed to form a perfect duplex structure after hybridization with target to avoid self-complementarity and fringe regions, which are deleterious for the analytical performance, and a hairpin capture probe containing 25-nt with a short 4-nt stem ($\Delta G = -2.07 \text{ kcal.mol}^{-1}$), to improve the selectivity, and a 62-nt signalling probe ($\Delta G = -6.00 \text{ kcal.mol}^{-1}$).

3.2. Optimization of the dual magnetogenoassay

The fundamentals of the simultaneous determination of soybean RR1 and RR2 events are displayed in Scheme 1. In brief, monodisperse Fe₃O₄@Au MNPs functionalized with pure MCHac were prepared (Scheme S1) and used as supports for the immobilization of aminated CP for both events, through EDC/NHS coupling reaction (Scheme 1, step 1). The average size of the Fe₃O₄ cores was 4.2 ± 0.6 nm, and the gold coating through reduction of HAuCl₄ led to an increase in the average diameter to 10.2 ± 1.3 nm, confirming the core-shell morphology of the resulting Fe₃O₄@Au nanomaterial and, consequently, its ability to be functionalized with other molecules while maintaining the magnetic properties of the cores [17]. Sandwich-type hybridization assays for each target were performed using signalling probes labelled with FITC (for RR1 event) or Dig (for RR2 event), followed by dual enzymatic labelling in two separate vial tubes (Scheme 1, steps 2 and 3). Afterwards, Fe₃O₄@Au MNPs biofunctionalized with DNA from RR1 and RR2 were magnetically captured on the corresponding working electrodes of the SPdCEs, and the chronoamperometric measurement of oxidized TMB reduction was carried out at the electrode surface (Scheme 1, steps 4 and 5).

First of all, a single assay using RR2 DNA sequences was carried out on SPCE under the previously optimized variables shown in Table S2 [17]. A linear dependency between the current and the RR2 concentration in the range of 0.1–1 nM was obtained, demonstrating that this assay design also works for the individual detection of the RR2 event. The regression equation is: j ($\mu\text{A}/\text{cm}^2$) = $13.3 (\pm 0.6)$ [RR2] + $0.06 (\pm 0.06)$, $R^2 = 0.994$. Afterwards, the dual magnetoassay was performed under the same conditions (Table S2) except for the enzymatic reaction, which was shortened to 30 s, in order to minimize cross-talk between electrodes, one of the major problems in multiplex

electrochemical approaches [21]. This assay was performed at 1 nM concentration for each target, and the results shown in Fig. 1 indicated a different sensitivity for each target, evidencing a current density about 3× higher for the RR2 event. This is an unexpected result, considering that the hairpin structure of the RR2 CP makes the hybridization more selective but more difficult. Of note, the RR1 CP also presents a small hairpin (Fig. 2) but in the closest region to the electrode surface, which seems to cause a stronger steric hindrance. The effect of the SP-tag at 3'-end FITC and Dig (Fig. 2, B) on sensitivity improvement cannot be excluded as a plausible explanation. González-Álvarez et al. compared the three most common DNA tags and showed that the Dig-antiDig-POD labelling system presents the best detectability [22], which is in accordance with our results and those of other recently reported studies [13].

A comparison between single and dual assays (Fig. 3) shows a slight decrease in the current density of the dual magnetoassay, which was ascribed to the shorter enzymatic reaction (30 s), so the enzyme reaction duration was increased to 1 min. For both events, the current density increased with the increase in the enzymatic reaction time, showing that 30 s are not sufficient to reach the steady state of the enzymatic reaction. Therefore, 1 min was the duration of the enzymatic reaction chosen for the subsequent experiments.

