# A Novel Photoelectrochemical Biosensor for Cystic Fibrosis Detection

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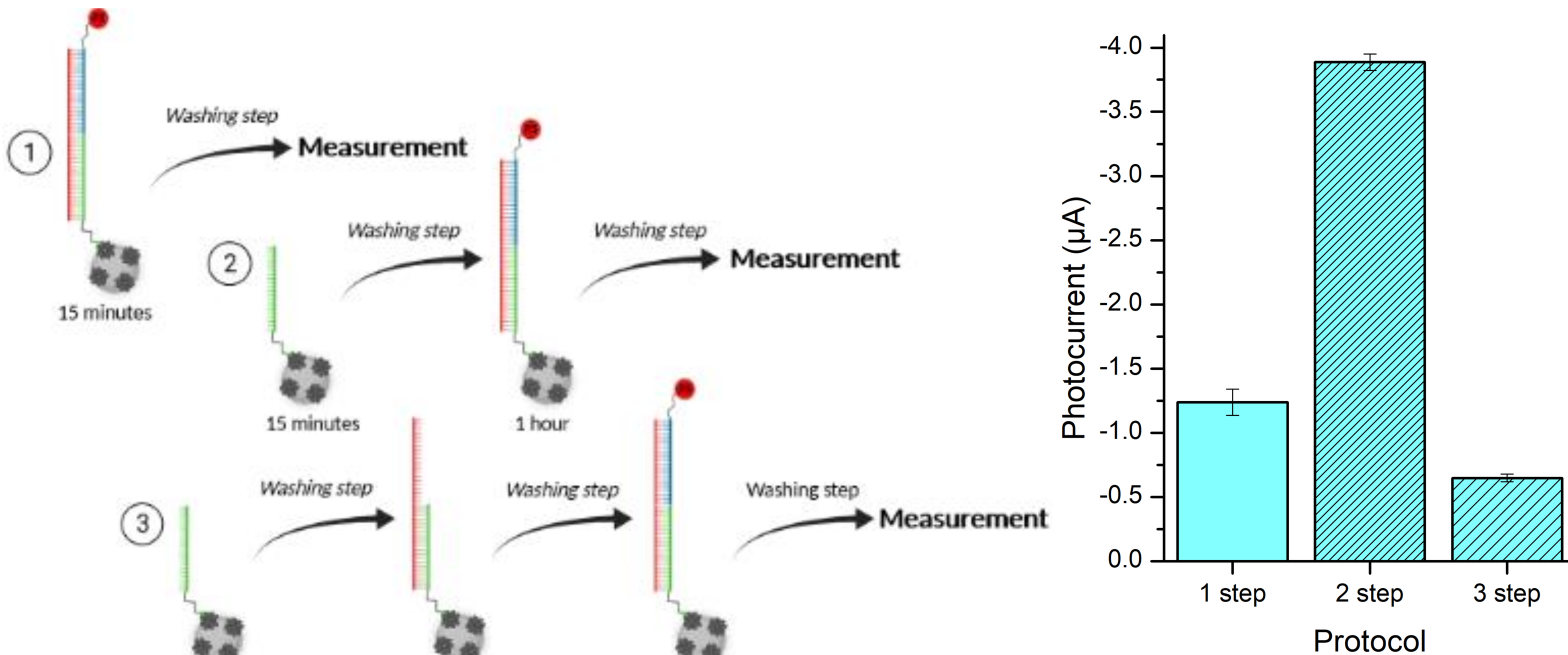
## INTRODUCTION

Nucleic acids and corresponding mutations are crucial in the diagnosis of a broad range of genetic diseases such as cystic fibrosis [1]. Different electrochemical sensors have been developed for the detection of nucleic acids to meet the demand for point-of-care diagnostics. However, these technologies present different drawbacks such as the need of a well-defined orientation of DNA strands on the electrode surface, need of trained personnel, time-consuming sample preparation [2]. This work presents a proof-of-concept photoelectrochemical (PEC) biosensor for the detection of the most common DNA mutation related to cystic fibrosis: ΔF508. This groundbreaking platform exploits a sandwich assay combining photosensitizers (PSs), that produce singlet oxygen ($^1O_2$), as a label in the detection strategy, a redox reporter (i.e. hydroquinone (HQ)) and magnetic beads, used to attract the synthetic DNA sequences close to the electrode surface, enhancing the sensitivity [3]. Using this sensor, after a first optimization of the protocol, we explore the effect of different buffers on the resulting photocurrent and we demonstrate the specific detection of the desired target (508) while avoiding unwanted interactions with random sequences.

