Fe_3O_4@Au nanoparticles-based magnetoplatform for the HMGA maize endogenous gene electrochemical genosensing

Juliana Beatriz Sousa[a,b], Joilson Ramos-Jesus[c], L.C. Silva[d], C. Pereira[e], N. de-los-Santos-Álvarez[f], Rosana A.S. Fonseca[d], R. Miranda-Castro[f], C. Delerue-Matos[b], J. Ribeiro Santos Junior[a], M. Fatima Barroso[b,*]

a Programa de Pós-graduação em Biotecnologia - RENORBIO, Pró-reitoria de pesquisa e pós-graduação, Universidade Federal do Piauí - UFPI, Teresina, Brasil

b REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Porto, Portugal

c Núcleo de Pesquisa em Biodiversidade e Biotecnologia, UFPI, Parnaíba, Brazil

d Instituto de Ciências Biológicas – ICB/UPE, Recife, Brazil

e REQUIMTE/LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

f Dpto. Química Física y Analítica, Universidad de Oviedo, Oviedo, Spain

Corresponding author: MFB MFB@isep.ipp.pt

Abtract

This work addresses a technological advance applied to the construction of a magnetogenoassay with electrochemical transduction for the maize taxon-specific (HMGA gene) detection using gold-coated magnetic nanoparticles as nanosized platform.

Superparamagnetic core-shell Fe3O4@Au nanoparticles (10.4 ± 1.7 nm) were used to assemble the genoassay through the covalent immobilization of HMGA DNA probes onto carboxylated self-assembled monolayers at the nanoparticles surface.

A hybridization reaction using sandwich format was selected to prevent inefficient hybridization connected with stable secondary DNA structures using also fluorescein isothiocyanate as DNA signaling tag. The labelling of the hybridization reaction with enzymes allowed the chronoamperometric measurement of the peroxidase activity linked to the nanoplatform located on gold surface.

Using this electrochemical magnetogenoassay a linear concentration range from 0.5 to 5 nM and a LOD of 90 pM with a RSD<1.2% was calculated. Certified maize was evaluated without further purification after PCR amplification. This work highlights the efficacy of the electrochemical magnetogenoassay for the HMGA detection, showing its potential as alternative procedure for the verification of the compliance of the legislation.

Keywords: Fe_3O_4@Au nanoparticles, electrochemical genosensors, GMO, maize, hmga gene

1. Introduction

Recently, the quality and the safety of food have become a topic of public consciousness. Guaranteeing food safety is currently the focus of authorities in the food supply chain [1]. Therefore, food regulations attempt to offer a high level of protection and to guarantee transparent information to the consumers [1,2]. In this context, determination of food authentication, i.e. verification that a foodstuff follows its label description (food composition, ingredients, and origin [3]) is an important issue and, in consequence, there is a pressing need for accurate food authentication techniques [4]. The analytical methods used for species/ingredients identification mainly rely on proteins and/or DNA detection. The protein-based methodologies include immunological, electrophoretical and chromatographic protocols [5,6]; while the DNA-based methods are mainly based on the use of polymerase chain reaction (PCR). Nevertheless, DNA has been the biomolecule of choice due to its relatively high stability, specificity and easy to amplification [7,8].

Although PCR has been considered the gold standard methodology for the DNA identification, this technique is time-consuming and costly. Thereupon, researchers are encouraged to develop new alternative methodologies, such as low-cost and portable equipments capable of performing accurate and fast analysis [1]. In this sense, the application of electrochemical genosensors/genoassays for these purposes seems to be a good alternative and represents a promising technique to explore.

Briefly, an electrochemical genosensor/genoassay is based on the formation of a duplex between a single-stranded DNA (ssDNA) probe, attached to a solid support acting as the immobilized recognition element, and a complementary DNA sequence, the target sequence or analyte. The resulting hybridization event is subsequently revealed onto an

electrochemical transducer in such a way that the recorded electrochemical current is related to the concentration of the DNA analyte under study [9,10].

Until now, some electrochemical genosensors have been developed to detect DNA oligonucleotides allowing the plant species identification, namely hazelnut [11], soybean [8], peanut [12] and transgenics [13].

Recently, several reports have been indicating the development of genosensors with highest performance and sensitivity. These researches comprise the use of novel assay formats, amplification schemes and transduction mechanisms [10,14]. So, the combination of nano-based tools and biosensors have obtained a significant interest in a variety of fields including food monitoring. Due to their unique properties, nanomaterials have been proposed as a novel category and applied to biosensors, namely as surface modifications.

Magnetic nanoparticles (MNPs) are appropriate platforms for such bioassays due to their easy functionalization through the attachment of organic or biological molecules to the MNP surface, which increases the sensitivity and bonding strength of the target to the solid support [15]. Moreover, the potential of their magnetic properties to perform their separation from solvents and other chemicals, eliminating tedious and costly separation processes [16], making these nanomaterials a good choice for the development of analytical protocols that are faster, simpler and more precise that existing methodologies [17].

The purpose of this work concerns the development of an alternative methodology for the maize detection based on the electrochemical genoassay construction. For that goal, superparamagnetic core-shell gold-coated iron oxide MNPs (Fe3O4@Au MNPs) were used as versatile nanomagnetoplatform for the electrochemical genoassay to perform the maize endogenous gene identification (Scheme 1). This innovative methodology merges

the advantages of both biosensor technology and MNPs as magnetic nanoplatform to develop a simple, low-cost, robust and accurate electrochemical genoassay. This type of procedure can be a useful alternative analytical tool on food authenticity process and on the screening the presence of transgenics.

