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Electrochemical Detection of *Salmonella* via On-surface Isothermal Amplification of its Genetic Material onto Highly Stable and Reproducible Indium Tin Oxide Platforms

Susana Barreda-García [1,*], Rebeca Miranda-Castro [1], Noemí de-los-Santos-Álvarez [1] and María Jesús Lobo Castañón [1]

[1] Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo, Julián Clavería 8, 33006, Oviedo.

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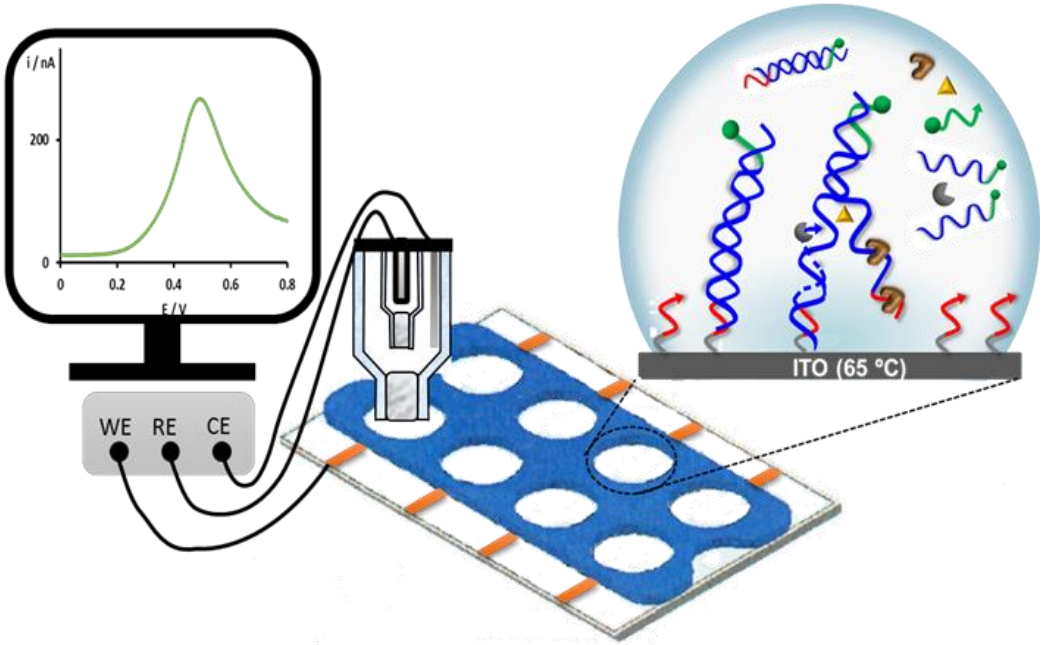
* Corresponding author: susanbarreda@gmail.com

Electrochemical Detection of *Salmonella* via On-surface Isothermal Amplification of its Genetic Material onto Highly Stable and Reproducible Indium Tin Oxide Platforms

Graphical Abstract



Abstract: *Salmonella* represents one of the major causes of foodborne diseases in humans, in addition to provoke important economic losses in the agri-food sector worldwide. Therefore, the surveillance and control of this human pathogenic bacterium in foodstuffs and biological fluids are necessary in order to prevent and diagnose the disease. Traditional culture-based methods require 5 to 6 days to obtain a definitive result. As a faster alternative, herein we report the integration of a nucleic acid-based sensor and an isothermal DNA amplification technique, helicase-dependent amplification (HDA), onto indium tin oxide (ITO) surfaces for the electrochemical/optical detection of a DNA sequence specific for the *typ*A gene of *Salmonella*. DNA amplification process occurs at 65 ˚C with the reverse primer covalently bound to the ITO surface, whereas forward fluorescein-tagged primer is incorporated in solution. As a result of the isothermal elongation step, fluorescein-tagged DNA duplexes are attached to the ITO surface. Then, an anti-fluorescein-enzyme conjugate is incorporated for subsequent detection of the enzymatic product. This developed integrated sensing platform allows the detection of *Salmonella* down to 100 genomes in just over 2 hours [1] without need of high-end benchtop instrumentation. Furthermore, the sensing phase maintains its performance even after 9 months storage.

[1] S. Barreda-García, R. Miranda-Castro, N. de-los-Santos-Álvarez, A.J. Miranda-Ordieres, M.J. Lobo-Castañón, *Chem. Comm*. 53 (2017) 9721-9724.

Keywords: *Salmonella;* genetic material; helicase-dependent amplification

Introduction

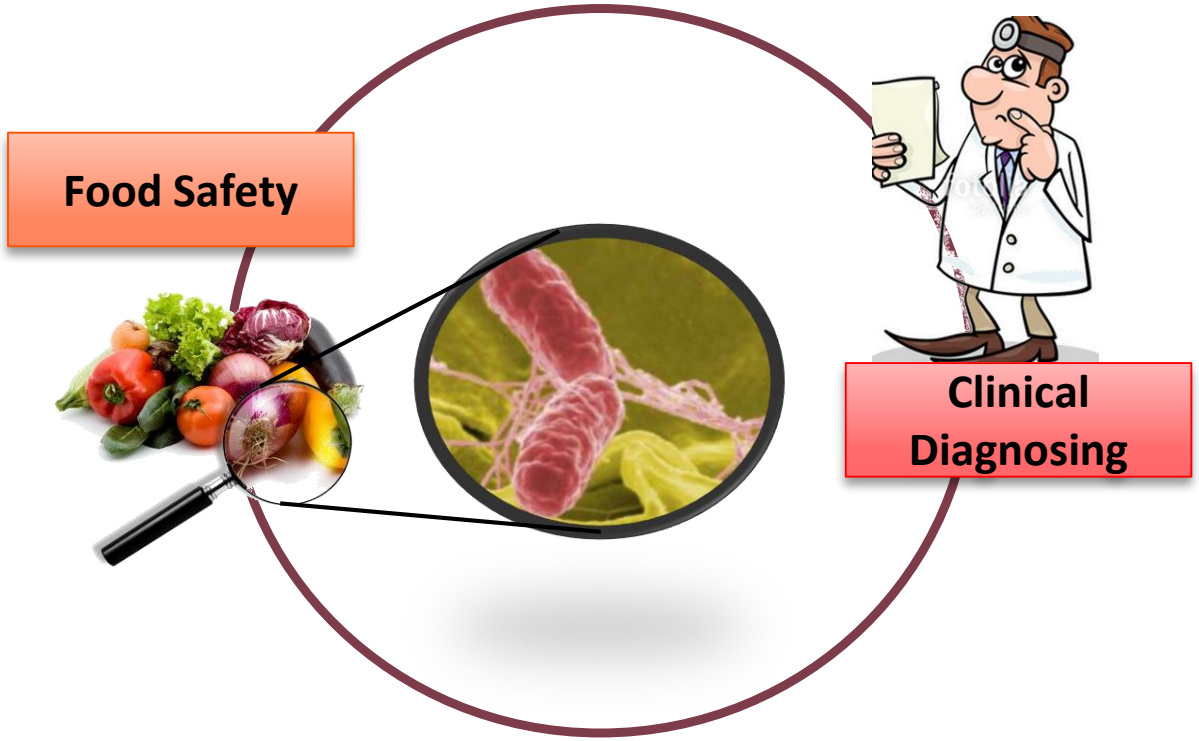
Salmonella spp. pathogens

constitutes one of the major causes of foodborne diseases

in humans worldwide.