3.3. Analytical characteristics of the dual magnetogenoassay

Under the optimized conditions, the analytical response of the dual magnetoassay was tested with different concentrations of RR1 and RR2 (Fig. 4). A linear relationship between current density and target DNA concentration was found between 0.10 and 2.5 nM for RR1 and between 0.10 and 1.0 nM for RR2 according to the following equations: j ($\mu\text{A}/\text{cm}^2$) = 25.1 (± 0.2) [RR1] + 0.5 (± 0.6) ($R^2 = 0.9996$); j ($\mu\text{A}/\text{cm}^2$) = 52.3

$(\pm 0.4) [\text{RR2}] + 0.1 (\pm 0.2) (R^2 = 0.9997)$. The limits of detection (LOD) achieved were 0.10 nM for RR1, and 0.09 nM for RR2, and these were calculated using the following equation: $\text{LOD} = 3 s_b/m$, where “ s_b ” is the standard deviation of the blank and “ m ” is the slope of the calibration plot. The higher slope for RR2 confirms that this approach is more sensitive for this event, which can be attributed to differences between the secondary structures of the capture probes as well as the tags used, as already discussed in section 3.2.

The comparison between the dual assay and the RR1 and RR2 single assays (Table 1) shows that the current density is higher in the dual assays, but the RR1 to RR2 ratio is maintained at about 0.5, which confirms that there is no cross-talk between the working electrodes due to product diffusion, in line with other dual approaches using TMB or other soluble peroxidase substrates [23-25]. It is important to note that the amount of MNPs added to the working electrode is different in dual and single assays, about 8.9 and 18.8 μg , respectively. Taking into account that the geometric area of the single platform is about 2.7-fold higher than that of each dual working electrode, we obtain a nanoparticle density of ~ 1.48 and $1.89 \mu\text{g}/\text{mm}^2$ for single and dual electrodes, respectively, which can account for the higher current density of the dual approach. The precision of the dual measurements was evaluated using 1 nM of each target. The relative standard deviation (RSD) values obtained were 7.7% for RR1 and 3.7% for RR2.

In general, analytical characteristics of single and dual assays are quite similar. Though the slightly lower reproducibility of the dual assay can explain the smaller LOD of the single assays, the analytically useful concentration range for single and dual assays is identical for RR2, while wider in the case of the RR1 single assay. This good performance might be attributed to the high stability and dispersion of the nanomaterial

in the solvent, allowing a well-oriented DNA immobilization as well as avoiding non-specific DNA absorption.

The specificity of the proposed methodology was evaluated with a non-complementary sequence of another GM event, the maize MON810 event. Current intensities indistinguishable from the blank response were obtained (Fig. 5), revealing that the assays are specific for these events, with no cross-reactivity with other common transgenic events.

To the best of our knowledge, electrochemical multiplexing for GMO detection has been scarcely addressed, which shows the relevance of the proposed methodology. An approach based on logical analysis involving chronoamperometric genosensors with enzymatic amplification achieved a LOD higher (225 pM) than those reported herein [14] although detection was performed on a single electrode where three hybridizations (promoter, coding and species gene regions) and two enzymatic reactions had to occur simultaneously to render a positive identification/quantification. A very sensitive approach, with an analytical range between 0.1 pM and 10 nM, required two levels of amplification with exonucleases (target recycling) and bio-barcode with AuNPs labelled with electroactive species. Our approach is simpler, faster and more cost-effective [15]. Finally, a genomagnetic assay on microparticles achieved LOD in the fM range, but it is not a fully simultaneous approach because two enzymes that work at different pH are used for detection, so separate measurement is mandatory [13].

4. Conclusions

Core-shell Fe₃O₄@Au MNPs (10.2 ± 1.3 nm with 4.2 ± 0.6 nm cores) modified with SAMs, containing a high degree of monodispersion and colloidal stability, proved to be a suitable nanosupport for electrochemical genosensing because of the relative simplicity of fabrication that yields highly reproducible SAM surfaces ready for fast

DNA immobilization using well-established chemistry. Moreover, their magnetic properties allowed fast and efficient separation by an external magnet, which contributed to the good performance of the resulting dual magnetogenoassay. Contrarily to most dual/multiplex assays' recognition of both GMO events, the magnetogenoassay was carried out on a single set of particles containing a capture probe for both targets, simplifying the procedure, simplifying the protocol and reducing the analysis time. The use of different tags on the signalling probe allowed the simultaneous detection with a single enzyme on a disposable device. Using dual electrodes the analytical signal did not degrade. On the contrary, higher current densities arising from a higher nanoparticle density entrapped on the electrode surface were obtained. The sensitivity of the genoassay was more pronounced in the case of MON89788, though the design is fully parallel in terms of length of target and probes. This behaviour is related to the secondary structure of the capture probes and/or to a higher affinity of the digoxigenin tag for its Fab fragment, as was previously suggested. Limits of detection at the sub-nM level were achieved for both assays, similarly to the single platform. The reported methodology provides a portable, miniaturized, selective, and low-cost tool for the detection of multi-events, representing a step forward in the simultaneous determination of several GMOs using a single transducer.

