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2-17 November 2020

Improving the Analytical Performance of Weak Aptamers: DNA Isothermal Amplification Approaches

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OUTLINE

1. INTRODUCTION

- Aptamers
- Isothermal DNA amplification

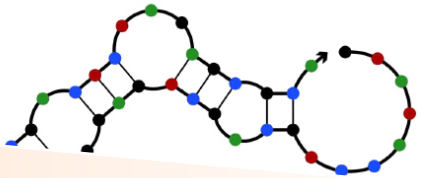
2. OBJECTIVES

3. EXPERIMENTAL SECTION

- Rolling circle amplification
- Terminal deoxynucleotidyl transferase

4. CONCLUSIONS

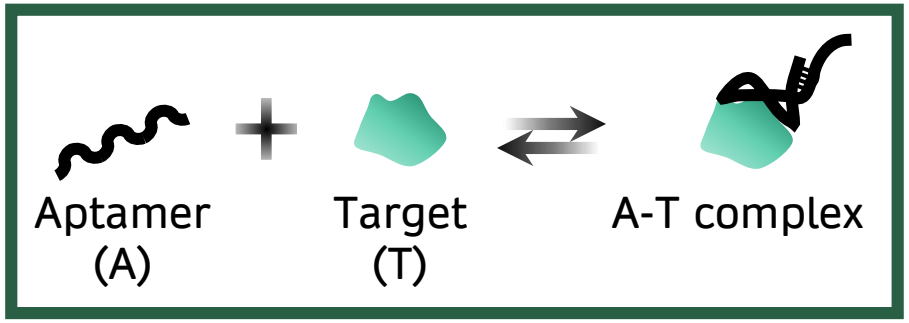
INTRODUCTION
APTAMERS



Aptamers, also known as **chemical antibodies**, are short, synthetic, single-stranded **DNA or RNA oligonucleotides** able to adopt special 3D structures that allow them to bind with **high specificty** almost **any type of target** (ions, proteins, cells…), mimicking the antigen-antibody reaction.

Characteristics

- Chemical and thermal stability
- Chemical synthesis with low batch-to-batch variations
- Easy to label with marker molecules (fluorophores, enzymes…)
- Easy to manipulate with molecular biology tools (polymerases, ligases…)

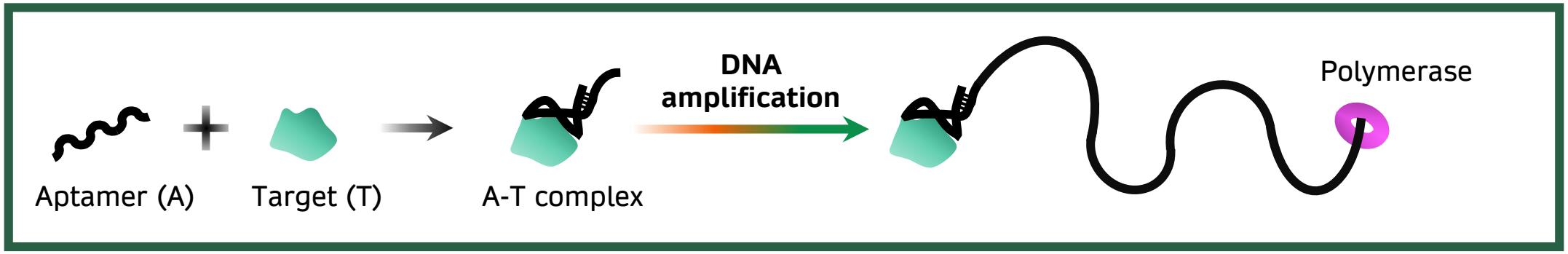


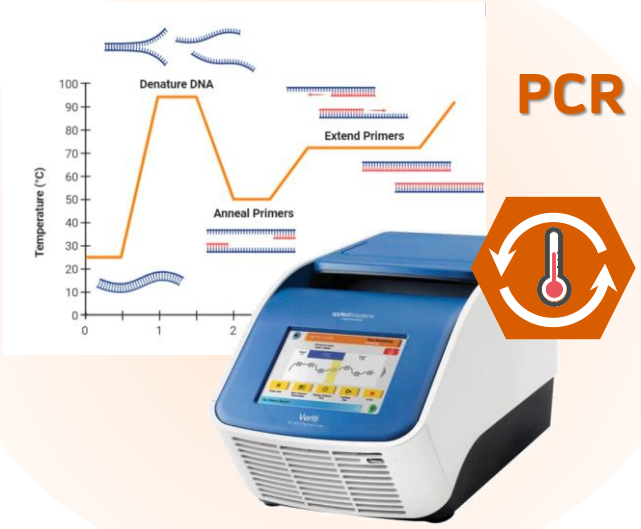
Equilibrium dissociation constant, K_d → pM-nM

$$K_a = \frac{[A\text{-}T]}{[A]\,[T]} = \frac{1}{K_d}$$

INTRODUCTION

ISOTHERMAL DNA AMPLIFICATION





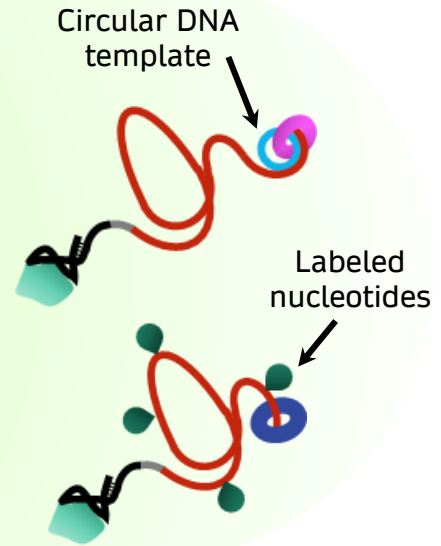
PCR *vs* **Isothermal DNA amplification**

- No thermal cycling required
- More eligible to miniaturization

CONST

Rolling Circle Amplification (RCA)

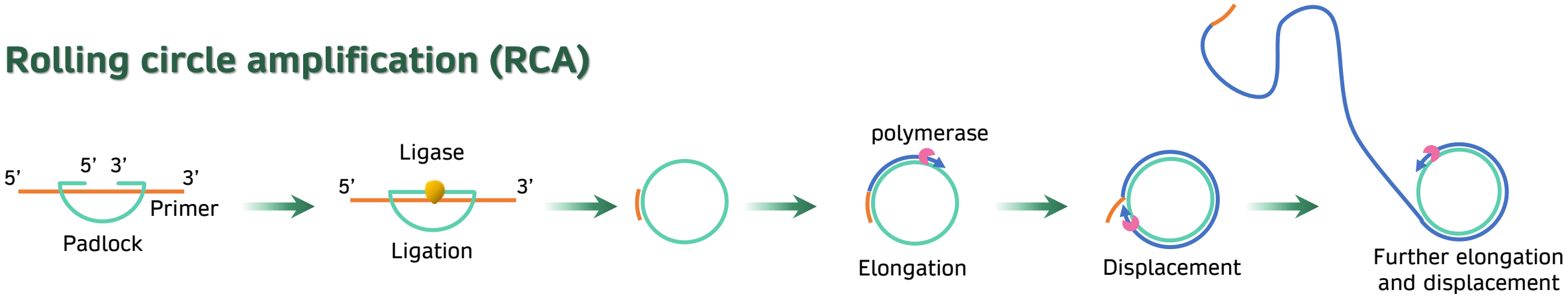
Terminal deoxynucleotidyl Transferase (TdT)