Introduction

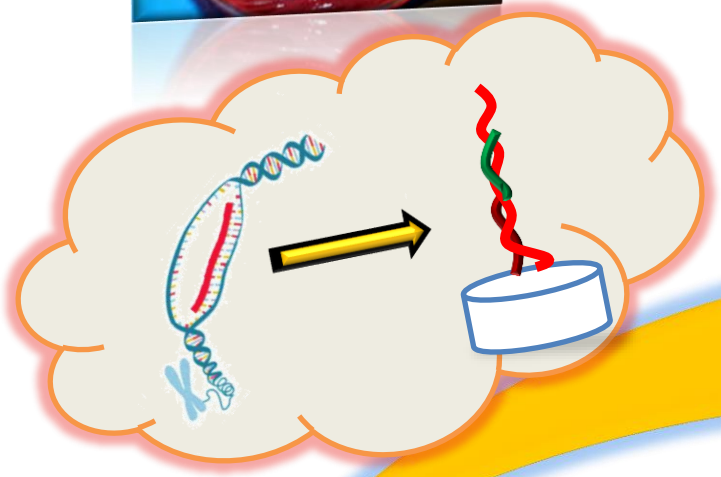
The surveillance and control of this human pathogenic bacterium in foodstuffs and biological fluids are necessary in order to prevent and diagnose the disease.



Introduction

Traditional microbiological methods for Salmonella detection in food require 5 to 6 working days to obtain a positive result

Decentralized genetic testing



Amplification

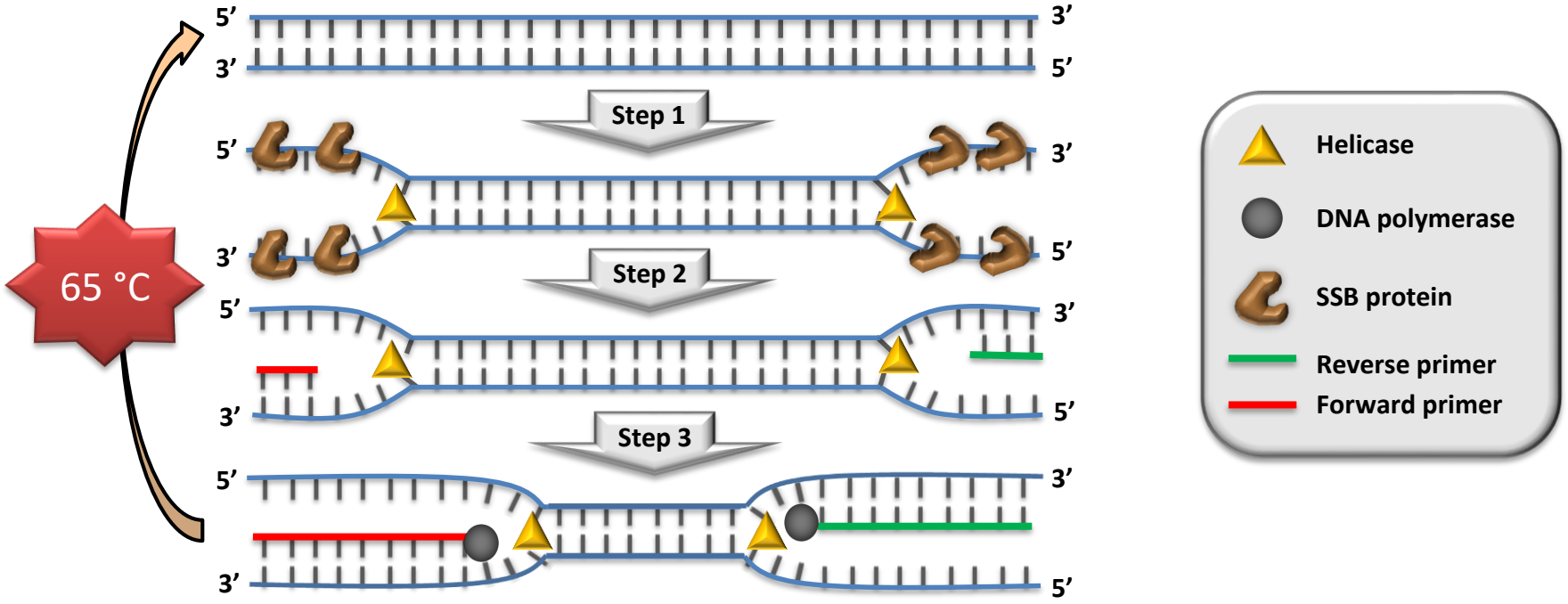
Hybridization

Detection

Isothermal nucleic acid amplification + sensing phase thermally stable

Introduction

HELICASE DEPENDENT AMPLIFICATION (HDA)



Biosens. Bieolectron. 68 (2015) 122-128
Anal.Chem. 87 (2015) 8547-8554
Anal. Bioanal. Chem. 408 (2016) 8603-8610

HDA + electrochemical detection matches real-time PCR

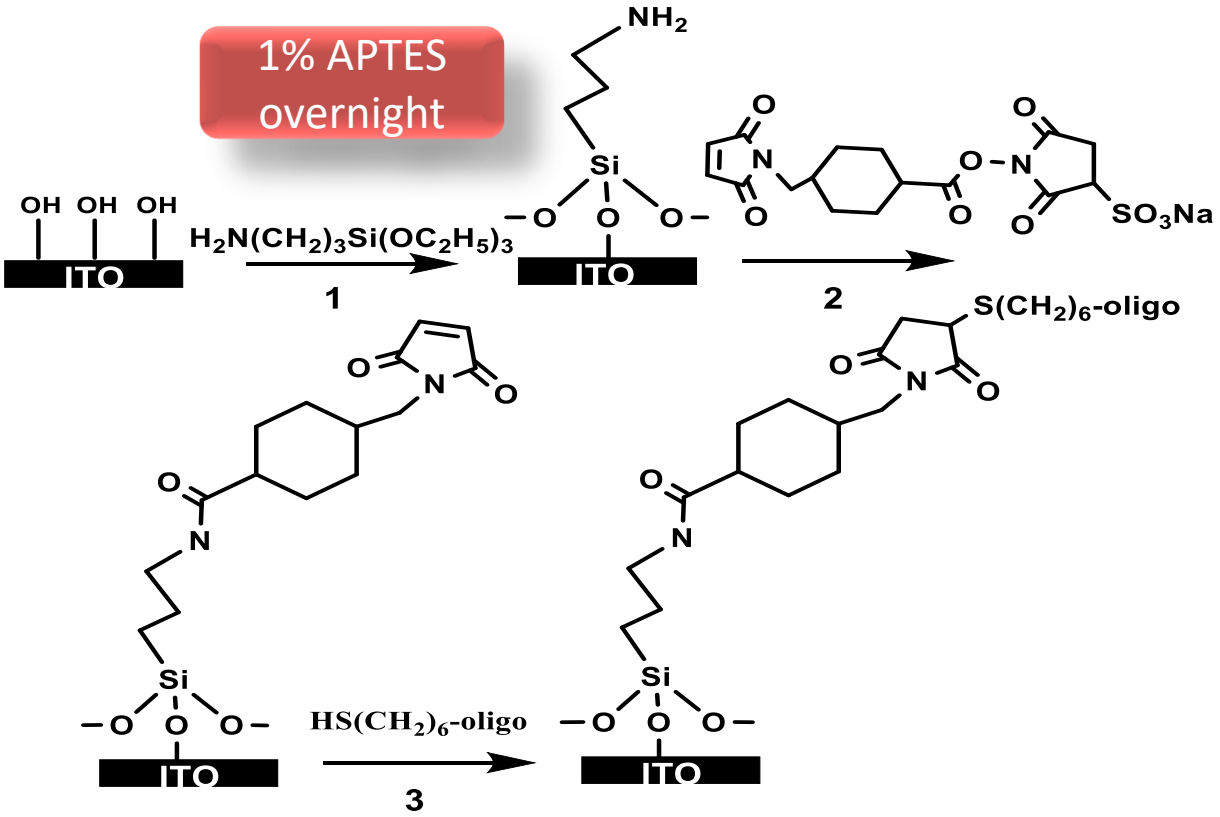
Introduction

Objective

To develop a simple and robust platform for the quantification of DNA sequences specific of *Salmonella* by integrating *on-surface* HDA and electrochemical detection at indium-tin oxide (ITO) surfaces