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Clara Pereira completed her PhD in Chemistry in 2011, at the Faculty of Sciences of Porto University, Portugal. Currently she is Auxiliar Researcher at REQUIMTE/LAQV, Chemistry and Biochemistry Department, Faculty of Sciences, Porto University, Portugal. Her research areas include the fabrication and characterization of nanomaterials with tuned properties – magnetic nanomaterials, metal, silica and titania nanoparticles, carbon-based nanomaterials and their hybrids – and their application in energy, functional and smart textiles, eco-sustainable catalysis and magnetic resonance imaging. She is author/co-author of more than 40 peer-reviewed papers, 4 book chapters and more than 140 communications in national and international conferences.

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Noemí de-los-Santos-Álvarez is an Associate Professor at Universidad de Oviedo (Spain). Her initial studies were conducted on electrocatalytic processes and, during her postdoc stage at Cornell University she switched to the characterization of intermetallic materials for fuel cells. Her current interests are focused on electrochemical genosensing and aptamer selection for cancer biomarkers, allergens or viruses to be used in biosensors.

Cristina Delerue-Matos is a Full Professor of Instituto Superior de Engenharia do Porto. She is a member of REQUIMTE, the largest network in Chemistry and Chemical Engineering established in Portugal. Her scientific expertise allowed dealing with the topic Green Chemistry – Clean Technologies and Processes with a wide range of tools and from different perspectives. Besides working on various research projects, she possesses expertise in analytical chemistry, especially in electrochemistry. Recently, the scientific

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Figure Captions

Fig. 1. Chronoamperometric responses obtained with the dual magnetogenoassay for each soybean event with the duration of the enzymatic reaction of 30 s.

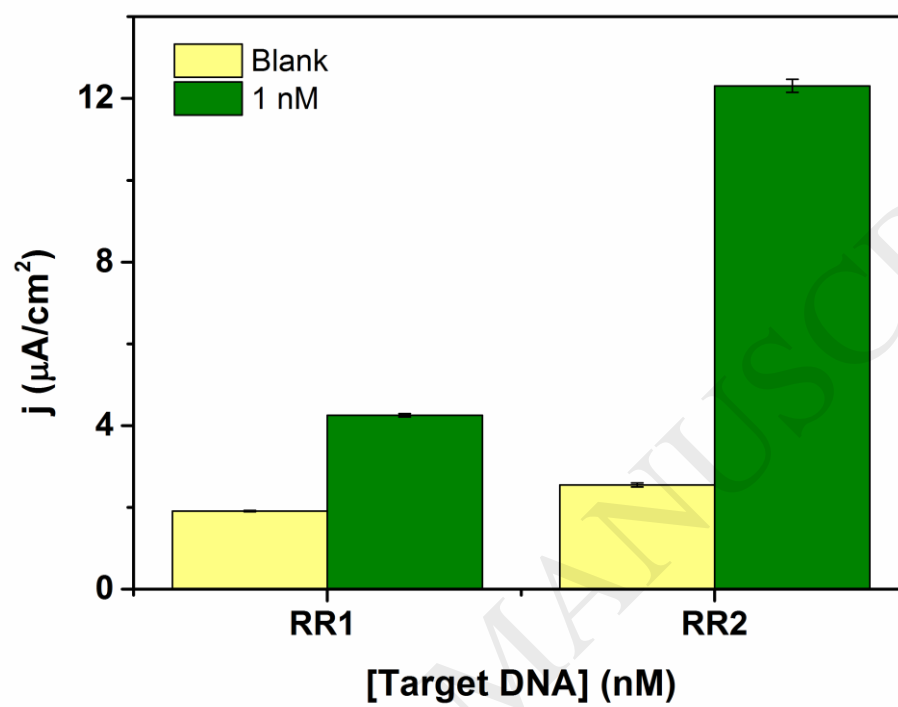


Fig. 2. (A) Secondary structures of (I) RR1 and (II) RR2 capture probes at 25 °C and 0.3 M Na⁺ from mfold web server; (B) Chemical structure of the tags fluorescein isothiocyanate (FITC) and digoxigenin (Dig).