The Fe_3O_4@Au MNPs were synthesized by using a sequential strategy involving the formation of iron oxide cores and their subsequent coating with a gold shell [18]. After covering of the Fe_3O_4@Au MNPs surface with a mixture of thiolated compounds containing terminal carboxyl groups, they were used to perform the hybridization recognition reaction in a sandwich format for electrochemical detection.

For that, a DNA capture probe targeting the endogenous maize gene (high mobility group proteins, HMGA) was immobilized onto the modified Fe_3O_4@Au surface. Then, a DNA sandwich format assay was performed on this modified support and the electrochemical detection was carried out after enzymatic labelling of the duplex formed on the surface and deposition of the modified MNPs (Scheme 1) on a gold homemade working electrode (Fig. S1 and Fig. S2).

The electrochemical magnetogenoassay was successfully applied for the cereal species detection by targeting the presence of maize in transgenic maize flour after PCR amplification. So, the utility of this approach was demonstrated as an analytical device to be used in food safety, namely in the determination of food authenticity (food composition) and as reference gene detection to screen the presence of maize in genetically modified foodstuffs.

2. Experimental

2.1. Reagents and solvents

All used reagents were of the highest analytical grade. Blocker casein solution 1% (w/v) (ready to use) in PBS buffer was purchased from Termo Scientific. Tween® 20

surfactant (grade for synthesis, 96–100%) and absolute ethanol (analytical grade) were from Merck. Anti-fluorescein-peroxidase fragment (Anti-FITC-POD) was obtained from Roche Germany and the bovine serum albumin (BSA) was from Acros Organics. 6-mercaptohexanoic acid, (MHA, 90%), 6-mercapto-1-hexanol (MCH, ≥97%), thioctic acid (TOA, 98%), 3,3′,5,5′-tetramethylbenzidine (TMB aqueous solution containing H2O2), N-hydroxysuccinimide (NHS, 98%), 1-ethyl-3- [3-dimethylaminopropyl] carbodiimide (EDC, ≥98%), 1-hexadecanol (95%), oleic acid (90%), 1-methyl-2-pyrrolidinone (NMP, ≥99.0%), ethanolamine (99%), iron(III) acetylacetonate ([Fe(acac)3], 97%), hydrogen tetrachloroaurate(III) trihydrate (HAuCl4·3H2O, ≥99.9%, Au 48.5–50.25%), oleylamine (70%), 20×saline sodium phosphate-EDTA (20×SSPE) pH 7.4 and 4- (2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES buffer ≥99.5%) were purchased from Sigma-Aldrich. Ultra-pure water was purified from a Millipore-Q purification system (18.2MΩcm). Several washing buffers were used: i) 2×SSPE solution; ii) SSPE-T (2×SSPE, 0.01% Tween® 20); iii) HEPES-T (0.1M HEPES, 0.01% Tween® 20); iv) HEPES (0.1M with 0.5M NaCl, pH 7.4) and v) PBS-C (1×PBS solution containing 1% casein).

Specific oligonucleotide sequences of the maize endogenous gene, HMGA: DNA capture probe, DNA target and DNA signaling probe (Table 1), were obtained as lyophilized desalted salts from Sigma-Aldrich. DNA stock solutions (100 μM) were prepared in Milli-Q water and stored at −20 °C. DNA working solutions were prepared by diluting an amount of DNA stock solution in 2×SSPE. Certified reference material (CRM) of genetically modified maize event MON810 10% (w/ w) was obtained from the Institute of Reference Materials and Measurements through Sigma-Aldrich.

2.2. Instrumentation

Electrochemical measurements were performed by using an Autolab PGSTAT12, potentiostat/galvanostat from Metrohm Autolab controlled by GPES 4.9 (EcoChemie, The Netherlands) using a conventional three electrode configuration: a home-made gold working electrode was obtained by physical vapor deposition of gold wire (99.99%), an Ag|AgCl|KCl (saturated) reference electrode and a platinum counter electrode.

A 12-tube mixing wheel (Dynal MX1) for solution mixing in Eppendorf tubes and the magnet (DynaMag-2) for magnetic separation were purchased from Thermo Fisher Scientific.

PCR amplifications were performed on a thermal cycler (GeneAmp® PCR System 2700 thermocycler - Applied Biosystems, Spain).

2.3. Electrochemical genoassay procedure

Briefly, the functionalized Fe3O4@Au were activated using a solution of EDC and NHS. Then, the modified MNPs were bioconjugated by incubating the Fe3O4@Au with DNA capture (NH2-CP) probe. After, 1M ethanolamine was put into contact with the NH2-CP-MNPs to block the unreacted carboxylic groups. Finally, the modified MNPs were dispersed in SSPE buffer and immediately used to perform the sandwich assay.

The sandwich format assay was performed in two consecutive hybridization steps: Firstly, for the homogeneous hybridization, it was mixed the signaling probe labeled with FITC (FITC-SP) and the desired DNA target. Then, the CP-modified Fe3O4@Au MNPs were added to each Eppendorf tube to allow the heterogeneous hybridization reaction.

After, the core-shell nanoparticles functionalized with the DNA duplex were resuspended in anti-FITC-POD conjugate in PBS-C buffer. The electrochemical measurements were performed on a home-made gold electrode. The detection of the

enzymatically oxidized product (TMB-H_2O_2 K-Blue reagent) was performed by chronoamperometry.

2.4. Real sample preparation

DNA from CRM was extracted and purified using a Nucleospin® food kit. DNA quantity and purity was determined by UV spectrophotometry. The PCR amplification was carried out in 25 μL of total reaction volume containing 2 μL of DNA extract, 1×buffer, 1.25 U of ImmolaseTM DNA polymerase, 200 μM of each dNTP, 6.5mM of $MgCl_2$ and 0.3 μM of each primer Mail-F/Mail-R. The amplification assays were performed according to the following program: initial denaturation at 95 °C for 10 min, 40 cycles at 95 °C for 15 s, and 60 °C for 60 s; with a final extension at 72 °C for 7 min. In both types of amplifications, the success of the amplification reaction was verified by electrophoresis.

