





# Design of an Electrochemical Genosensor for *BDNF* Gene Polymorphism Sequence Detection Using an Enzymatically Labeled DNA Probe <sup>†</sup>

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**Abstract:** The *BDNF* gene is associated with high degrees of variability in antidepressant treatments. The Val66Met polymorphism is widely known as a source of this variability, warranting growing interest in genotyping patients that undergo antidepressant treatment to better suit their needs. This paper reports on an electrochemical genosensing platform, based on gold electrodes, capable of detecting this polymorphism, through the use of synthetic enzymatically labeled DNA probes for two different *BDNF* alleles. The sensor showed promising results, and its applicability to real samples is currently being tested.

**Keywords:** chronoamperometry; depression; electrochemical genosensor



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## 1. Introduction

Major depressive disorder (MDD) is an incapacitating psychiatric illness affecting around 280 million people worldwide [1]. Antidepressant drugs (Ads) have remained the main pharmacological treatment for this condition since their discovery in the 1950s [2]. Despite the good tolerability and high efficacy of current medications, along with the progressive development and introduction of new Ads throughout the decades, treatment-resistant depression, side-effects that result in treatment abandonment, and insufficient remission rates remain causes of public health concern [3,4]. In fact, 50% of patients with MDD do not respond positively to first-line treatments or respond only minimally to monotherapy [5]. Evidence suggests that neuroplasticity-related genetic factors play a significant role in the pathophysiology of depression and AD response [6,7]. *BDNF* is an important neurotrophin involved in neuroplasticity processes and other brain-related disorders [8]. The *BDNF* gene is one of the most thoroughly studied genes regarding depression and AD response [9–11]. Furthermore, the rs6265 (Val66Met) non-synonymous single nucleotide polymorphism (SNP) in this gene has been established as a source of genetic variation [12]. Pharmacogenetic studies reveal great heterogeneity regarding this SNP and AD response, with some studies showing better remission rates in Met allele carriers [13],

while others have shown better outcomes in Val/Val genotypes [14]. As a result, there is a growing interest in patient genotyping to detect specific SNPs, such as Val66Met, to predict the most favorable treatment outcomes. Current SNP detection methodologies are vast, usually being based on the polymerase-chain reaction (PCR) technique [15]. Although this approach has been extensively optimized, this methodology also presents some disadvantages such as requiring expensive equipment and highly trained personnel, as well as being time-consuming. As a result, cheaper and more flexible genotyping tools, like electrochemical genosensors, are attractive alternatives for the potentially faster and accurate detection of SNPs, namely the Val66Met SNP.

Electrochemical biosensors have been shown to present several advantages over conventional molecular biology techniques, such as low operation costs, fast experiment processing times, and portability. More specifically, electrochemical genosensors are highly specific and sensitive platforms that can be used to detect a quantitative signal derived from a hybridization event between an immobilized single-stranded DNA probe (ssDNA) on the electrode and its complementary DNA. Considering these devices as alternative and promising tools to standard methods, this work aims to report on the first disposable electrochemical genosensor device on a gold electrode for the sensitive and selective detection of the Val66Met SNP, by targeting two specific 74 bp fragments of the coding sequence: one for the Val allele genotype (target C) and the other for the Met allele genotype (target T). Additionally, electrochemical detection was carried out by employing chronoamperometric detection techniques and the sensor was successfully applied for the detection of the Val66Met SNP.