- Optimizing the sensing phase formation and the hybridization assay
- Evaluating the genosensor response and stability
- Integrating on-surface HDA and electrochemical detection

ITO modification with oligonucleotides



1. (3-aminopropyl)triethoxysilane (APTES)
2. Sulfosuccinimidyl 4-(N-maleimidemethyl)cyclohexane-1-carboxylate
3. Thiolated DNA capture probe (25 mer)

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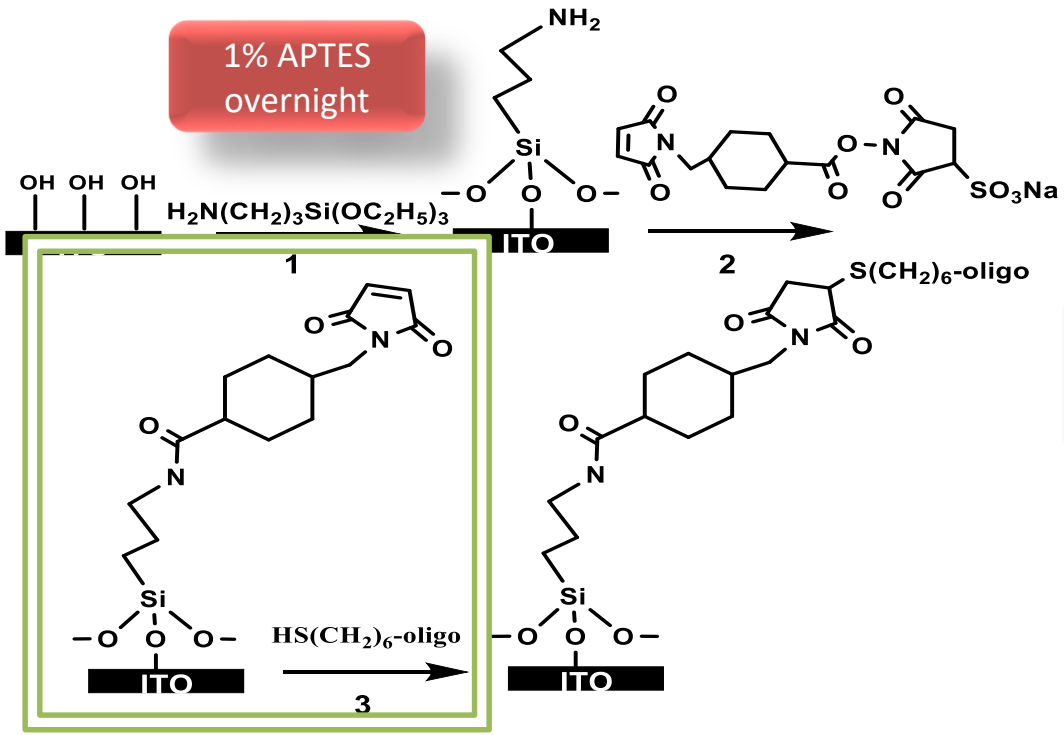
ITO modification with oligonucleotides



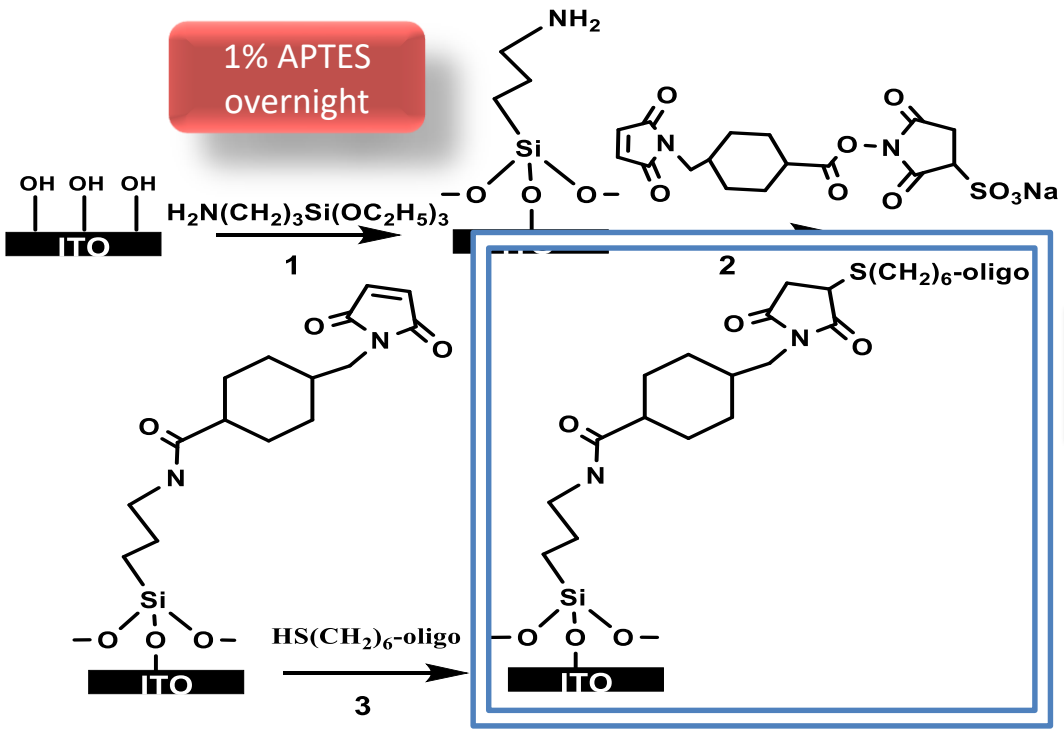
Quantification of Active Sites

Cyclic voltammetry after linking 6-(ferrocenyl)hexanethiol

$\Gamma_{active\ sites} = 1.7\times10^{14}$ molecules/cm^2

1. (3-aminopropyl)triethoxysilane (APTES)
2. Sulfosuccinimidyl 4-(N-maleimidemethyl)cyclohexane-1-carboxylate
3. Thiolated DNA capture probe (25 mer)