Details about all the procotols used (Home-made gold working electrode construction; Synthesis and functionalization of the Fe_3O_4@Au MNPs; Electrochemical genoassay procedure and the real sample preparation) are described in detail in Supporting information.

3.2. Optimization of the experimental conditions

In this study, core-shell Fe_3O_4@Au MNPs were used as nanoplatform for the construction of the magnetogenoassay for the maize endogenous gene detection. Fe_3O_4 MNPs with 4.8 ± 0.5 nm average particle size were produced by using a thermal decomposition of [$Fe(acac)_3$] in the presence of oleic acid and oleylamine. After this synthesis, a gold coating was carried out by deposition of gold from a Au (III) salt on the MNP surface followed by reduction of Au(III) to Au (0).

Previous studies performed in our group indicated that a Fe_3O_4:Au precursor molar ratio of 1:7(Fe_3O_4: Au 1:7) resulted on the complete encapsulation of the Fe_3O_4 NPs with a

Au shell, while preserving the superparamagnetic properties of the magnetic cores, thus being suitable for the development of an electrochemical magnetogenoassay [18]. In this context, in this work, a similar Fe_3O_4:Au precursor molar ratio was used, resulting in well-dispersed Fe_3O_4@Au MNPs (Fig. S4). NPs were well-dispersed with no signs of aggregation, which presented a quasispherical morphology with an average particle size of 10.4 ± 1.7 nm [18].

3.2.1. Optimization of experimental variables

Self-assembled monolayers (SAMs) of alkanethiols on gold-coated MNPs have been widely applied in chemical and biological biosensing. The main merits of SAMs based on Au–S bond are: i) formation of well defined structures that are easy to prepare; ii) incorporation of a wide range of functional groups and iii) high stability due to the covalent sulfur – gold bond, and iv) reproducibility [25–27].

In this work, the binary SAM (composed by thiolated carboxylic acid and thiolated alcohol) assembled on the Fe_3O_4@Au surface was optimized in terms of composition and ratio between thiols. First, a 3:1M ratio of 1-mercapto-6-hexanol (MCH): thioctic acid (TOA) or 6-mercaptohexanoic acid (MHA) was tested employing 0.75mM of alcohol and 0.25mM of carboxylic acid.

The effect of using different thiolated carboxylic acids, TOA or MHA, was evaluated by analyzing the efficiency of the hybridization through recording the electrochemical cathodic current of the TMB enzymatically oxidized.

Under the same analytical conditions (0.025 mg of Fe_3O_4@Au, 1 μM of DNA capture probe and 5 nM of DNA target) the MHA: MCH mixture was found to give the highest signal to blank ratio (S/B). As it is possible to observe in Fig. 1, the use of MHA as a component of the mixed SAM increased the S/B ratio from 1.8 to 4.0. Althought the signals corresponding to the blank experiments (electrochemical current obtained in the

absence of the DNA target) were similar with both thiolated carboxylic acids, the use of MHA as binary layer component promoted an increase in the specific signal. According to Campuzano and collaborators [28] the improved performance of the MHA over the TOA can be ascribed to the preferential orientation adopted by this linear thiol that may minimize nonspecific adsorption of biomolecules while maintaining favorable orientation of the capture probe and good permeability of small molecules, such as the TMB substract [28]. Therefore, the binary SAM used in the following assays was obtained from a mixture of MHA: MCH.

The effect of the binary SAM composition on the electrochemical responses was subsequently studied. Two different MHA: MCH molar ratios namely 3:1 and 6:1 were tested by using a DNA capture probe of 1 μM and a DNA target of 2.5 nM. Fig. 2 shows that increasing the amount of thiolated carboxylic acid groups immobilized on the Fe_3O_4@Au surface (ratio 6:1) the electrochemical current measured after hybridization reaction also increases. Likewise, the S/B ratio increases from 1.9 to 3.9 when using a MHA: MCH ratio of 3:1 and 6:1, respectively. The amount of thiolated carboxylic acids assembled on the Fe_3O_4@Au at this ratio leads to more DNA capture probe immobilization with a suitable spatial distribution not to preclude the efficiency of HMGA hybridization reaction, a well-known but deleterious behavior when high DNA density is achieved [27]. For the optimization of the next steps a mixture 6:1 of MHA: MCH was used.

Bovine serum albumin (BSA) is a protein widely used as surface blocking agent in sandwich-like genoassay protocols to improve the S/B ratio. However, in this work, the addition of BSA, at room temperature, on the homogenous hybridization step (step which involves the partial hybridization between the DNA target and the DNA signaling probe) BSA while a S/B ratio of 2.6 was calculated in the presence of BSA. In

fact, in the absence of BSA, the homogenous hybridization reaction was set at 98 °C for 5 min and in an ice bath for 5 min (denaturation of DNA), and then 30 min at room temperature. It seems that the use of this denaturation procedure improves the efficiency of the homogenous reaction. The hybridization between the two partial complementary DNA sequences involves both the opening of the stem-loop structure of the DNA probes as well as the formation of the probe-target duplex which is favored by the use of high temperature. So, the next steps were performed in the absence of any blocking agents, namely the BSA presented a negative effect by decreasing the electrochemical current. As indicated in Fig. 3, a S/B ratio of 5.6 was obtained in the absence of BSA while a S/B ratio of 2.6 was calculated in the presence of BSA. In fact, in the absence of BSA, the homogenous hybridization reaction was set at 98 °C for 5 min and in an ice bath for 5 min (denaturation of DNA), and then 30 min at room temperature. It seems that the use of this denaturation procedure improves the efficiency of the homogenous reaction. The hybridization between the two partial complementary DNA sequences involves both the opening of the stem-loop structure of the DNA probes as well as the formation of the probe-target duplex which is favored by the use of high temperature. So, the next steps were performed in the absence of any blocking agents, namely the BSA.