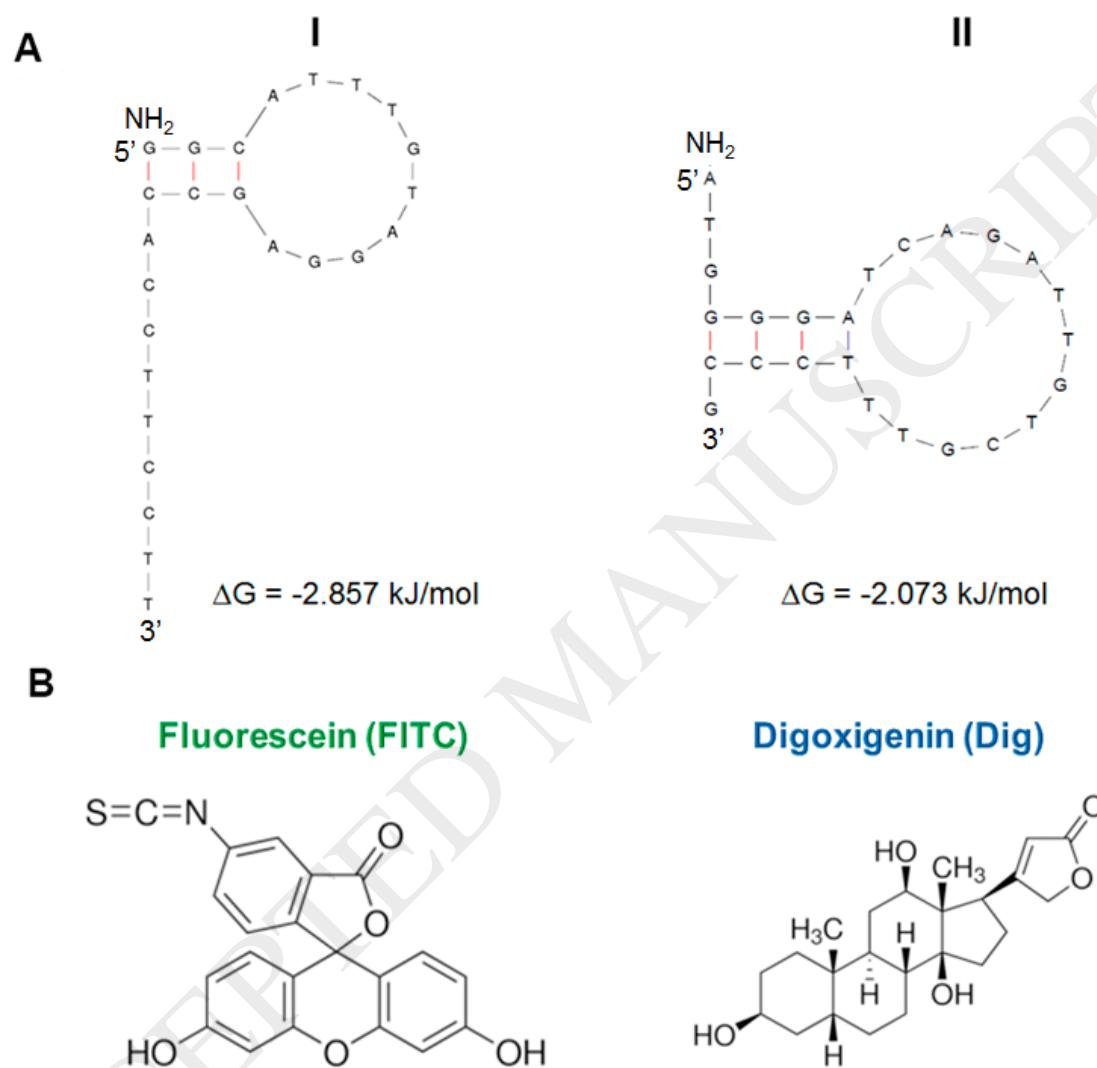


Fig. 3. Comparison of the chronoamperometric response obtained with single (SA) and dual (DA) assays with the duration of the enzymatic reaction of 1 min (SA/DA) and 30 s (DA) for RR1 (columns without pattern) and RR2 (columns with pattern) events.