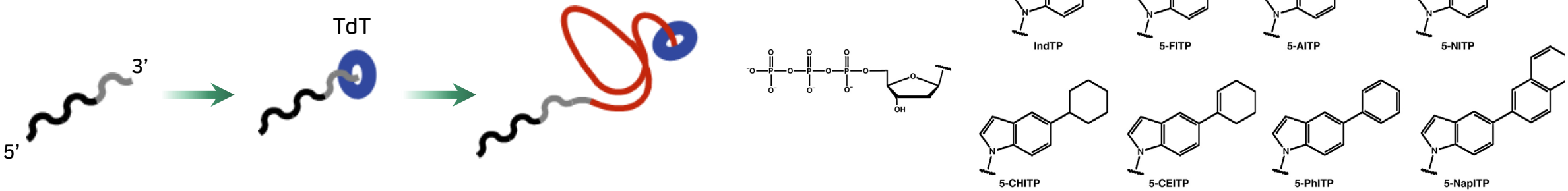
INTRODUCTION

ISOTHERMAL DNA AMPLIFICATION

Rolling circle amplification (RCA)



Terminal deoxynucleotidyl tansferase (TdT)



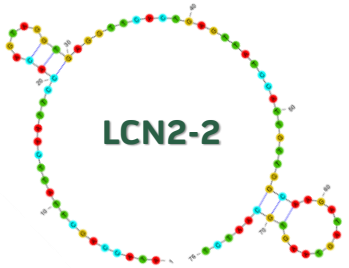
Motea, E.A.; Berdis, A.J. *Biochim. Biophys. Acta - Proteins Proteomics* **2010**, *1804*, 1151–1166.

OBJECTIVES

1. Study the affinity of aptamers evolved against **NGAL** and **AFP**.

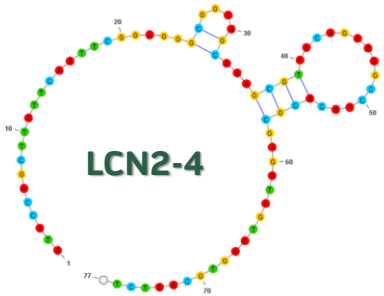
NGAL

Neutrophil gelatinase-associated lipocalin
Potential biomarker of pancreatic cancer



K_d: 2.24 pM

Lee, K.A. *et al. Sci. Rep.* **2015**, *5*, 10897.



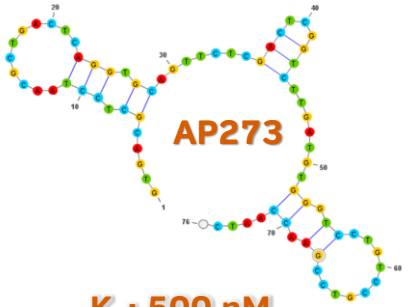
K_d: 60.9 pM

AFP

Alpha-fetoprotein
Biomarker of hepatocellular cancer

K_d: 2.37 nM

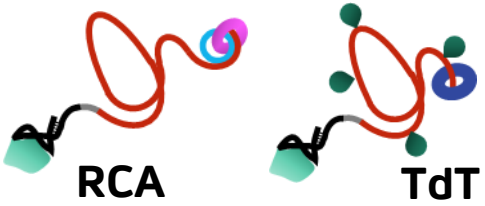
Huang, C.J. *et al. Biosens. Bioelectron.* **2012**, *35*, 50-55.



K_d: 500 nM

Dong, L. *et al. Sci. Rep.* **2015**, *5*, 15552.

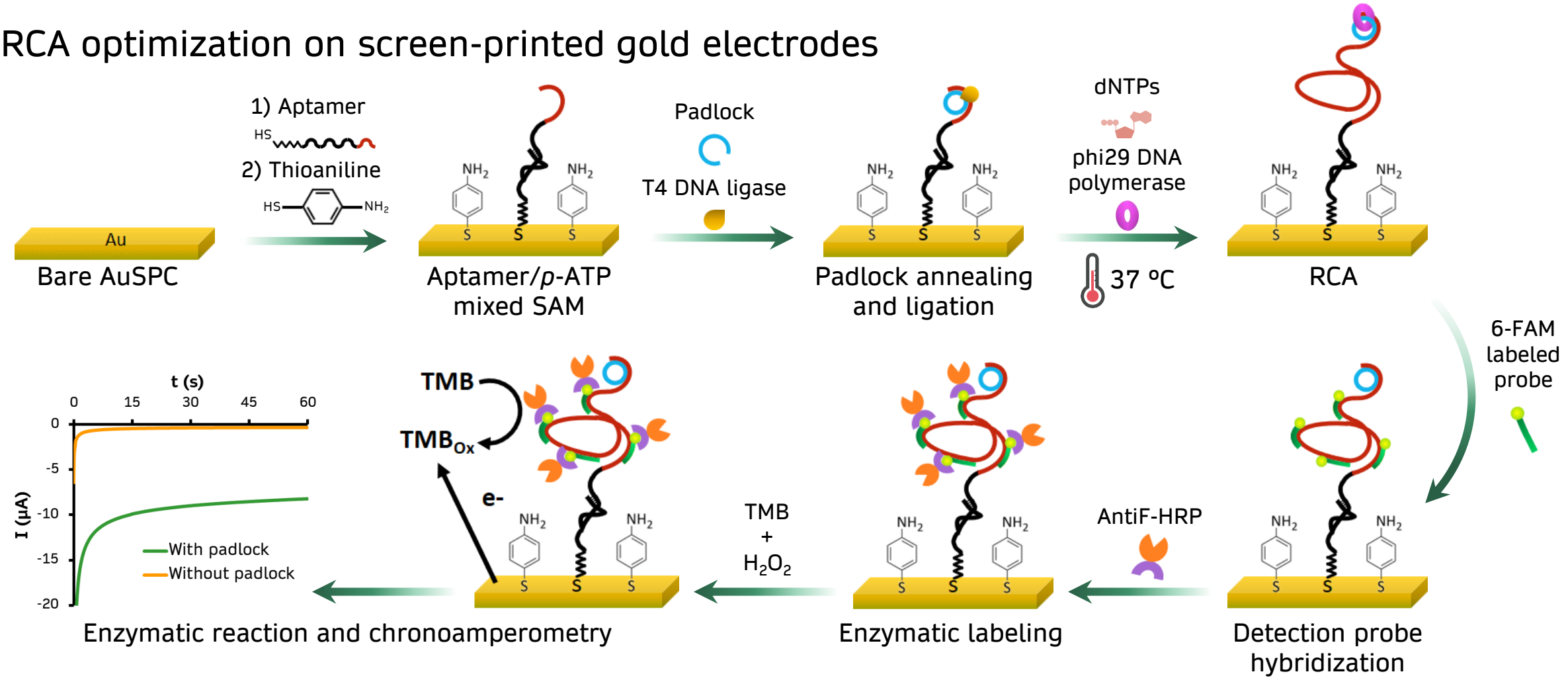
2. Evaluate the implementation of **DNA isothermal amplifications**, either **RCA** or **TdT**, to enhance the performance of these receptors.