## 2. Methods

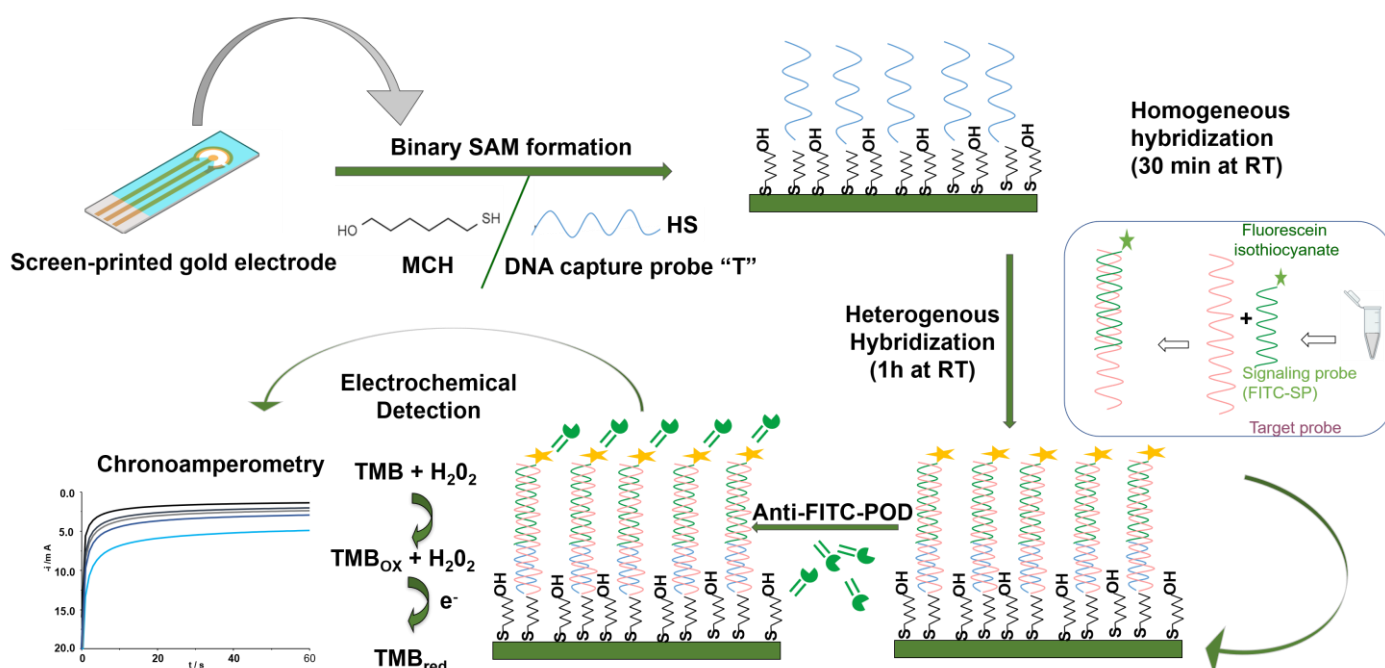
The experimental procedure consisted of the immobilization of a synthetic DNA-capture probe (39-mer), coupled with a thiol group complementary to the target T, on a disposable screen-printed gold electrode (SPGE). To enhance the sensor's general features, a sandwich hybridization format of the target C and T was carried out using a fluorescein isothiocyanate (FITC)-labeled signaling probe to which anti-fluorescein antibodies labeled with horseradish peroxidase (POD) enzymes were attached. The enzymatic increase in the signal was obtained through chronoamperometry using a POD/H<sub>2</sub>O<sub>2</sub> system.

The optimization of the developed genosensor protocol involved, briefly, four phases, a pretreatment stage, a sensing phase, the sandwich format hybridization, and the electrochemical detection, as seen in Figure 1. The pretreatment stage comprised the cleaning of the SPGEs with ethanol and Milli-Q ultrapure water.

Secondly, in the sensing phase, a self-assembled monolayer (SAM) was formed, to guarantee the favorable alignment of the SNP DNA capture probes. The SAM involves the linear capture probe and MCH immobilization onto the SPGE's working electrode.

Then, the sandwich format hybridization assay was attained through a two-step hybridization reaction. The adoption of the sandwich hybridization technique increases the selectivity of the sensor. Firstly, homogeneous hybridization occurs when the DNA-signaling probe interacts during 30 min with the DNA target. Afterward, the heterogeneous hybridization reaction is promoted, by adding the mixture of the homogeneous hybridization step to the modified SPGEs, linking the target/signaling probe solution to the immobilized DNA-capture probes.