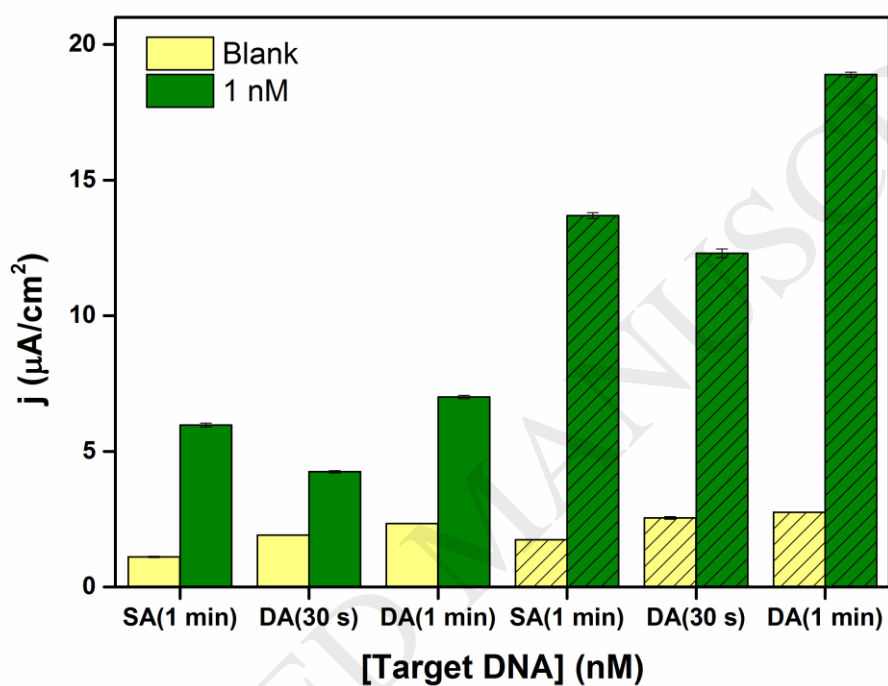


Fig. 4. Linear dependency between the current density obtained by chronoamperometry at 0 V and increasing concentrations of RR1 and RR2 DNA targets. Insets: Chronoamperograms obtained with (1) 0 (black), (2) 0.1 nM (red), (3) 0.25 nM (light green), (4) 0.5 nM (orange), (5) 1 nM (violet), (6) 2.5 nM (magenta) of RR1 (A) and RR2 (B) events, respectively, at 0 V for 60 s under the optimized conditions.