EXPERIMENTAL SECTION

ROLLING CIRCLE AMPLIFICATION (RCA)

RCA optimization on screen-printed gold electrodes



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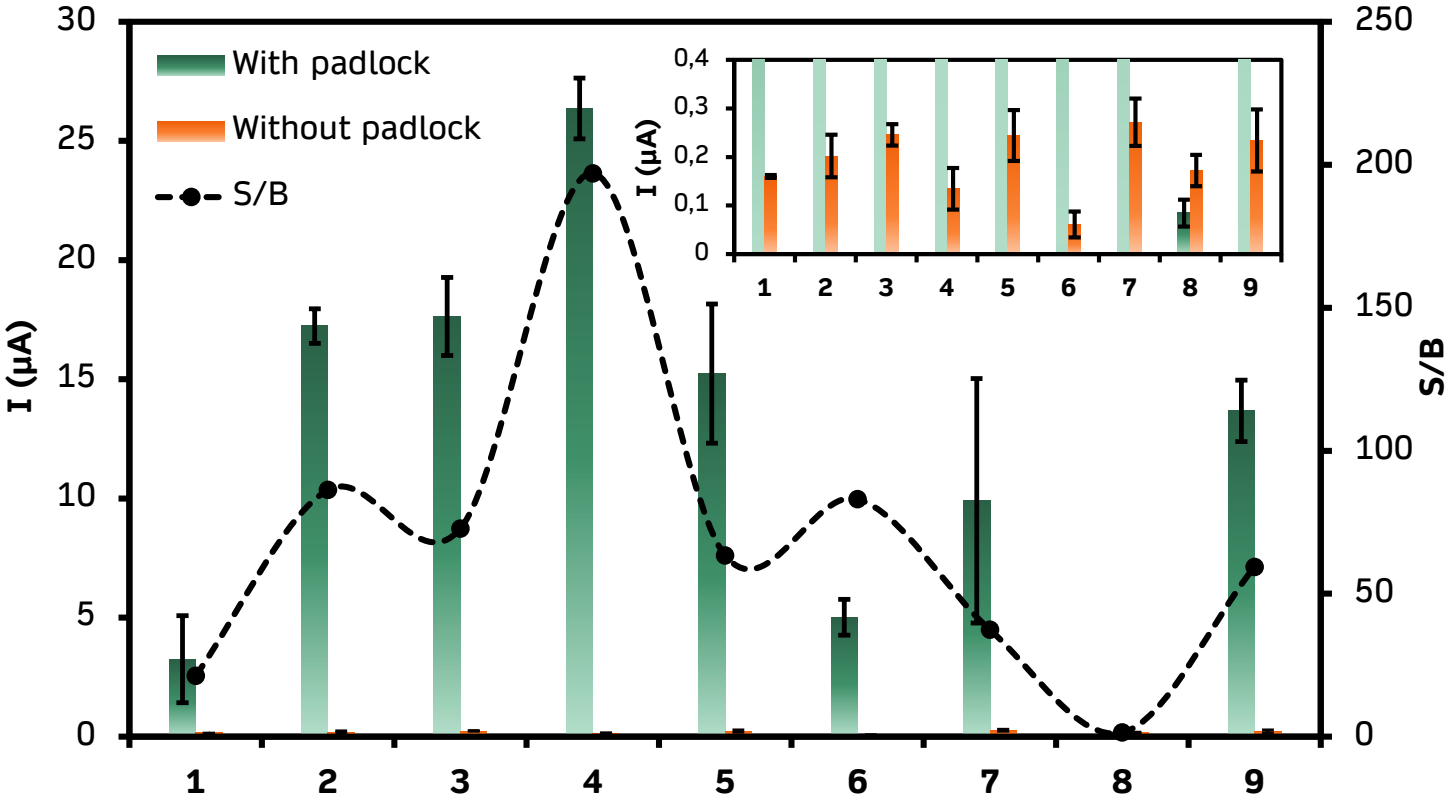


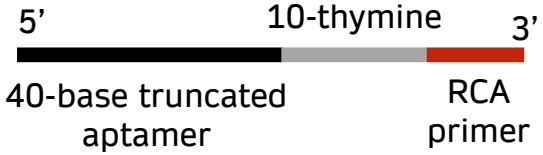
Lorenzo-Gómez, R. *et al. Talanta* **2019**, *197*, 406-412.

Variables	Exp. 1	Exp. 9
[Padlock] (nM)	100	10
[T4 DNA ligase] (weiss U/μL)	1	0.125
[phi29 DNA polymerase] (U/μL)	1	0.25
[Labeled probe] (nM)	500	100
Annealing + ligation (min)	120	30
RCA (min)	60	15
Signal probe hybridization (min)	60	30
Total time (h)	4.5	1.5

EXPERIMENTAL SECTION

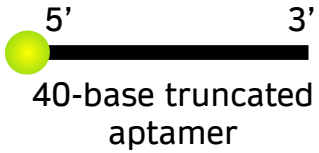
ROLLING CIRCLE AMPLIFICATION (RCA)