## Optimization

### 1. Optimization of the protocol




**Scheme 2.** Illustration of the three different protocols.

**Figure 1.** Protocol influence on the final photocurrent.

*After investigating the difference on the final photocurrent between the three protocol, the one including two incubations was selected: i) 15 minutes incubation between magnetic beads functionalized with streptavidin and capture probe; followed by ii) 1 hour incubation adding target and detection probe with the resulting hybridization and formation of the sandwich assay. After a washing step in a magnetic rack, the beads were transferred at first in the measuring buffer (0.1M KCl, 0.01M $KH_2PO_4$ at pH7), with 1 mM HQ, and then on the working electrode.*

### 2. Optimization of the second incubation time

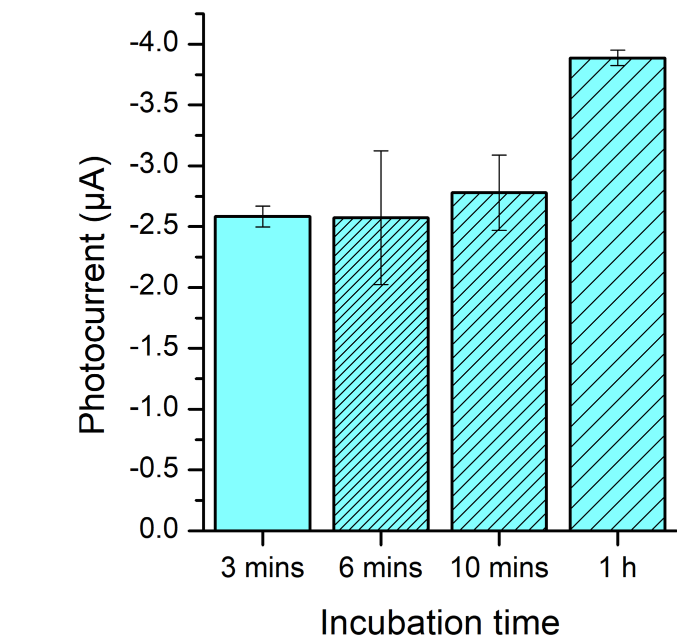



**Figure 2.** Influence of the second incubation time on the photocurrent. Lower incubation times don't affect the hybridization efficiency of the probes, resulting in a similar photocurrent.