ITO modification with oligonucleotides



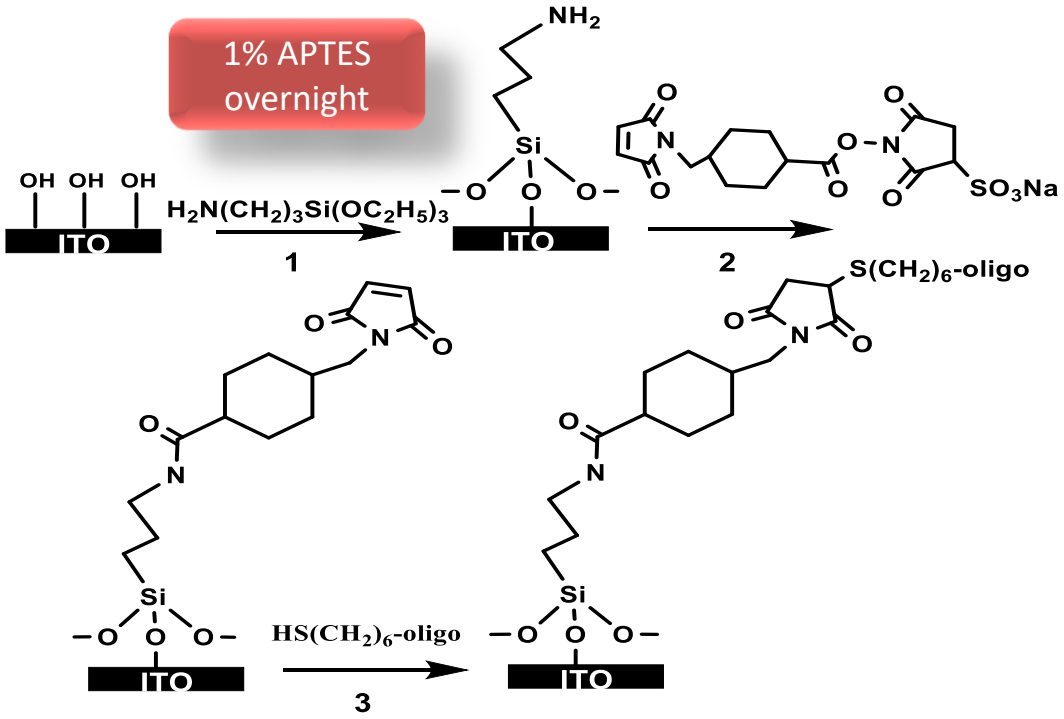
Quantification of bound ssDNA

Chronocoulometry after interacting with $Ru(NH_3)_6^{3+}$

$\Gamma_{ssDNA} = 2.5\times10^{12}$ molecules/cm^2

1. (3-aminopropyl)triethoxysilane (APTES)
2. Sulfosuccinimidyl 4-(N-maleimidemethyl)cyclohexane-1-carboxylate
3. Thiolated DNA capture probe (25 mer)

ITO modification with oligonucleotides



$\Gamma_{active\ sites} = 1.7\times10^{14}$ molecules/cm^2

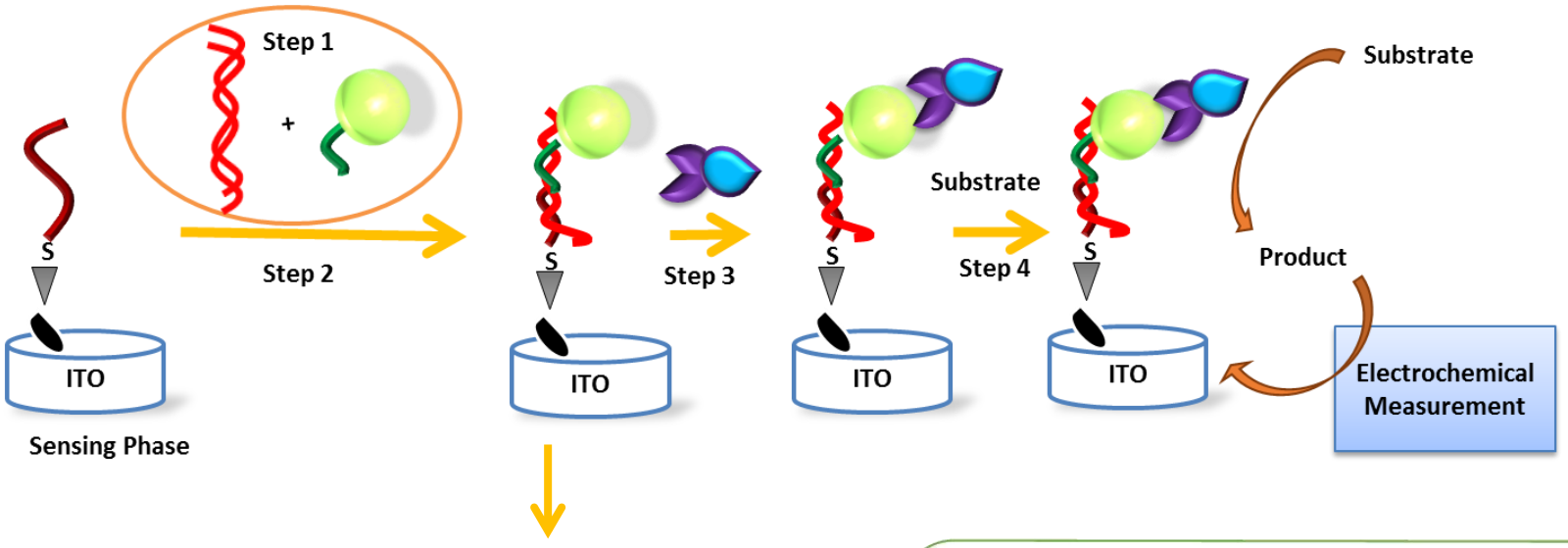
1.5 %

$\Gamma_{ssDNA} = 2.5\times10^{12}$ molecules/cm^2

Medium surface density
Adequate DNA spacing
for hybridization

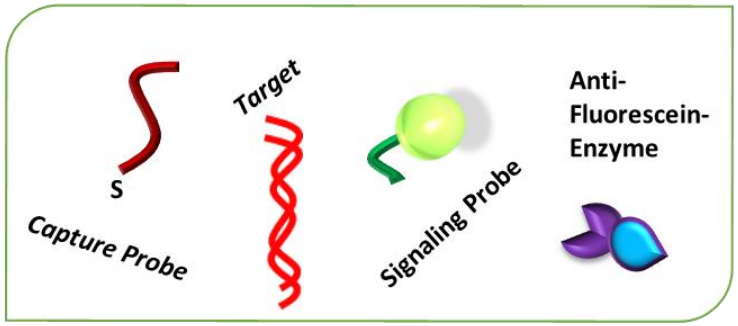
1. (3-aminopropyl)triethoxysilane (APTES)
2. Sulfosuccinimidyl 4-(N-maleimidemethyl)cyclohexane-1-carboxylate
3. Thiolated DNA capture probe (25 mer)