Aptamer adapted to RCA scheme

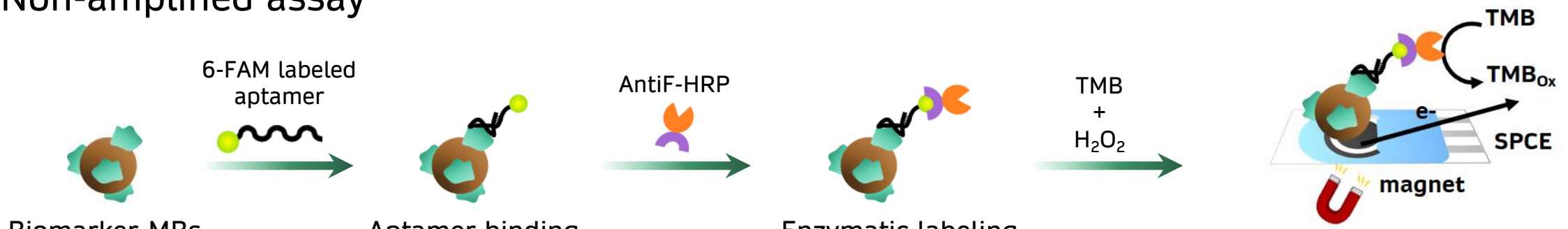


NGAL or AFP-modified magnetic beads (MBs)

6-FAM labeled aptamer



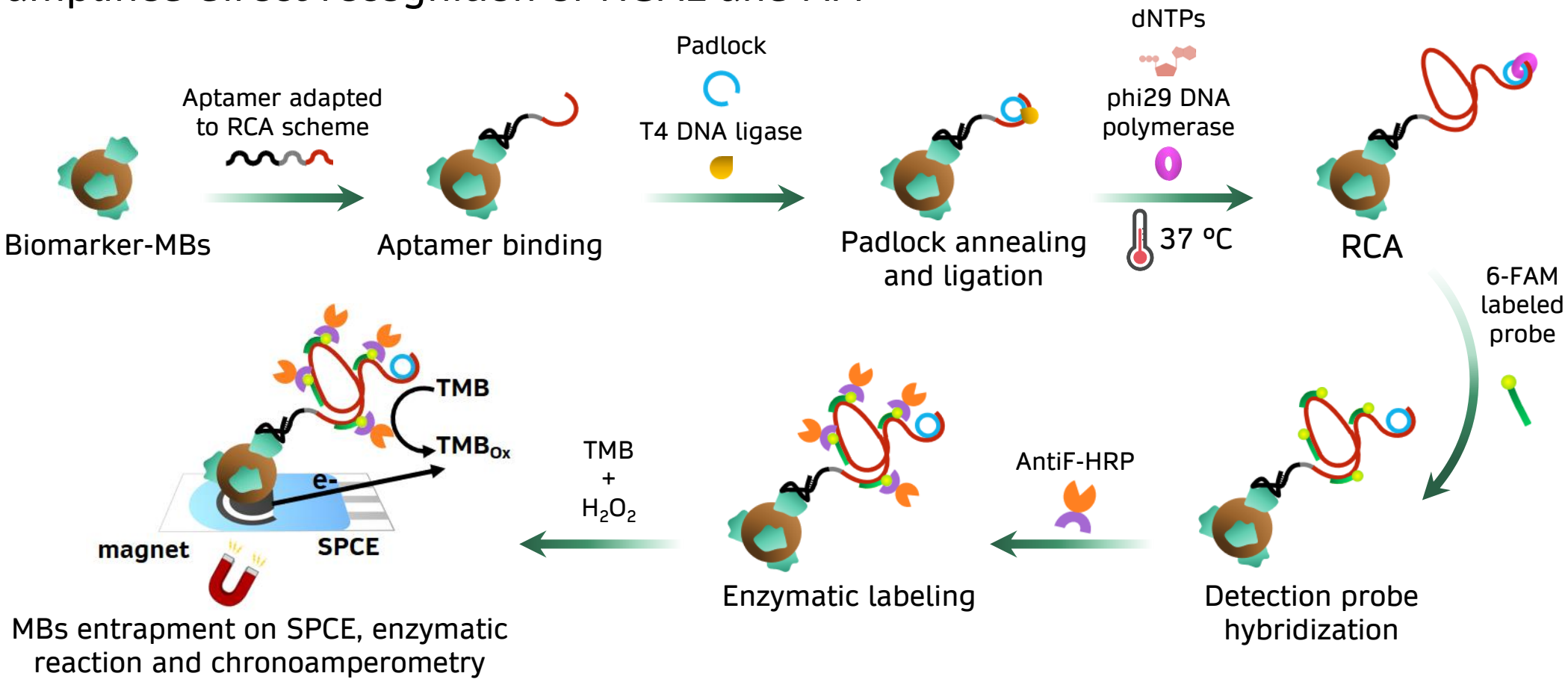
Non-amplified assay



EXPERIMENTAL SECTION

ROLLING CIRCLE AMPLIFICATION (RCA)

RCA-amplified direct recognition of NGAL and AFP



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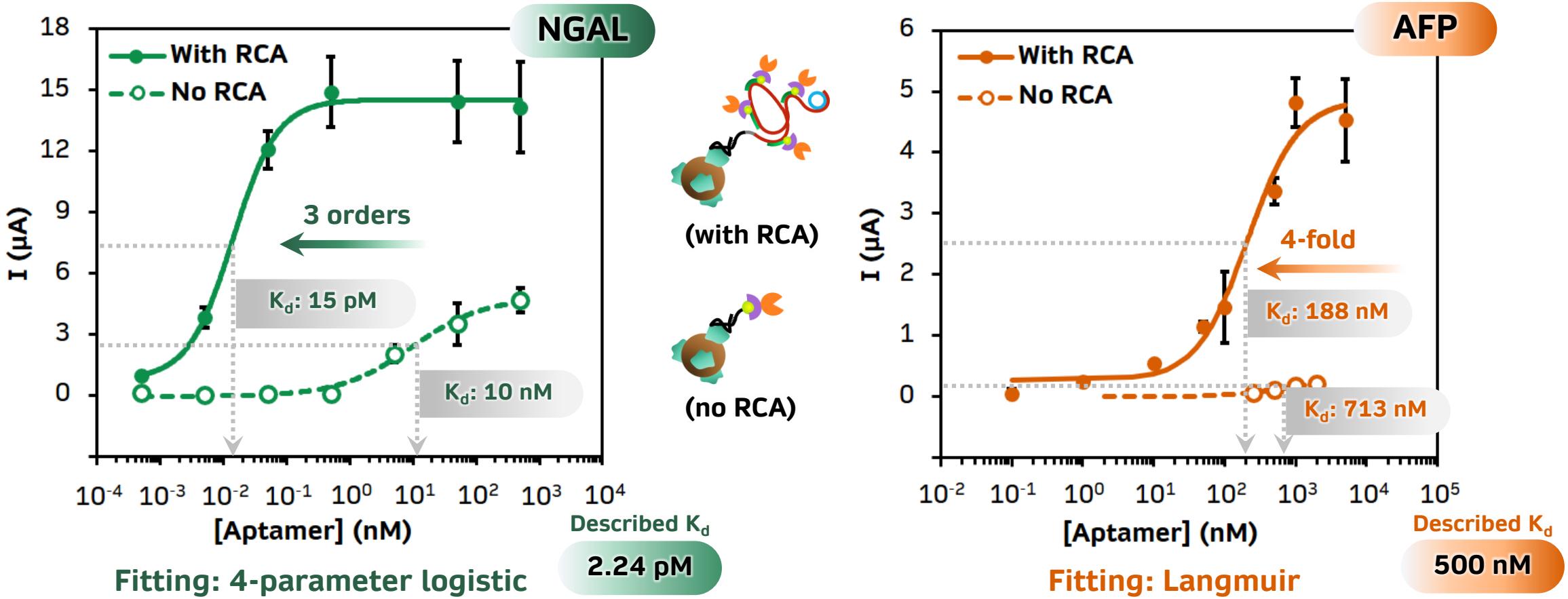