The amount of Fe3O4@Au nanoparticles used in the genoassay plays a crucial role in the performance of the electrochemical measurement, so its effect was also studied. In order to evaluate this parameter different amount of Fe_3O_4@Au MNPs (from 0.0313 to 0.125 mg) were used on the genoassay procedure. As can be seen in Fig. 4, the electrochemical response measured for 2.5 nM DNA target increased when the amount of Fe_3O_4@Au MNP increased. Considering that the electrochemical current of the blank assay did not change significantly, higher S/B ratios were obtained when increased

amounts of modified Fe_3O_4@Au were deposited on the working electrode. S/B ratios values of 1.9, 2.5 and 6.7 were recorded for 0.0313 mg, 0.0625 mg and 0.125 mg of Fe_3O_4@Au, respectively. Thus, 0.125 mg of Fe_3O_4@Au nanoparticles was the amount selected for the subsequent studies.

Under optimized experimental conditions, the effect of increasing the synthetic HMGA DNA target concentration on the analytical signal was assessed by determining the chronoamperometric current from 0.25 to 10 nM (Fig. 5). A linear correlation between the blank-subtracted intensity (Inet) and the HMGA DNA target concentration was found in the interval from 0.5 to 5 nM (Fig. 5b inset). The regression equation is the following: I(net) (nA) = 544.8 (±16.2) [HMGA ssDNA] (nM) + 27.3 (±9.9); r = 0.9997. The limit of detection (LOD) and the limit of quantification (LOQ) calculated as three times and ten times the standard deviation of the blank assay divided by the slope of the calibration plot were 0.09 nM and 0.31 nM, respectively.

The precision of the magnetogenoassay was assessed by using 2 nM of maize HMGA DNA target. For that, the repeatability was determined by inter-electrode measurements and the reproducibility was evaluated by carrying out three measurements in five consecutive days. Repeatability and reproducibility expressed as relative standard deviation were 0.9% and 1.2%, respectively.

Liao and collaborators reported the use of a sensor Au array chip as electrochemical platform for the development of genosensors able to the multiplex screening of genetically modified DNA as well the endogenous soybean and maize gene, lectin and SSIIb, respectively (Table S1). This bioanalytical system allowed the discrimination of several different DNA sequence presenting a LOD of 0.225 nM [29]. We have previously reported the development of two genosensor platforms (for the MON810 detection), the conventional Au electrode and a novel Au 3D-nanostructure [10]. Using

the conventional and the Au 3D a LOD of 0.48 nM and 0.25 nM was found, respectively (Table S1). Although, both presented good analytical performances, the home-made 3D nanostructure exhibited less reproducibility. The synthesis of gold coated MNPs covered with SAM is carry out under much strictly controlled conditions and fill this gap yielding the highly stable and low polydisperse nanoplatform excellent for analytical purposes [18].

3.3. Application of the genoassay to detect HMGA gene in maize flour

The proposed electrochemical magnetogenoassay was applied to the detection of HMGA endogenous maize gene in amplified PCR products from maize flour. Adenaturation procedure of the HMGA amplified DNA was carried out by heating at 98 °C for 5 min and cooling in ice bath during 5 min. This procedure is mandatory due to the double stranded nature of the amplicons [8]. Then 20 μL of amplified HMGA endogenous gene, diluted 1:10, were added to the solution containing the signaling probe (homogenous hybridization). After the heterogeneous hybridization reaction (at room temperature for 60 min), the electrochemical signal was recorded. In Fig. S5 the analytical current obtained for the amplicon is clearly distinguished from the blank measurement and similar to a 2 nM target concentration. This result indicate that unpurified amplicons of maize gene can be detected using the magnetoassay.

A CRM containing soya flour but not maize was also subjected to the same PCR amplification procedure, acting as a control, and then evaluated with the magnetogenoassay for maize detection. The analytical current obtained was identical to that for the blank of the magnetogenoassay within the experimental error, which indicates that the influence of the PCR reagents is negligible on the analytical current and confirms that there is no cross-reactivity with other short ssDNA present in the sample.

4. Conclusions

In this work, we have shown that Fe3O4@Au nanoparticles covered by a mixed SAM are especially well-suited to become a routine nanoplatform for genosensing. Their stability and monodispersity allows to achieve a very high reproducibility, which is a characteristic extremely desirable that is precluding them to reach the market. Simple covalent immobilization of the DNA capture probe and a sandwich assay was performed on their surface and the electrochemical detection on a gold leaf obtained by direct current (DC Sputering) on a polyester film. A limit of detection in the pM range permitted the application of the assay to maize samples after PCR end-point amplification without further amplicon purification, which reduces the analysis time and cost. Supporting Information available:

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Figure caption

Scheme 1. Scheme of the electrochemical genoassay development: A) Fe_3O_4@Au synthesis; B) Fe_3O_4@Au functionalization; C) Genoassay protocol and D) Electrochemical detection

Fig. 1. Comparative hybridization efficiency, blank signal (in the absence of DNA target) and S/B ratio (blue dots, secondary axis) obtained with 0.025 mg of Fe_3O_4@Au nanoparticles in the presence of 5.0 nM of DNA target in 2×SSPE buffer using different thiolated carboxylic acids (MHA and TOA) as DNA anchoring compounds. Error bars were estimated from three parallel assays.