Genosensor for *Salmonella*



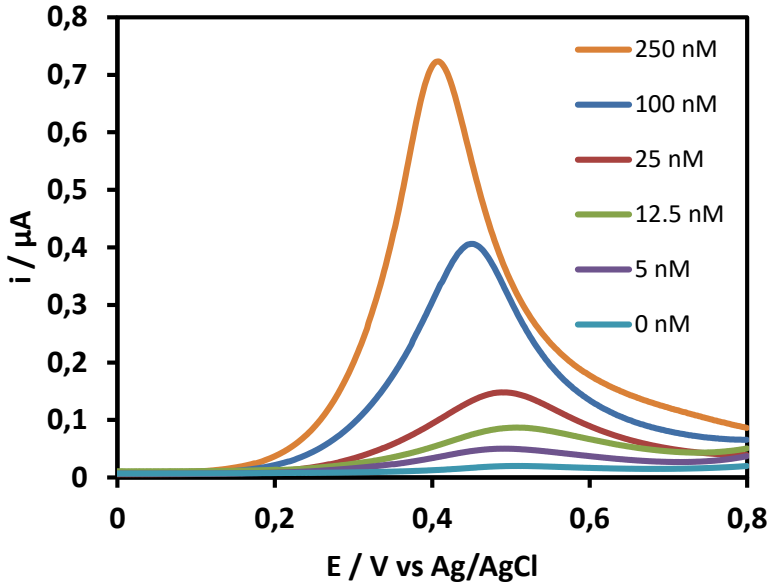
Fluorescence Spectroscopy

Hybridization efficiency: 50%

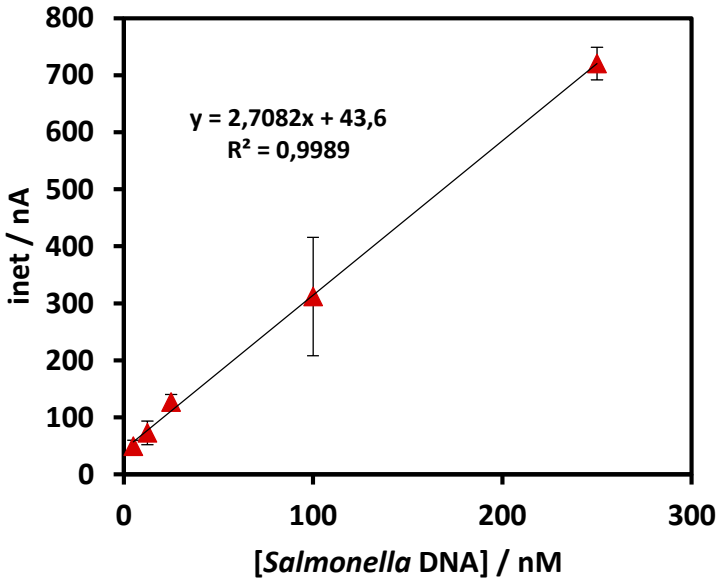


Genosensor for *Salmonella*

Voltammograms



Sensor Response to the Concentration of *Salmonella*



LOD : 2.5 nM

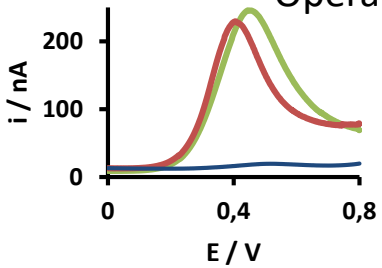
Linear range: 5 to 250 nM

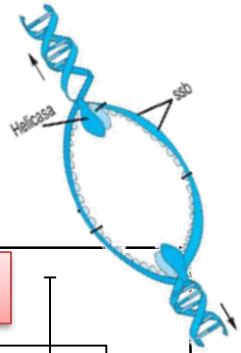
Reproducibility: 10 % (5 nM)

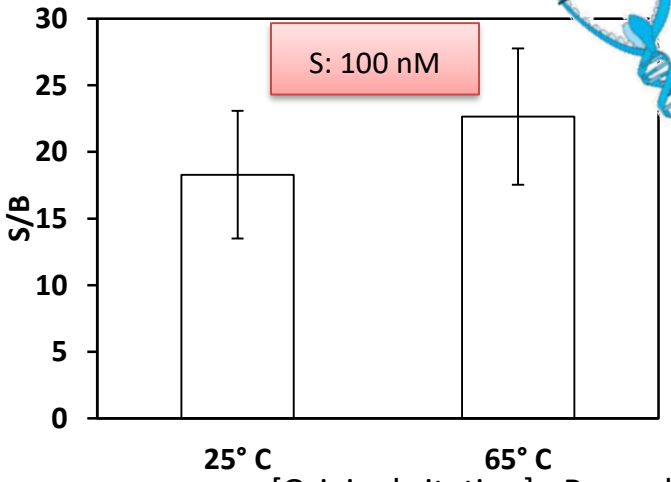
Genosensor stability

Thermal Stability

Operational Conditions



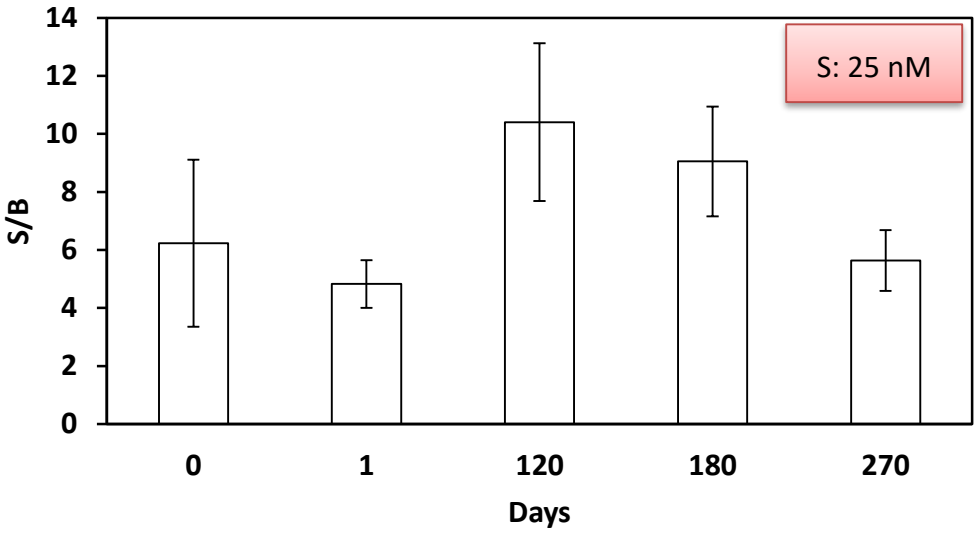




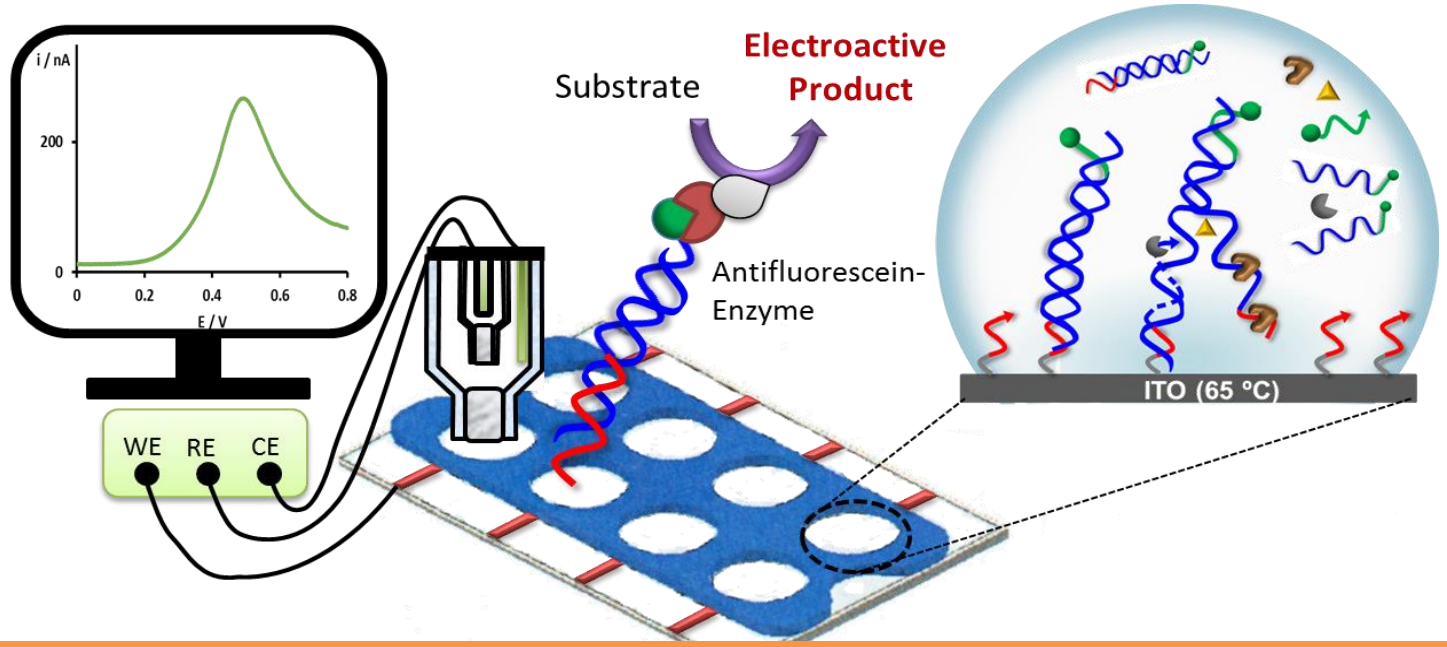
Storage Stability

Dry/ 4 ˚C

2.5% (BSA + Glucose)