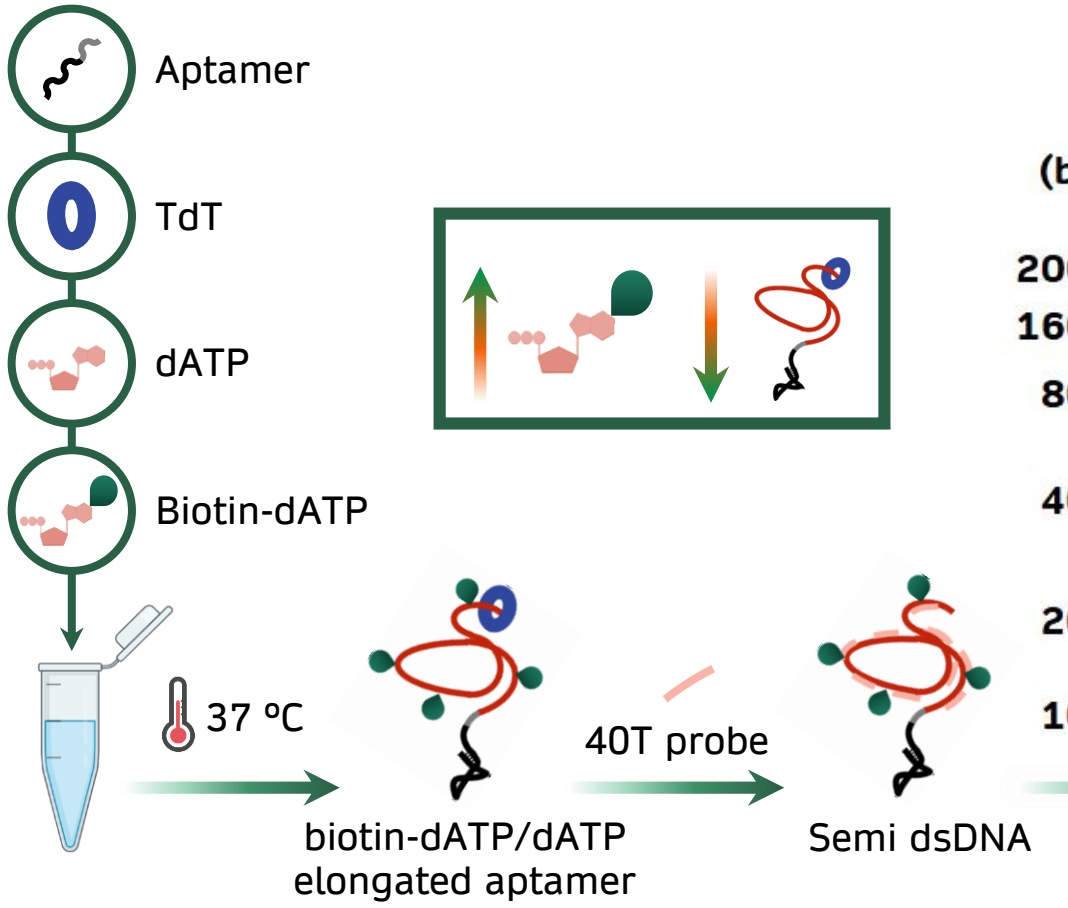
Lorenzo-Gómez, R. *et al. Talanta* **2019**, *197*, 406-412.

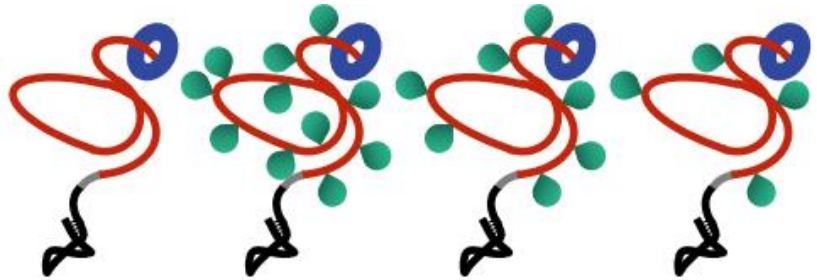
Lorenzo-Gómez, R. *et al. Biosensors* **2020**, *46*, 10.