### 3. Influence of the ionic strength of the buffer

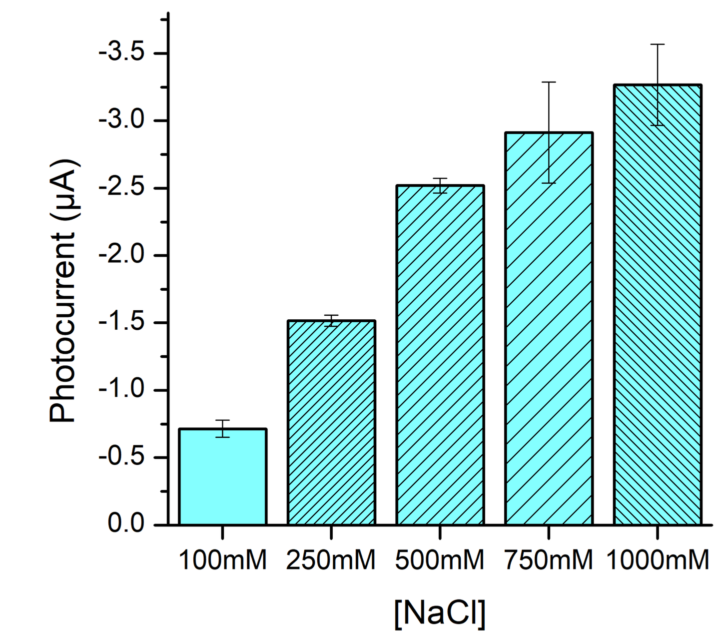
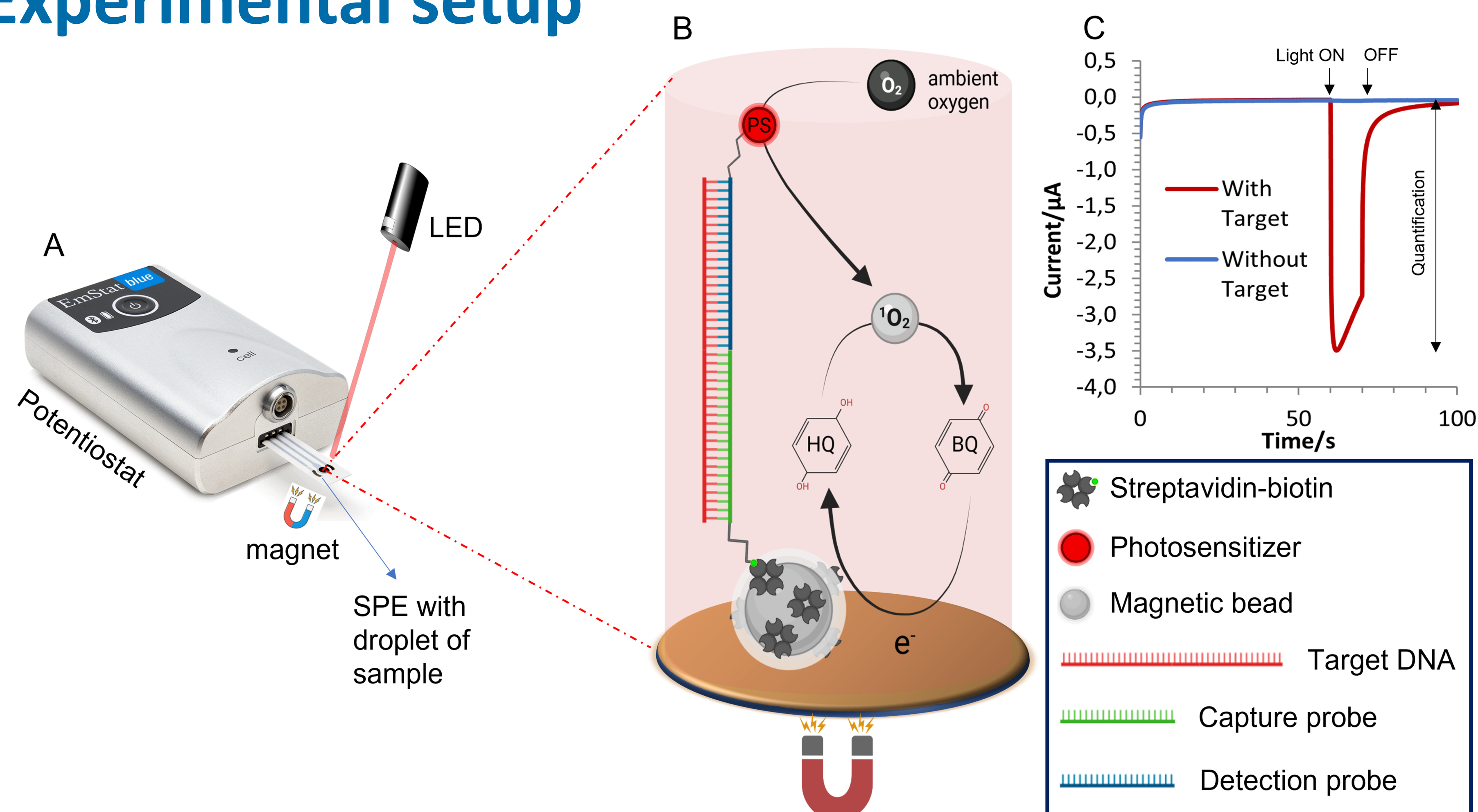



**Figure 3.** Effect of the ionic strength on the photocurrent. Hybridization buffers (5mM Tris-HCl, 0.5mM EDTA, 1M NaCl, 0.05% Tween 20 at pH7.5) with 100, 250, 500, 750 and 1000 mM NaCl were tested.

## CONCLUSION

- Proof-of-concept of a novel bio-inspired PEC platform for detection of cystic fibrosis is presented.
- The protocol was successfully optimized allowing fast (< 1 hour) analysis.
- The PEC platform is highly specific and selective, discriminating between ΔF508 and random sequences present in solution.

## Experimental setup




**Scheme 1.** Schematic representation of the experimental setup.

*As a platform for the detection strategy, disposable screen-printed electrodes (SPE) connected to a potentiostat were used. This PEC biosensor employ a sandwich assay for the detection of ΔF508 mutation. The capture probe (green), attached to magnetic beads via a streptavidin-biotin interaction, is complementary to part of the target DNA (red). The detection strand (blue) (which contains a PS as label) complements the overhang of the nucleic acid. Upon recognition of the target (red) and illumination of the SPE surface by a LED, $^1O_2$ is produced, acting as a strong oxidant. As a result, the redox reporter present in the solution (i.e. HQ) will be oxidized, forming benzoquinone (BQ), followed by electrochemical regeneration of the reporter (HQ) at the electrode surface by applying a potential (ca. -0.2 V versus silver reference electrode). This completes the electrocatalytic redox cycle resulting in an amplified PEC response. Since the analytical signal is only triggered by light, a background can be clearly distinguished by turning the light on and off.*