Fig. 2. Effect of the SAM molar ratio of MHA: MCH on the current intensity obtained with 0.025 mg of Fe_3O_4@Au nanoparticles in the absence (blank signal) or in the presence of 2.5 nM of DNA target and S/B ratio (blue dots, secondary axis) for each assay. Error bars were estimated from three parallel assays. [capture DNA]=1 μM, BSA 1%.

Fig. 3. Currents measured chronoamperometrically for the blank (in the absence of DNA target) and for 5.0 nM DNA target in 2x SSPE buffer in the absence or in the presence of BSA with 0.025 mg of Fe_3O_4@Au nanoparticles. S/B ratio (blue dots, secondary axis). Error bars were estimated from three parallel assays. [capture DNA]=1 μM

Fig. 4. Effect of the Fe_3O_4@Au nanoparticles amount on the current intensities obtained in the absence (blank signal) or in the presence of 2.5 nM of DNA target and S/B ratio (blue dots, secondary axis) for each assay. Error bars were estimated from three parallel assays.

Fig. 5. A) Chronoamperometric responses for different DNA target concentrations (0; 0.5; 1; 2; 5; 7 and 10 nM). B) Variation of the blank subtracted current with the DNA target concentration

Tables

Table 1:

Oligonucleotide sequences

DNA strand name	Oligonucleotídeos 5’ à 3’
Target (T) 79 nt	T TGG ACT AGA AAT CTC GTG CTG ATT AAT TGT TTT ACG CGT GCG TTT GTG TGG ATT GTA GGA CAA GGC TCC CTA TGT AGC´
Capture probe (NH_2-CP) (30 nt)	(NH_2) GCT ACA TAG GGA GCC TTG TCC TAC AAT CCA
Signalling probe (FITC-SP) (49 nt)	CAC AAA CGC ACG CGT AAA ACA ATT AAT CAG CAC GAG ATT TCT AGT CCA A (FITC)
Forward primer (Mail-F) (23 nt)	TTG GAC TAG AAA TCT CGT GCT GA
Reverse primer (Mail-R) (22 nt)	GCT ACA TAG GGA GCC TTG TCC T