On-surface HDA amplification



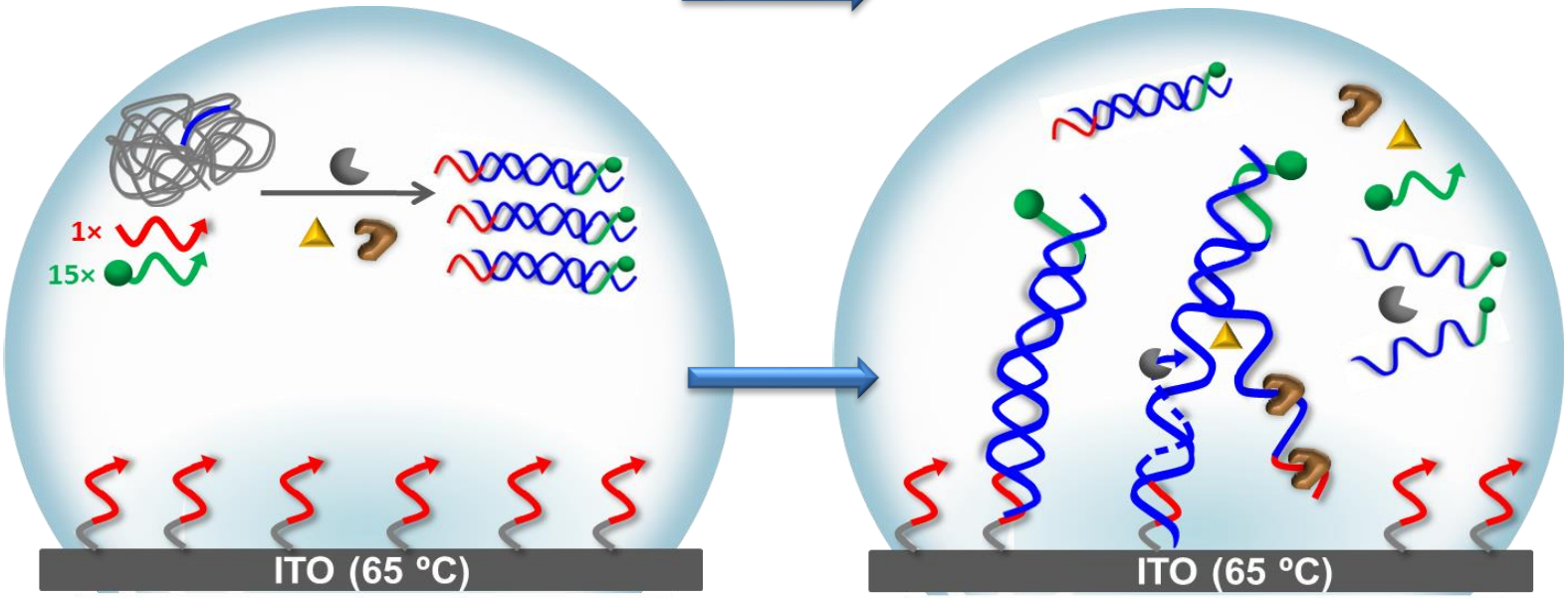
Function	Name	Sequence (5'→3')
Forward primer	6-FAM-FP_2	6-FAM-GGT CTG CTG TAC TCC ACC TTC AGC
Reverse Primer (solution)	RP	TTG GAG ATC AGT ACG CCG TTC T
Reverse primer (immobilized)	HS-T_{10}-RP_2	HS-C6-$(T)_{10}$ TTG GAG ATC AGT ACG CCG TTC TGA CGC T

On-surface HDA amplification

1. Asymmetric genome amplification in solution

RP depletion

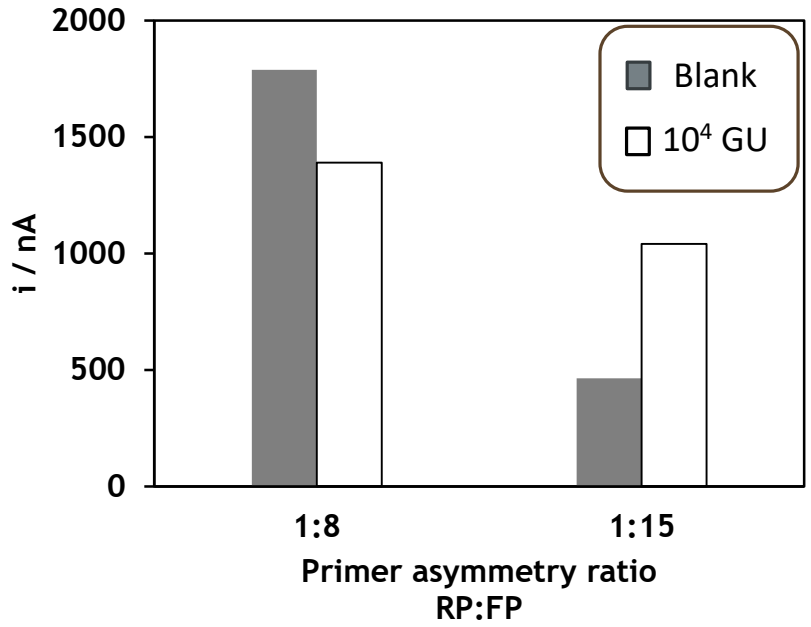
2. Elongation of anchored RP using as target 6-FAM-shortened amplicons



Salmonella genome
helicase
SSB protein
polymerase
RP
6-FAM-FP$_2$
HS-T$_{10}$-RP$_2$
86bp 6-FAM-amplicon

On-surface HDA amplification optimization

Asymmetry Ratio
for primers in solution



Minimizing non-specific amplification

FP: 75 nM

RP: lower amount

On-surface amplification for 90 min

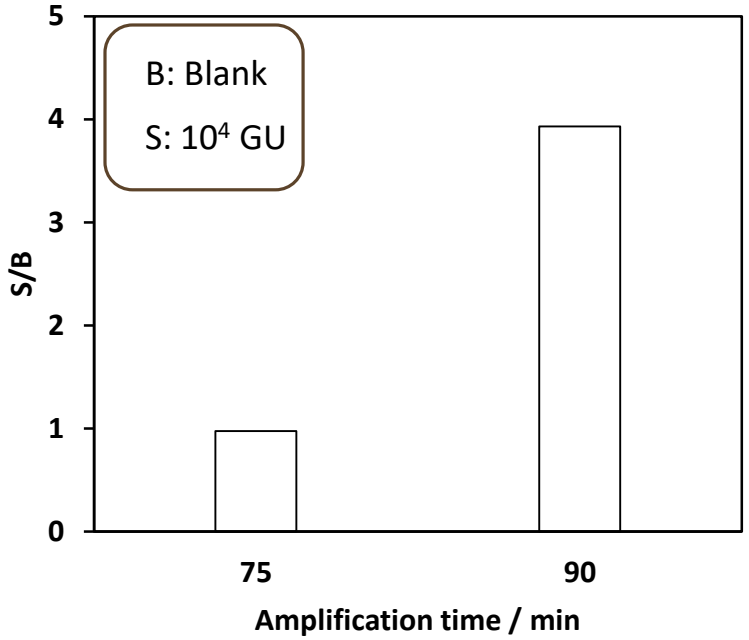
On-surface HDA amplification optimization