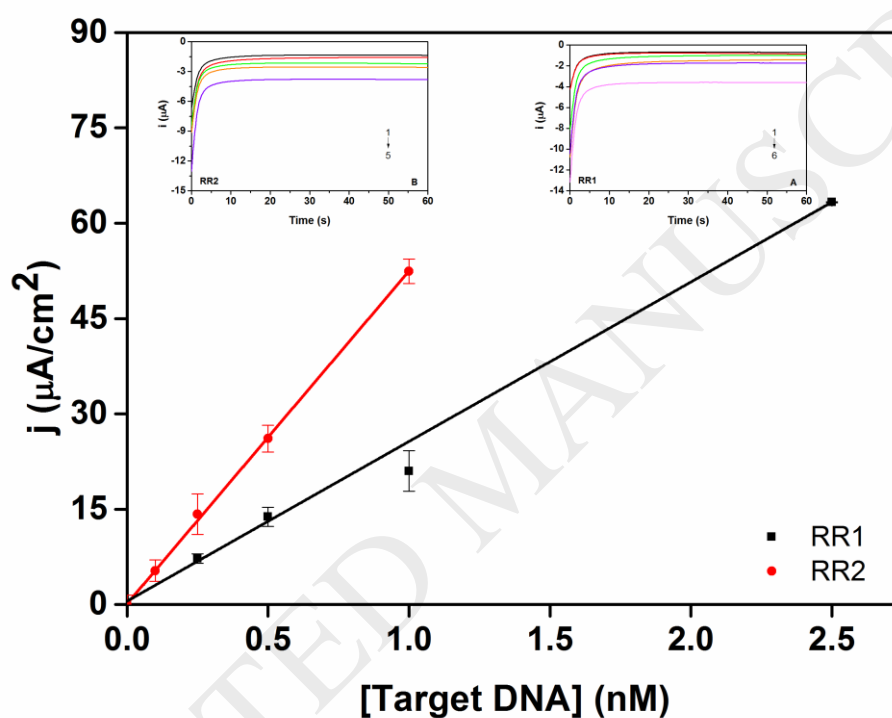
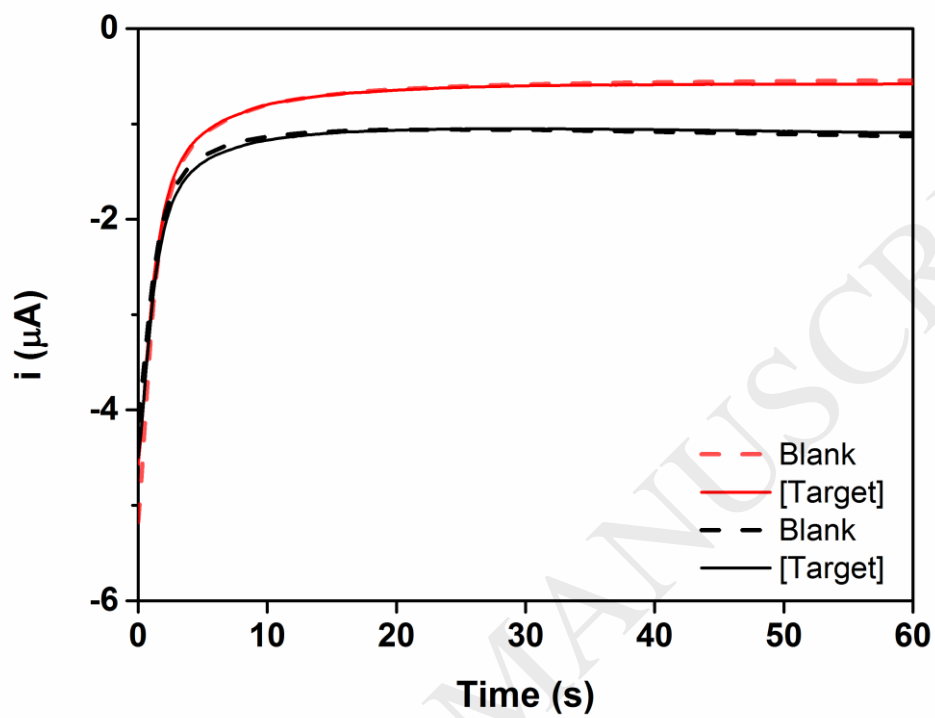


Fig. 5. Current intensities for the blank experiments and for non-complementary (MON810) DNA sequences (1 nM) of RR1 (black) and RR2 (red) events.



Scheme 1. Schematic illustration of the electrochemical dual magnetogenoassay design to simultaneously detect soybean GTS 40-3-2 and MON89788 events: **(A)** Core-shell $\text{Fe}_3\text{O}_4@Au$ MNPs synthesis, and surface functionalization with pure MCHac; **(B)** electrochemical dual magnetogenoassay procedure divided into five steps: **(1)** attachment of aminated CP from both events to the surface of $\text{Fe}_3\text{O}_4@Au$ MNPs via EDC/NHS reaction; **(2)** sandwich-type hybridization for each target in two consecutive steps: firstly, homogeneous hybridization between the target sequence and the SP labelling with FITC (RR1) or Dig (RR2), and subsequent heterogeneous hybridization with CP bound to the MNPs; **(3)** addition of the Fab-enzyme conjugates in two separate vial tubes; **(4)** magnetic capture of the biofunctionalized $\text{Fe}_3\text{O}_4@Au$ MNPs on the corresponding working electrodes of the SPdCEs and enzymatic reactions by TMB addition; **(5)** chronoamperometric measurement of TMB_{ox} reduction at the electrode surface.