*Amperometric responses (current obtained at a given potential) have been recorded to collect the reductive photocurrents.*

## Validation: negative controls and discrimination with random sequences

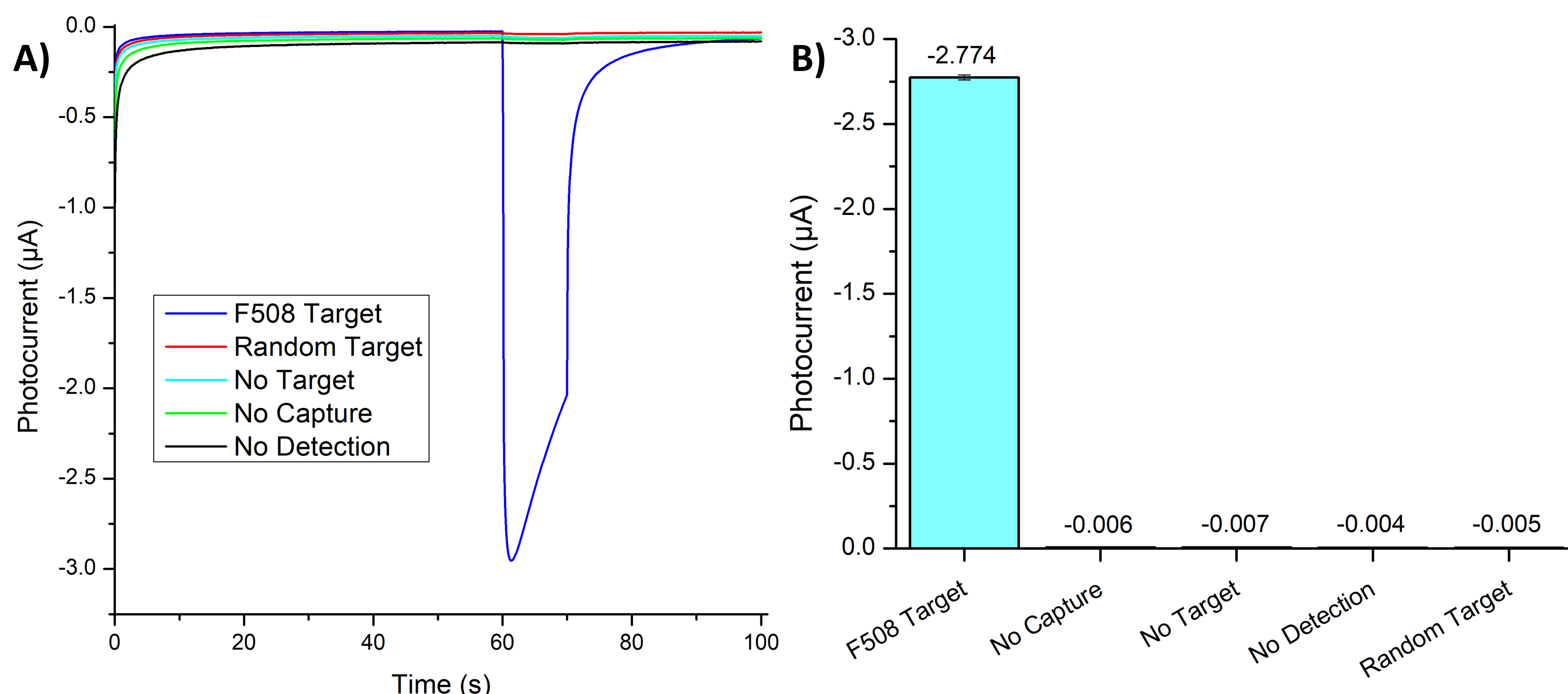



**Figure 4.** PEC measurements (in triplicate) obtained in the measuring buffer, containing 1 mM HQ: **A)** chronoamperograms under LED illumination; and **B)** histogram with the corresponding photocurrent responses.

The selectivity and specificity of the PEC biosensor was demonstrated by performing four different negative control experiments: i) absence of capture probe; ii) absence of target sequence; iii) absence of detection probe; and iv) presence of a random target, non complementary with both capture and detection probe. Only when the ΔF508 target is present a higher photocurrent has been recorded, while for all the other negative controls the photocurrent is negligible.

## REFERENCES

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