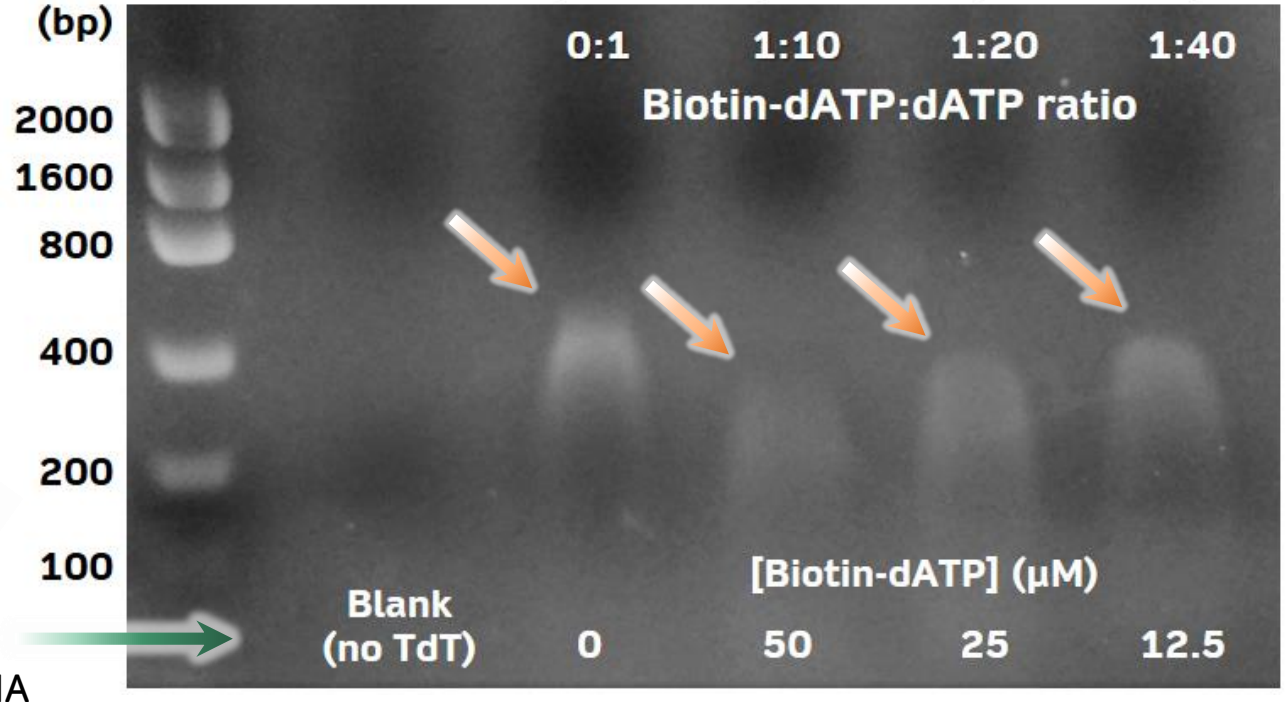
EXPERIMENTAL SECTION

TERMINAL TRANSFERASE (TdT)

TdT amplification study by gel electrophoresis







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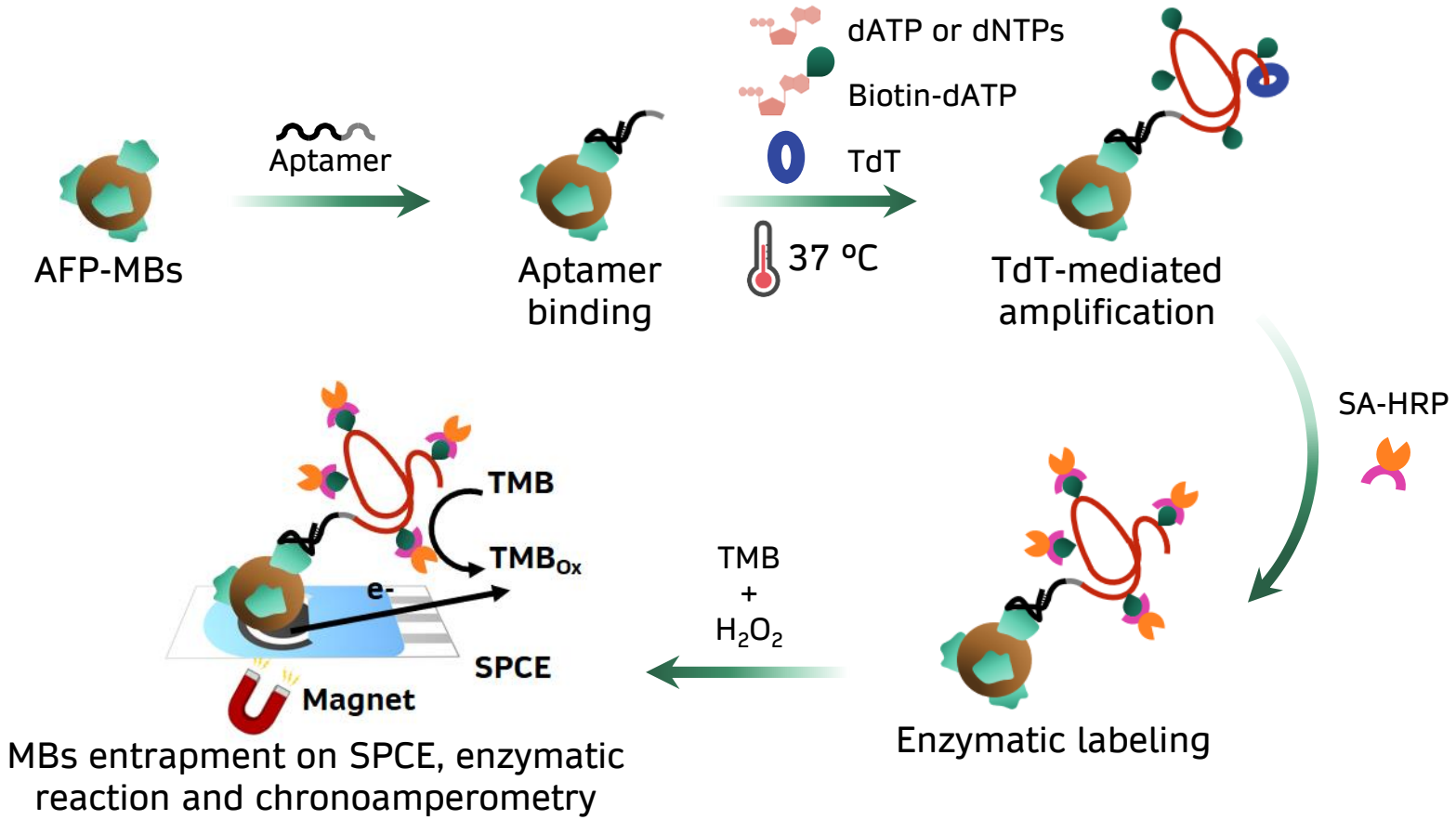
EXPERIMENTAL SECTION

TERMINAL TRANSFERASE (TdT)

TdT amplification study by direct assays on AFP-MBs with electrochemical detection



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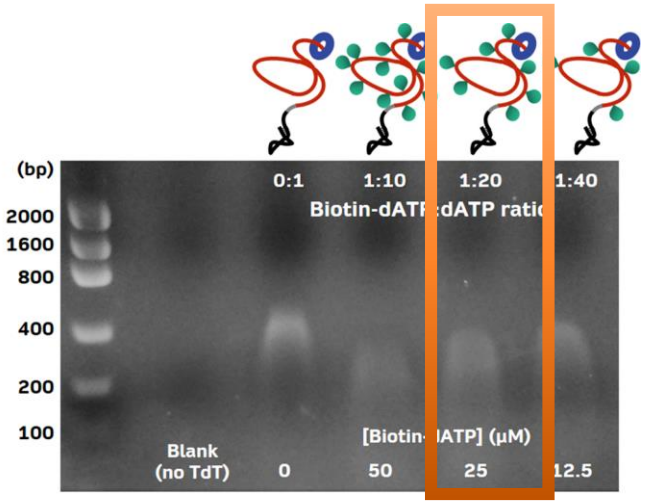