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Scheme 1

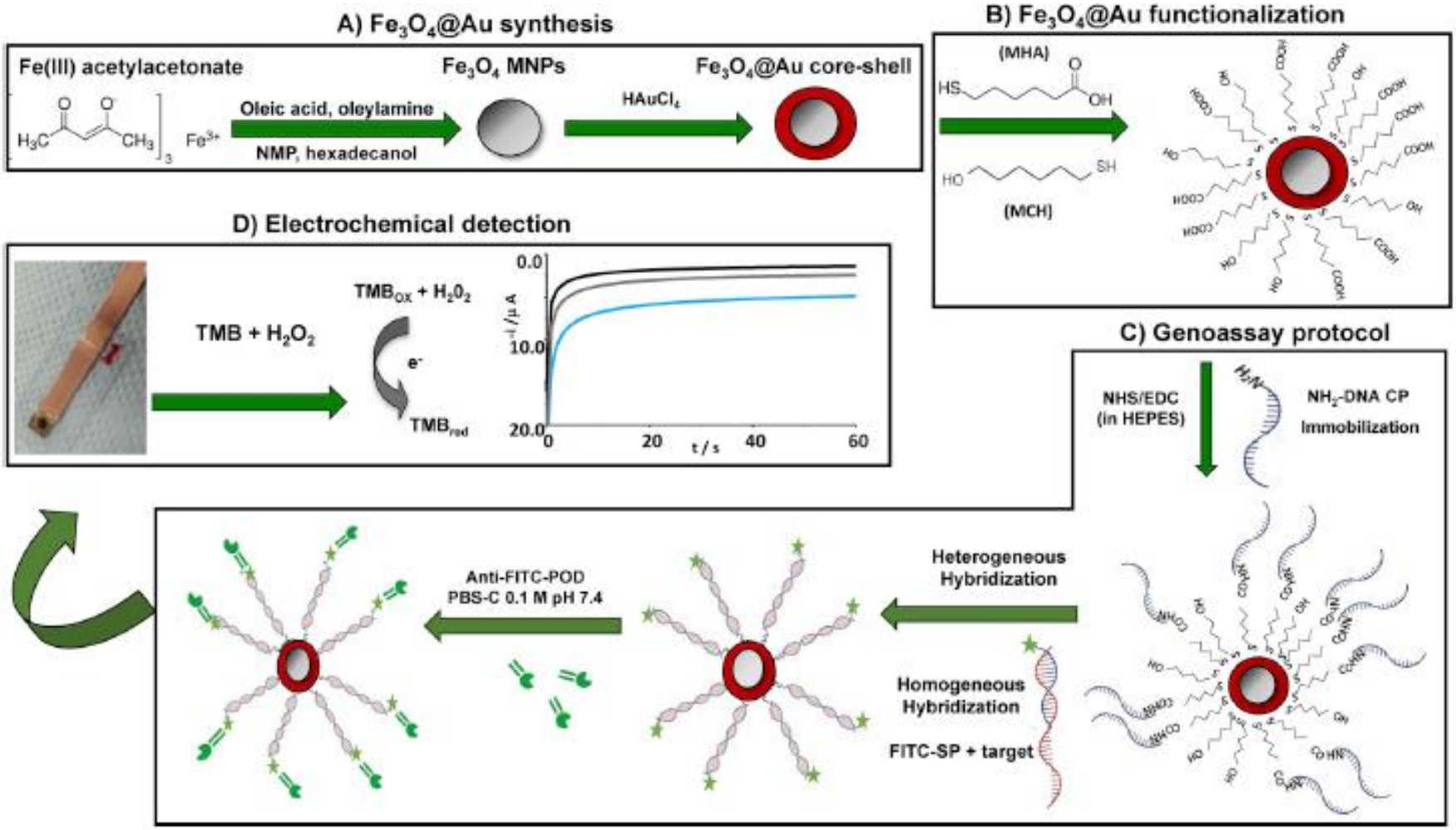

A) Fe_3O_4@Au synthesis
Fe(III) acetylacetonate
Fe_3O_4 MNPs
Fe_3O_4@Au core-shell
Oleic acid, oleylamine
NMP, hexadecanol
$HAuCl_4$
B) Fe_3O_4@Au functionalization
(MHA)
(MCH)
C) Genoassay protocol
NHS/EDC
(in HEPES)
NH_2-DNA CP
Immobilization
Heterogeneous
Hybridization
Homogeneous
Hybridization
FITC-SP + target
Anti-FITC-POD
PBS-C 0.1 M pH 7.4
D) Electrochemical detection
$TMB + H_2O_2$
$TMB_{OX} + H_2O_2$
e^-
TMB_{red}
0.0
10.0
20.0
$-i$ / µ A
0
20
40
60
t / s

Figure 1

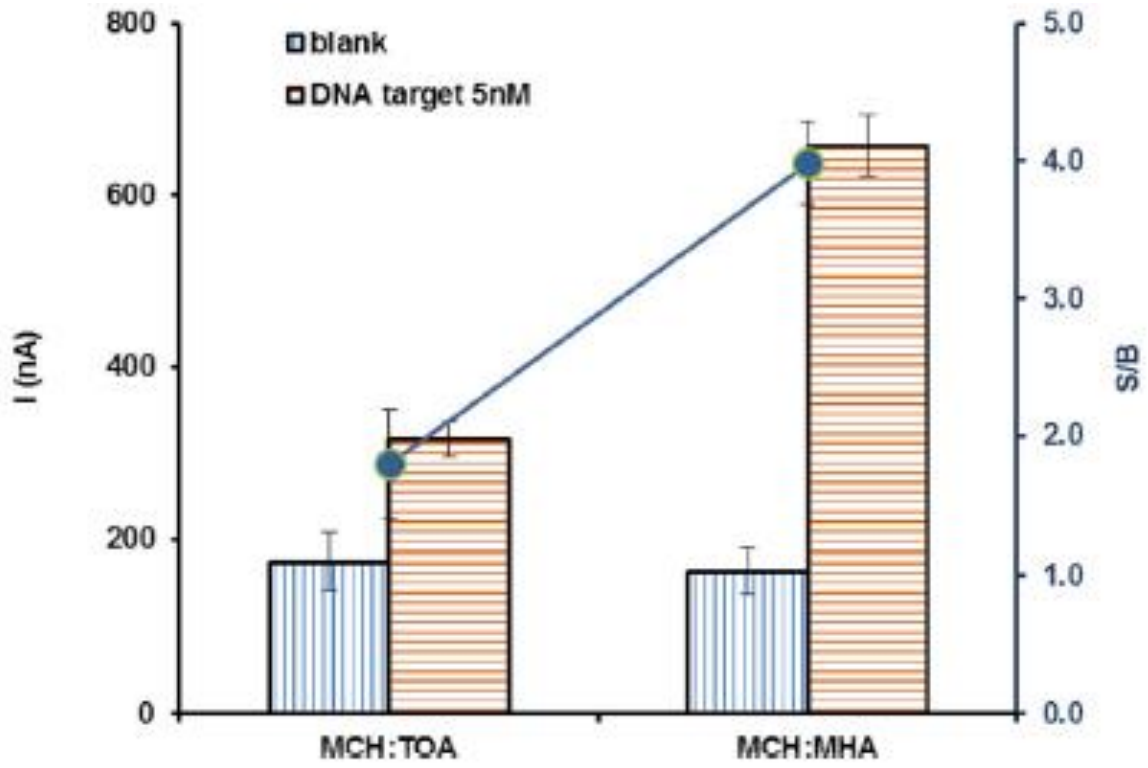

800
600
400
200
0
I (nA)
blank
DNA target 5nM
5.0
4.0
3.0
2.0
1.0
0.0
S/B
MCH:TOA
MCH:MHA