Amplification time

RP:FP / 1:15

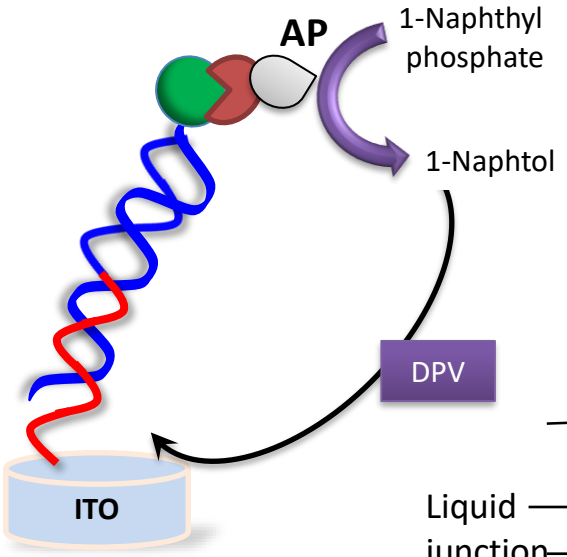
FP: 75 nM

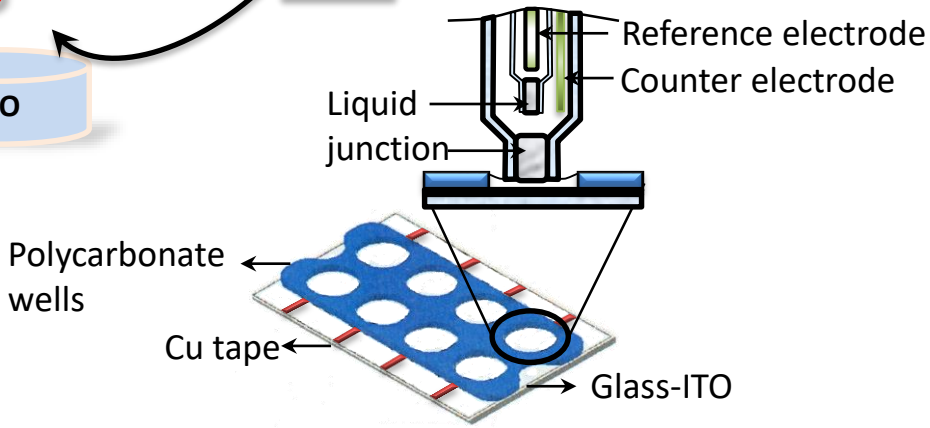
RP: 5 nM



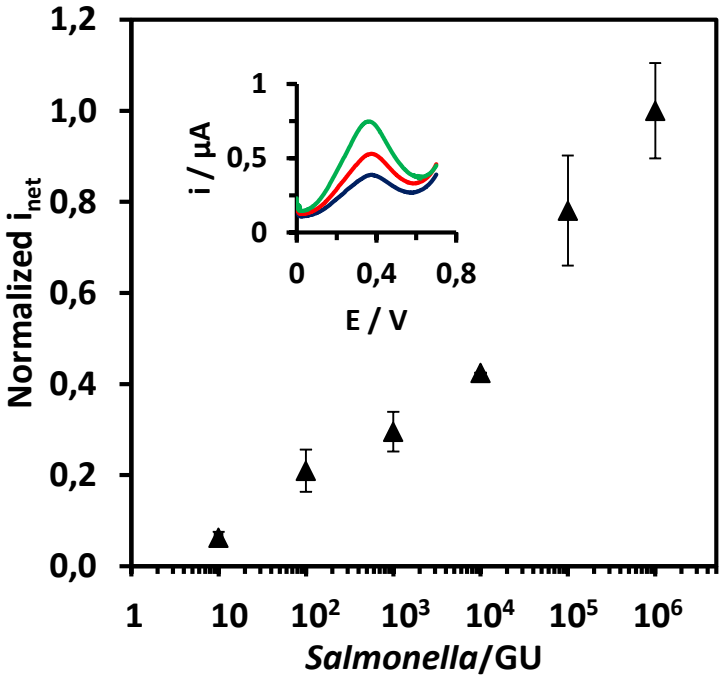
Analytical performance

Electrochemical Detection





LOD real-time PCR = 100 GU



LOD = 10 GU

RSD = 20 %

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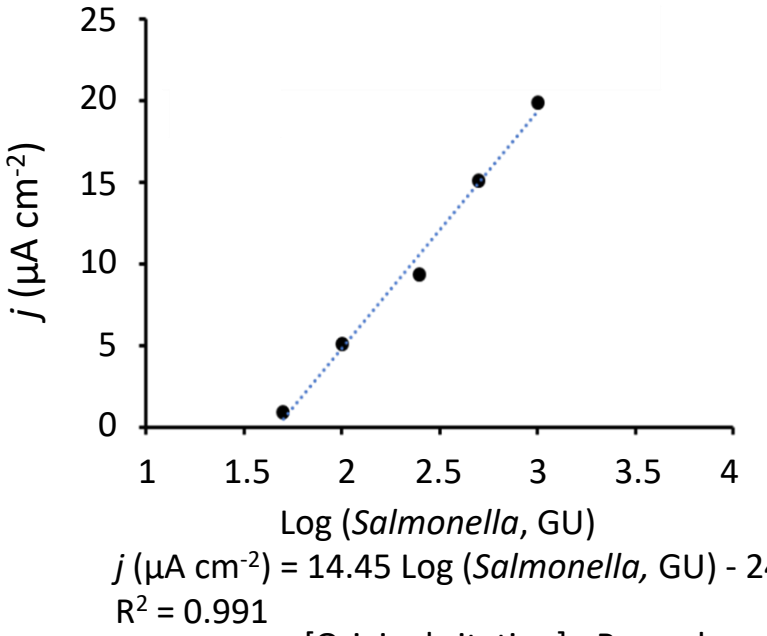
Analytical performance