To uncover the electrochemical signal, peroxidase in PBS/Casein solution was applied to the dsDNA SPGEs for 15 min. Lastly, commercial TMB/H<sub>2</sub>O<sub>2</sub> was added to the modified genosensor to generate an electroactive enzymatic oxidized product. Chronoamperometry at −0.1 V was used to detect the electroactive product. Three replicates were made for all measurements.



**Figure 1.** General procedure for the development of the electrochemical genosensor.

### 3. Results and Discussion

For the construction of the SNP-specific electrochemical genosensing platform, a DNA oligonucleotide sequence specific to Val66Met SNP genetic variation is needed. In our work, two 74-mer synthetic oligonucleotide sequences (one for the Val allele variant, target-C, and another for the Met allele variant, target-T) were selected.

Two complementary sequences to the DNA-target probes C and T were utilized: a 35 bp DNA-signaling probe and a 39 bp thiolated DNA-capture probe, complementary for either the target-C or target-T probes.

For the development of the SPGE-based genosensor, capable of differentiating the Val66Met genotypes, all the experiments were carried out using both DNA-target probes (Target-C and Target-T), with the DNA-capture T probe as the complementary probe.

Many of the experimental parameters, namely DNA-capture and DNA-target probe concentration, MCH concentration and incubation time, homogeneous and heterogeneous hybridization steps, anti-FITC-POD antibody concentration, and incubation time, involved in the development of the genosensor were optimized.

The experimental variables were optimized according to the largest ratio obtained between the chronoamperometric signals measured at  $-0.1$  V (vs. the Ag pseudo-reference electrode) for 0 (blank, B) and 0.2 nM of synthetic target DNA (signal, S): signal-to-blank or S/B ratio.

Table 1 summarizes all the optimized parameters, as well as the tested ranges and selected values, in the development of the genosensor.

After the optimization of the sensor, different concentrations of both DNA-target probes were tested, in order to establish a linear relationship between target concentration and current intensity. In fact, a calibration curve with a linear range of  $0.10 \text{ nmol L}^{-1}$ – $2.0 \text{ nmol L}^{-1}$  was obtained.

**Table 1.** Optimization of the developed electrochemical genosensor.

Parameters	Tested Range	Selected Value
DNA-capture probe concentration ( $\mu\text{M}$ )	0.25–1.0	0.5
MCH incubation time (min)	0–60	30
MCH concentration (mM)	0.25–1.0	0.5
Homogeneous hybridization incubation time (min)	15–60	30
Heterogeneous hybridization incubation time (min)	30–120	60
Homogeneous hybridization incubation temperature ( $^{\circ}\text{C}$ )	25; 98	25
Antibody concentration (U/mL)	0.5–2	1
Antibody incubation time (min)	15–60	15

#### 4. Conclusions

In this paper, the development of a novel, disposable electrochemical genosensor, capable of detecting the Val66Met BDNF gene polymorphism with high selectivity, is described. The genosensor was constructed through the use of an SAM interface in a sandwich format on a gold electrode. Moreover, two enzymatically labeled DNA probes of the rs6265 SNP, one for each allele, were successfully detected through chronoamperometric techniques, employing a  $\text{POD}/\text{H}_2\text{O}_2$  system, with distinct electrochemical currents achieved between the complementary probe (DNA-target probe T) and non-complementary probe (DNA-target probe C). Additionally, a calibration curve was obtained, linear in the range  $0.10 \text{ nmolL}^{-1}$ – $2.0 \text{ nmolL}^{-1}$ .

Some parameters, such as the detection limit, are currently being optimized. Additionally, the genosensor is also being tested on real samples, paired with PCR techniques, to warrant its real-world use.

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