Figure 2

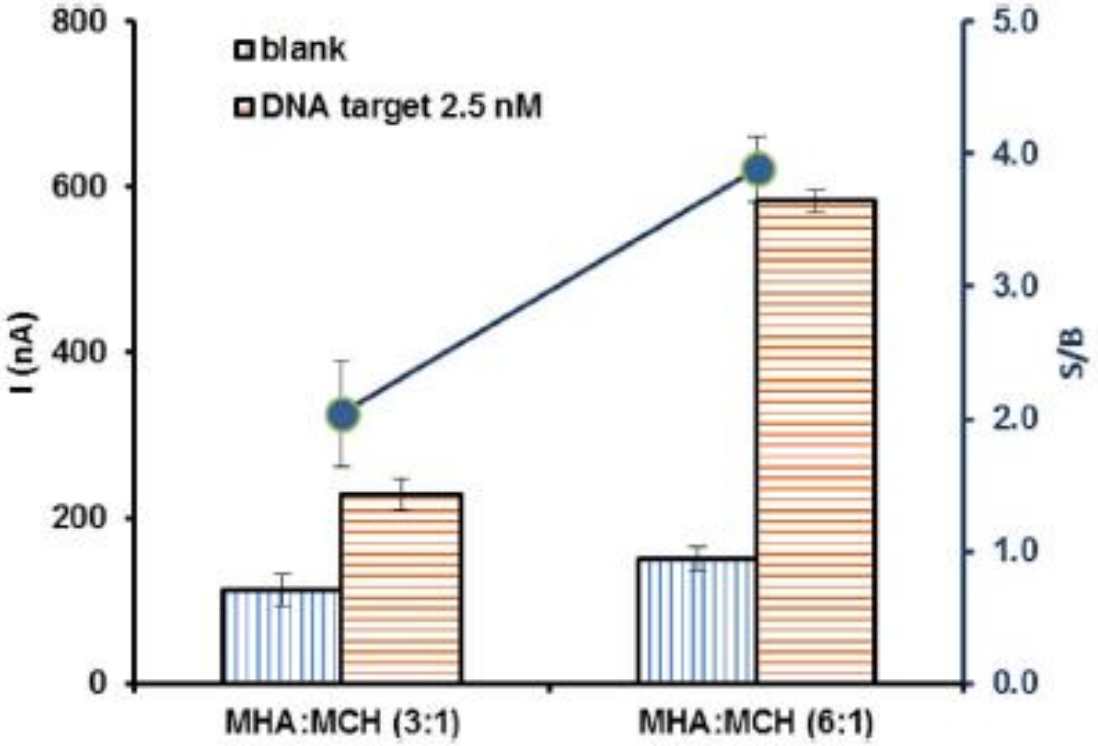

800
600
400
200
0
I (nA)
blank
DNA target 2.5 nM
5.0
4.0
3.0
2.0
1.0
0.0
S/B
MHA:MCH (3:1)
MHA:MCH (6:1)

Figure 3

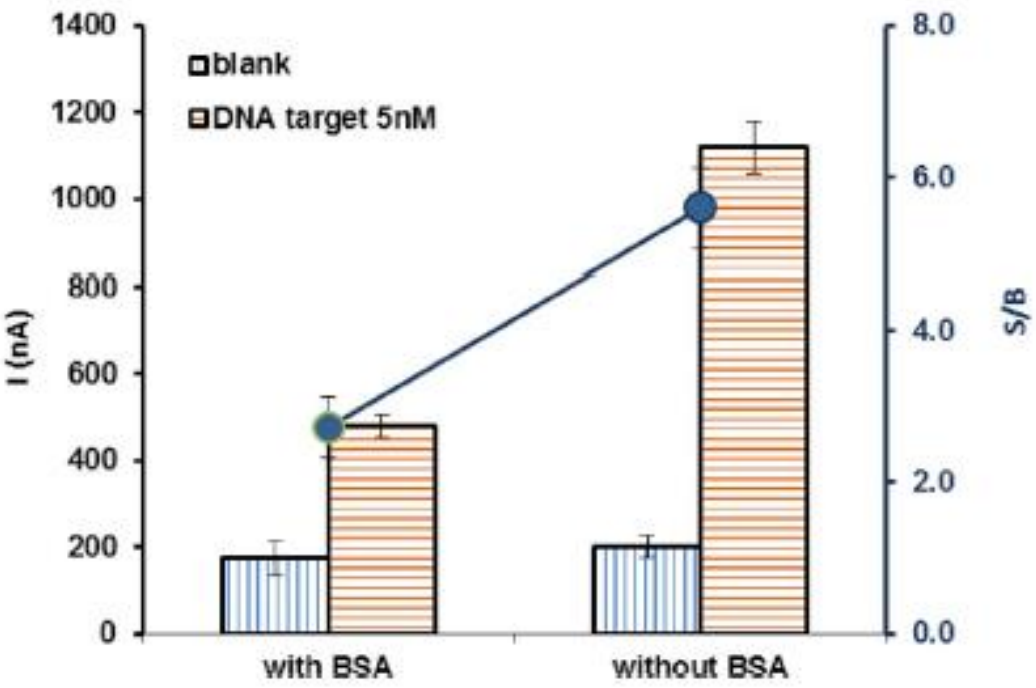

1400
1200
1000
800
600
400
200
0
I (nA)
blank
DNA target 5nM
8.0
6.0
4.0
2.0
0.0
S/B
with BSA
without BSA