Homogeneous amplification + genosensor

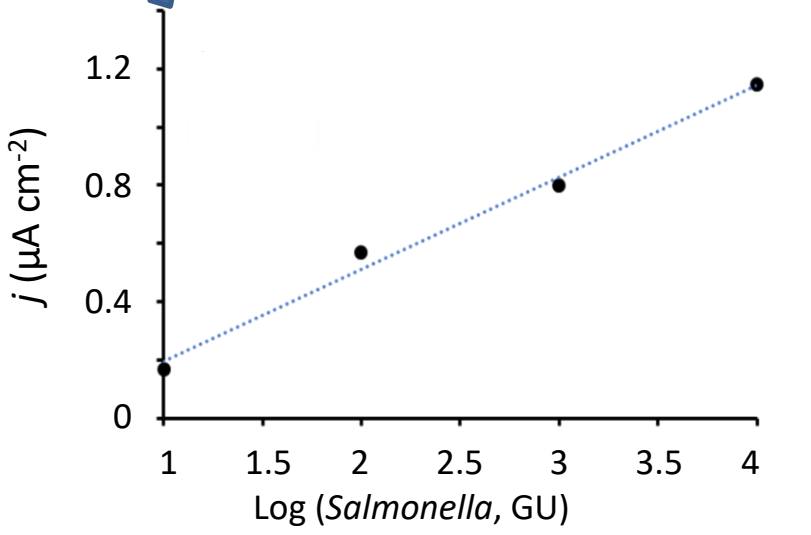
On-surface amplification

45 times higher slope → Highest efficiency



j (µA cm^{-2}) = 14.45 Log (*Salmonella,* GU) - 24

R^2 = 0.991



j (µA cm^{-2}) = 0.32 Log (*Salmonella,* GU) – 0.12

R^2 = 0.991

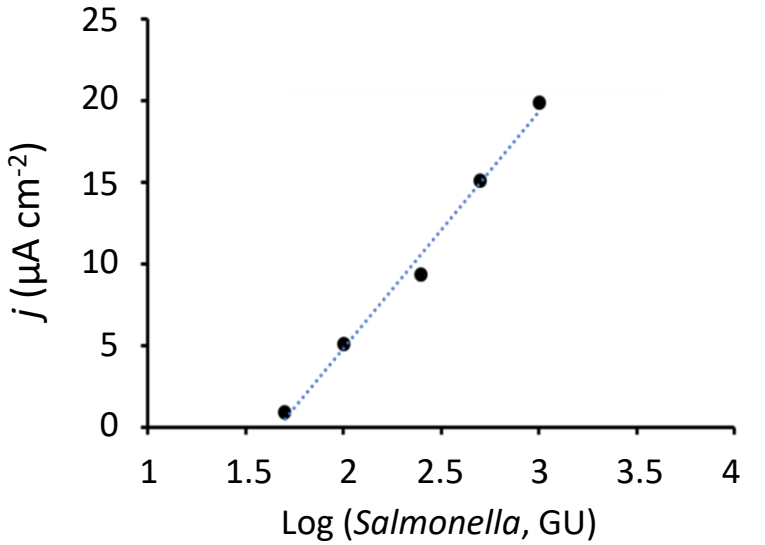
Analytical performance

Homogeneous amplification + genosensor

On-surface amplification

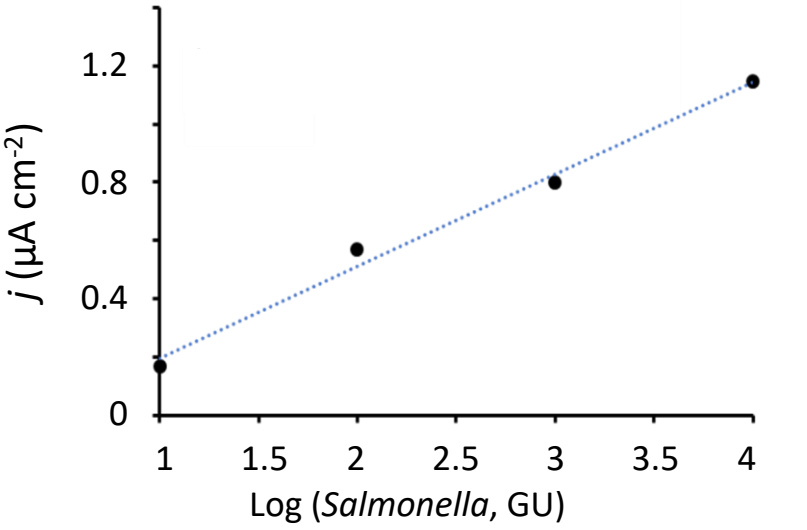
Reduction of non-specific amplification → **Better detectability than real-time PCR**

But…



j (µA cm^{-2}) = 14.45 Log (*Salmonella,* GU) - 24

R^2 = 0.991

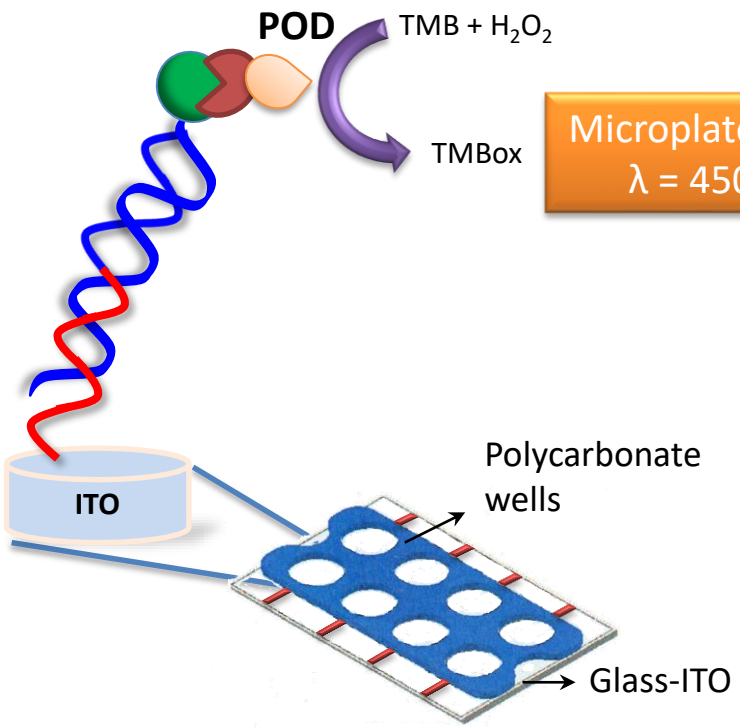


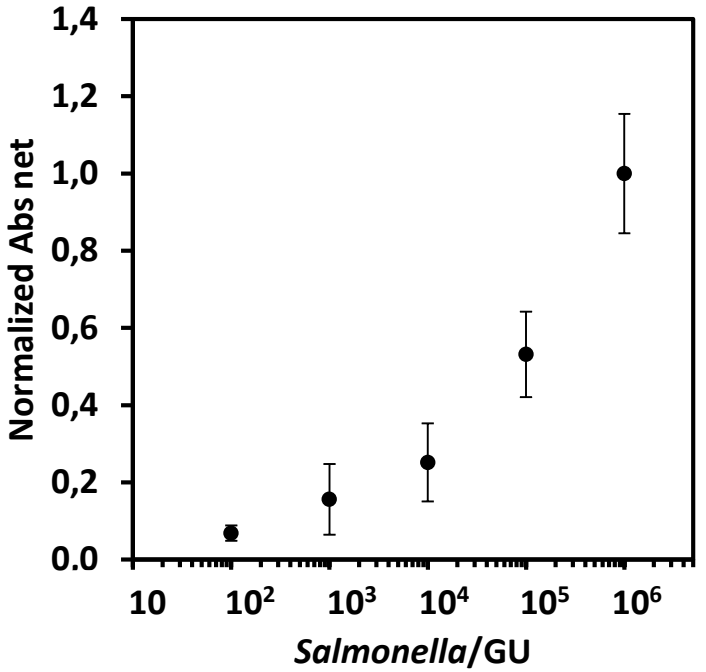
j (µA cm^{-2}) = 0.32 Log (*Salmonella,* GU) – 0.12

R^2 = 0.991

Analytical performance

Optical Detection





LOD = 100 GU

RSD = 30%

Conclusions

- A platform with wide applicability, useful for the quantification of genomic DNA by thiolated oligonucleotides (reverse primer) covalently immobilized on ITO surfaces
- It shows excellent thermal and storage stability
- Amplification and detection are performed on the same platform without thermal cycling
- Electrochemical detection of HDA amplification on ITO electrodes has better limit of detection than optical detection of the same assay
- Better detectability than real-time PCR, offering an excellent option for genetic detection at the point-of-need

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