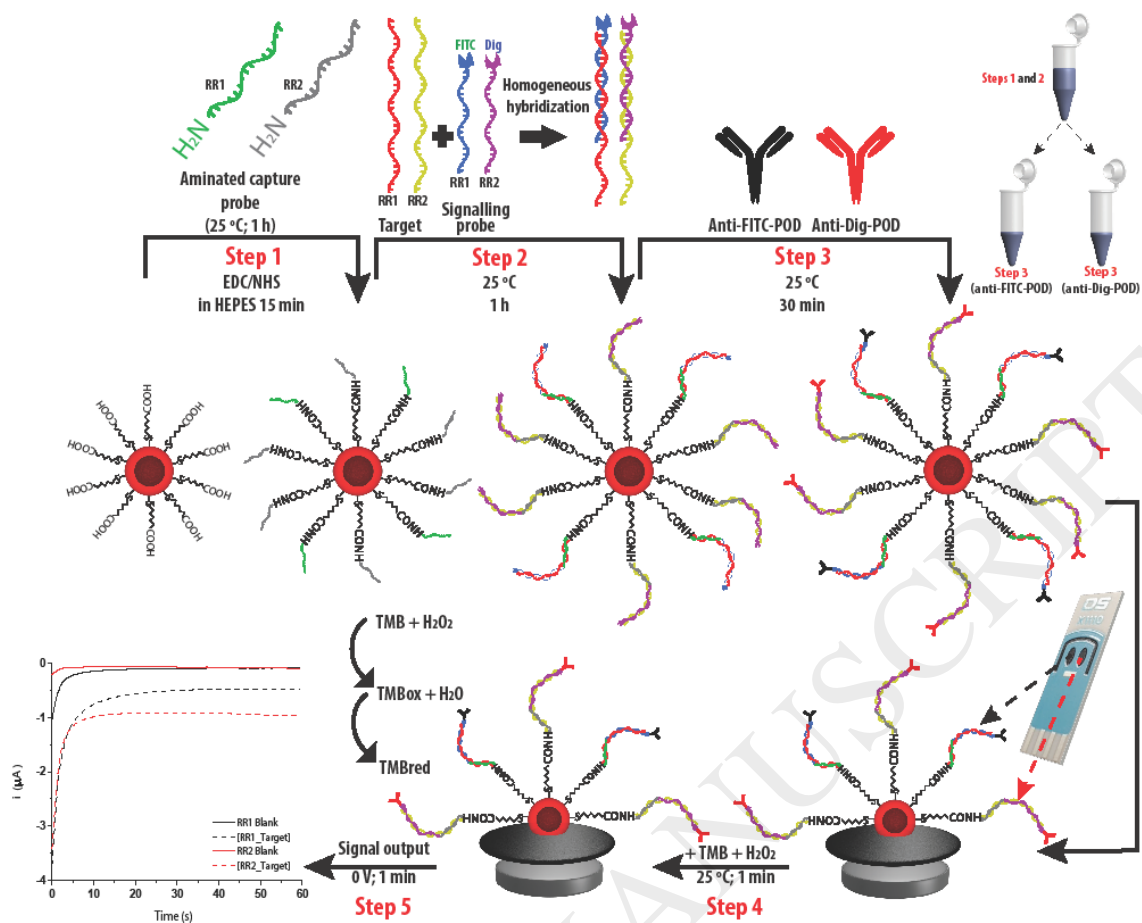


Table 1

Analytical characteristics of the dual and single magnetogenoassays for determination of soybean RR1 and RR2 events

	Dual Assays		Single Assays	
	RR1	RR2	RR1 ^[17]	RR2
Slope, $\mu\text{A cm}^{-2} \text{ nM}^{-1}$	25.1	52.3	6.73	13.3
Linear range, nM	0.1-2.5	0.1-1.0	0.1-10	0.1-1.0
R ²	0.9996	0.9997	0.9987	0.994
LOD, nM	0.1	0.1	0.02	0.02
RSD	7.7	3.7	3.9	7.3