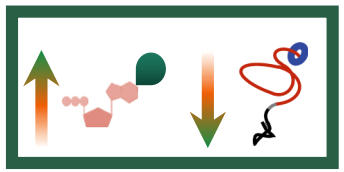

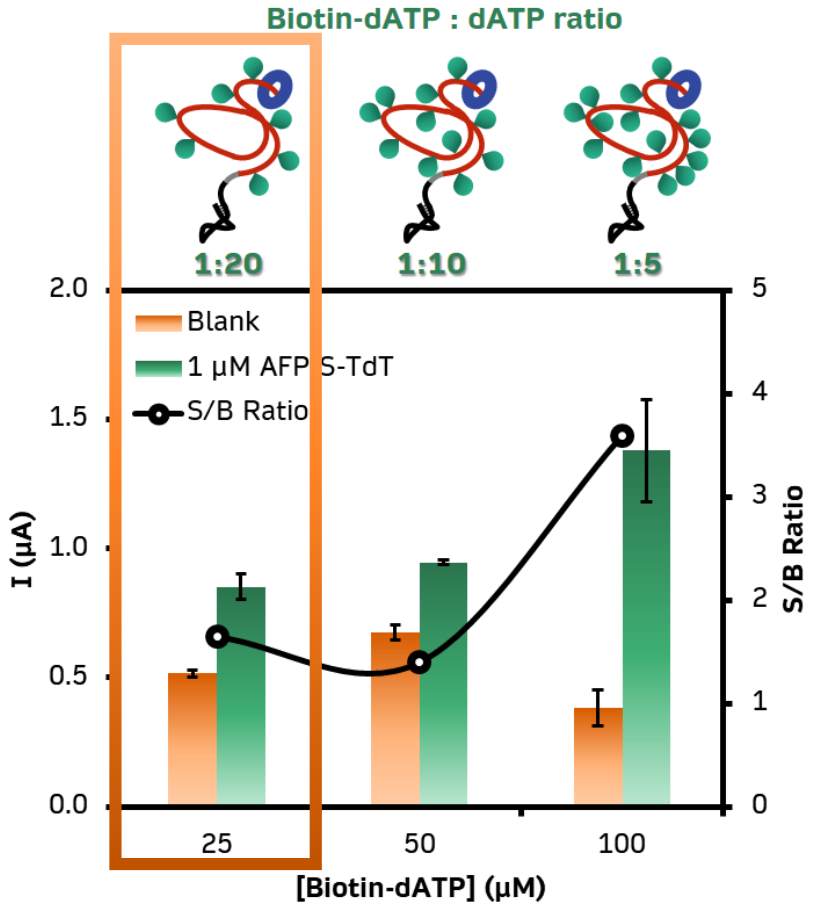

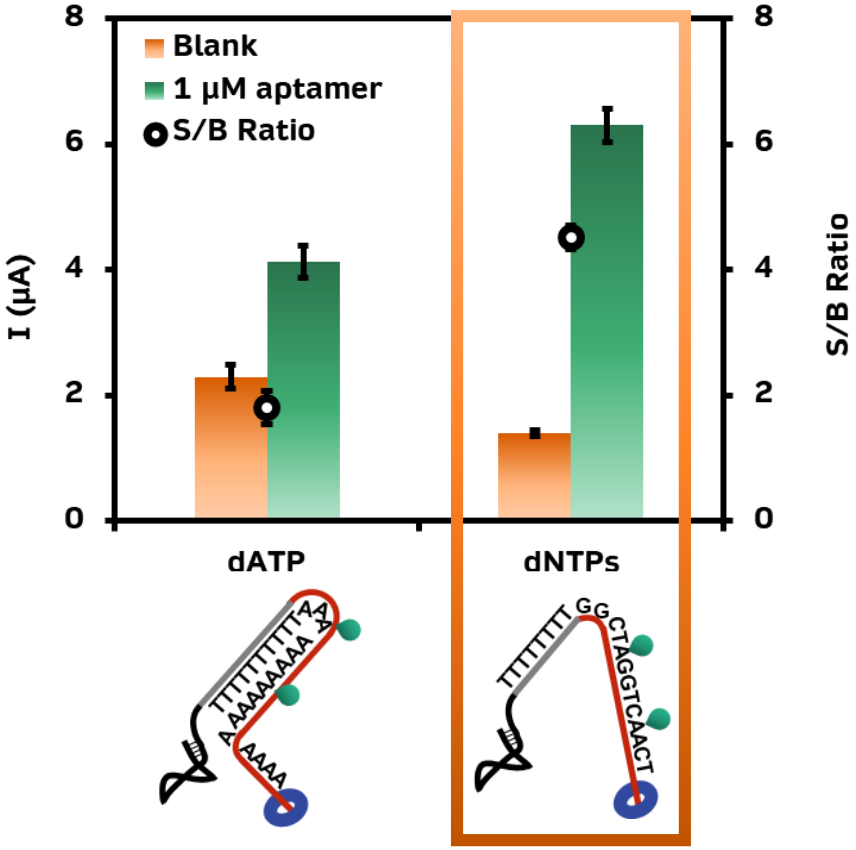

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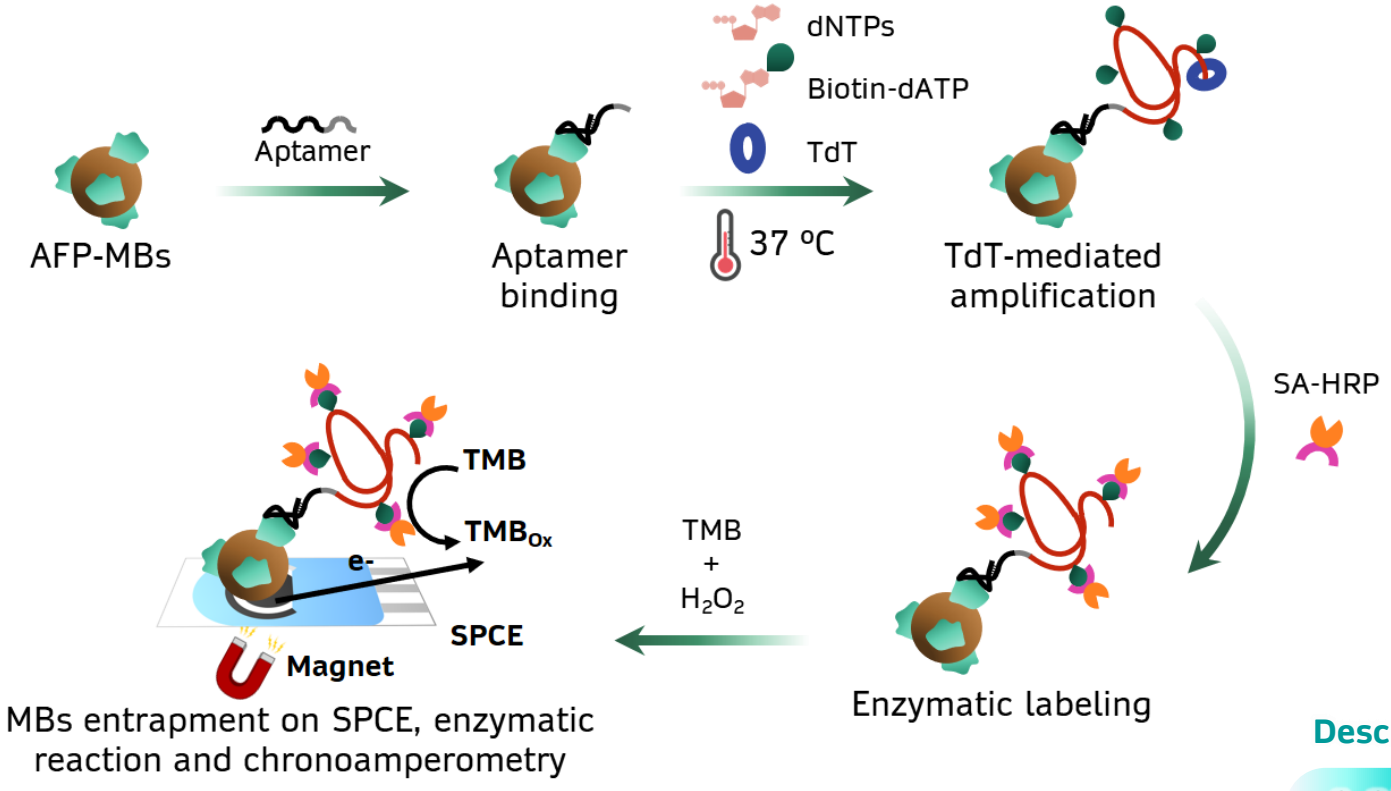