Figure 4

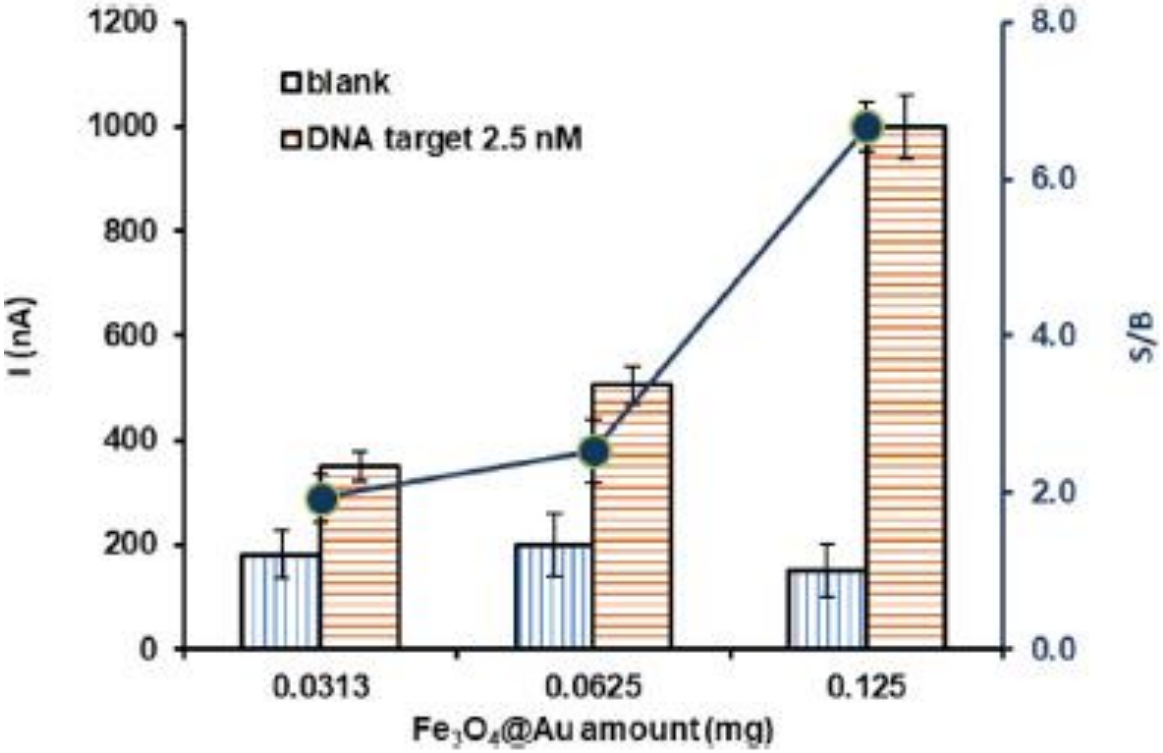
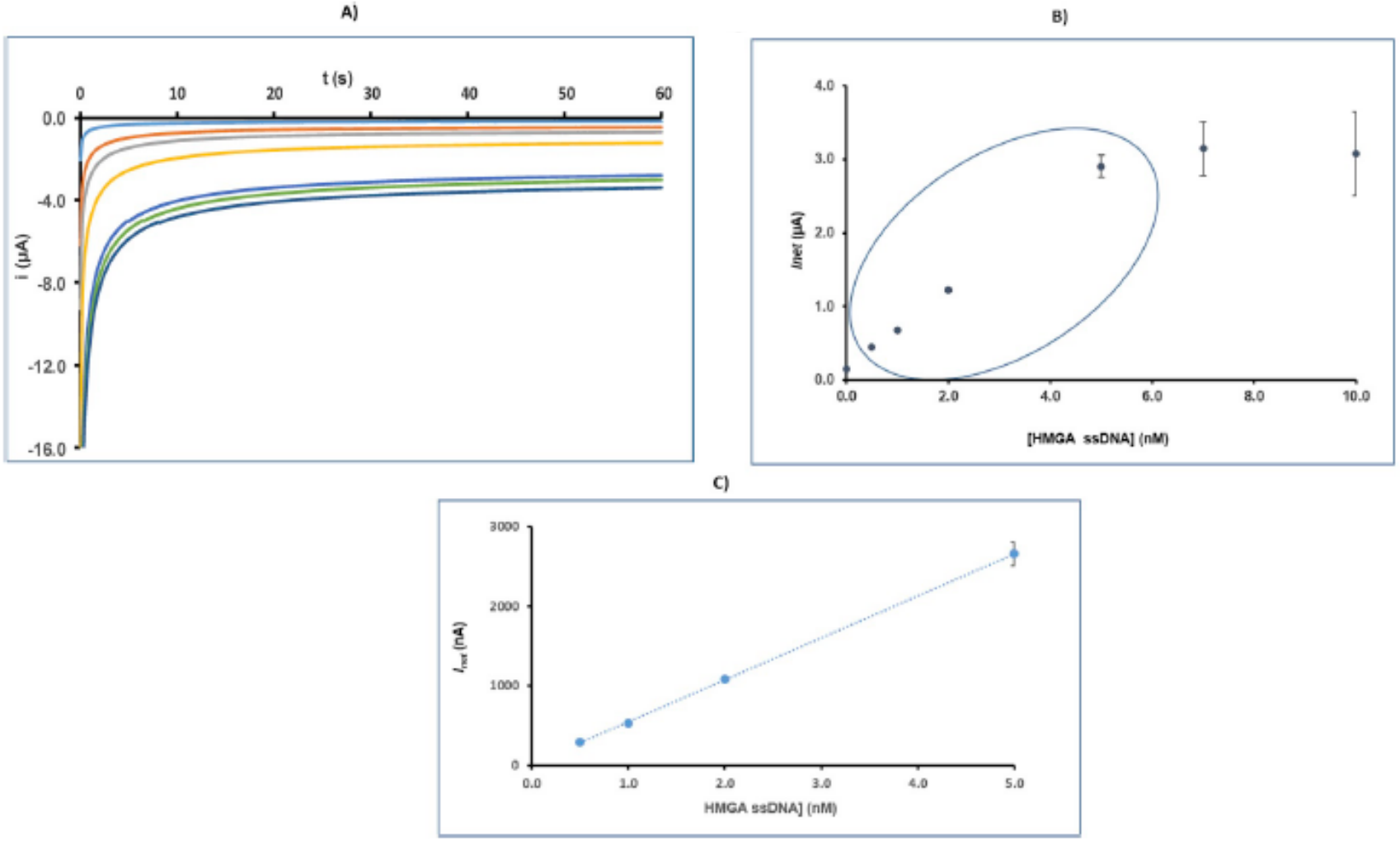

1200
1000
800
600
400
200
0
I (nA)
blank
DNA target 2.5 nM
8.0
6.0
4.0
2.0
0.0
S/B
0.0313
0.0625
0.125
Fe3O4@Au amount (mg)

Figure 5


A)
t (s)
0
10
20
30
40
50
60
0.0
-4.0
-8.0
-12.0
-16.0
i (μA)
B)
4.0
3.0
2.0
1.0
0.0
Inet (μA)
0.0
2.0
4.0
6.0
8.0
10.0
[HMGA ssDNA] (nM)
C)
3000
2000
1000
0
Inet (nA)
0.0
1.0
2.0
3.0
4.0
5.0
HMGA ssDNA] (nM)