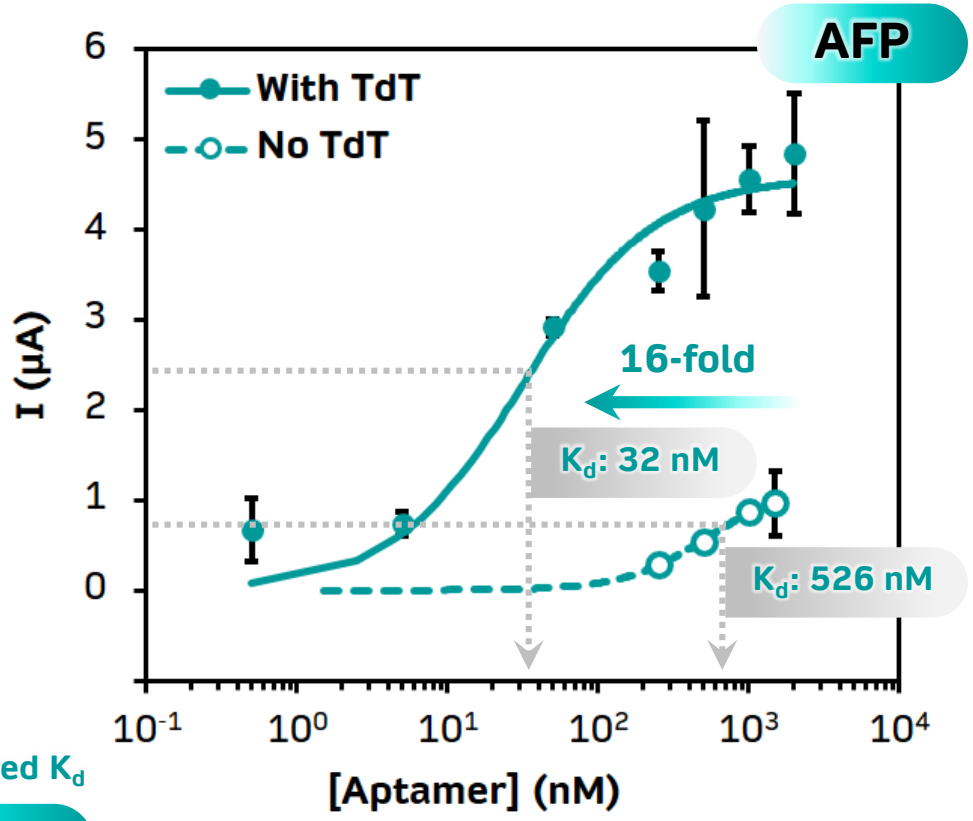

Described K_d

2.37 nM

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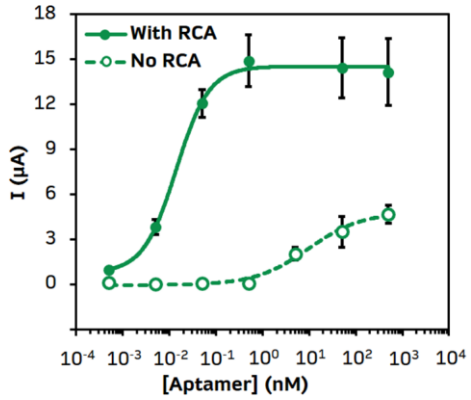
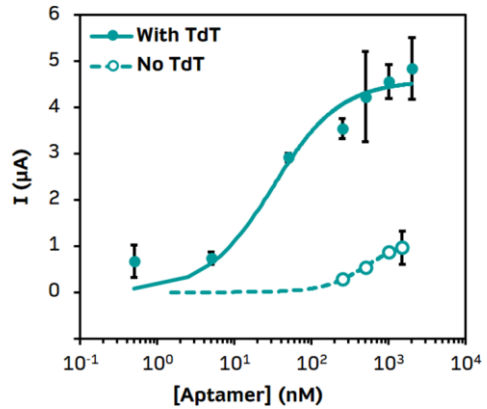
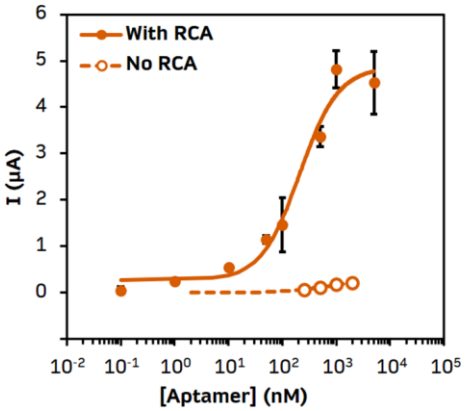
CONCLUSIONS

1. **DNA isothermal amplification** of aptamers can **enhance** the **performance** of **weak binding aptamers**, but their magnitude depends on the **true affinity** of the aptamer, which ultimately limits their **analytical usefulness**.

2. These findings are of general significance, as a number of **electrochemical biosensors** relying on the recognition by **poor aptamers** could **benefit** from these results.







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