Universidad de Oviedo

GIVING NEW USES TO GLUCOSE METERS: DETECTION OF PROSTATE CANCER

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Emerging techniques

LIQUID BIOPSY

- Potential supplement of a traditional biopsy for early cancer diagnosis
- Monitoring the level of tumor biomarkers present in body fluids

Point-of-care **(POC)** Testing,

"Medical diagnostic test carried out outside the clinical laboratory, close to the place where the patient is being treated"

- Immediate, portable, easy to use and low-cost analyses

Pregnancy test

- Human chorionic gonadotropin detection
- Semiquantitative results

Glucose meter

- Glucose detection
- Quantitative results

Personal glucose meter (PGM)

- Most commonly known and widely developed point-of-care testing
- Device to control blood glucose concentration in people with diabetes
- Electrochemical detection: oxidoreductase enzymes

- Promising portable meter for targets beyond glucose
- The aim of this challenge: to generate glucose as a final product
  - Encapsulation of glucose solutions in nanocontainers
  - Enzyme-catalyzed glucose production

New targets

- Prostate cancer
  - Most common cancer in men
  - Damages prostate tissue
  - The probability of getting it increases with age

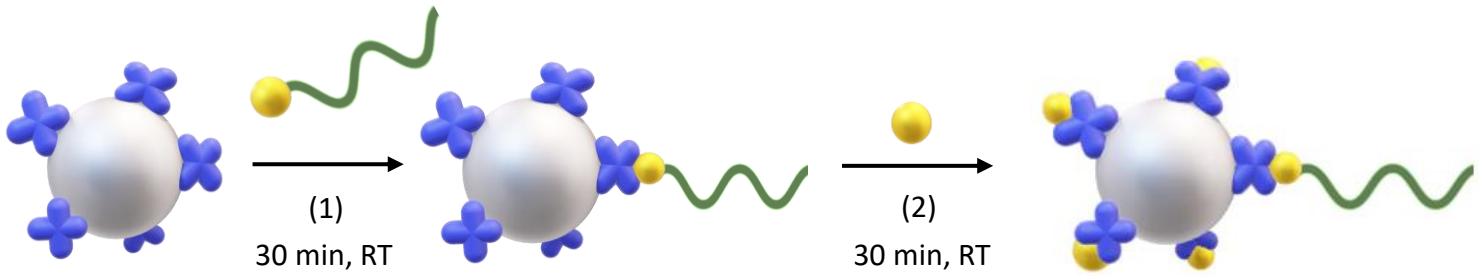
- Prostate Cancer Antigen 3 or PCA3
  - Urinary nucleic acid biomarker for prostate cancer diagnosis
  - Synthetic DNA target of PCA3 RNA

Objective

- The main objetive is to transform **personal glucose meter** into an electrochemical transducer of hybridization event, by introducing alkaline phosphatase onto magnetic beads as a **glucose-generating enzyme,** for the detection of the urinary biomarker for prostate cancer PCA3.

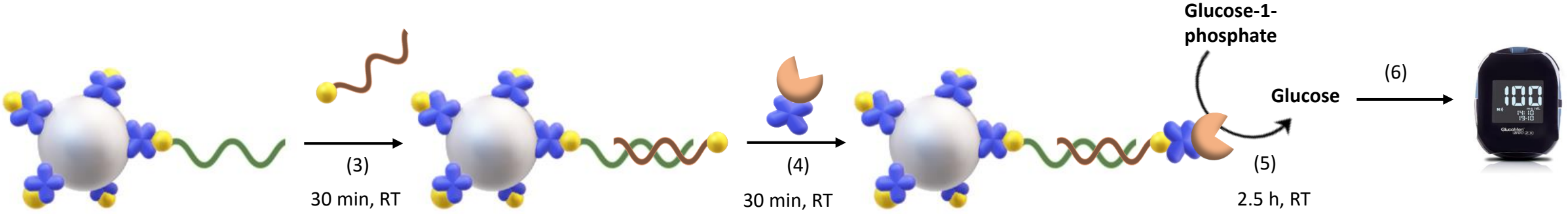
Proof of concept assay: A_{20}-T_{20}

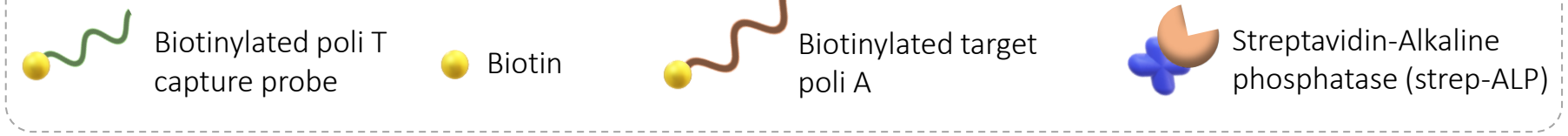
1. Magnetic beads modification: **Sensing layer construction**



Dynamic range:
20 to 600 mg glucose dL^{-1}

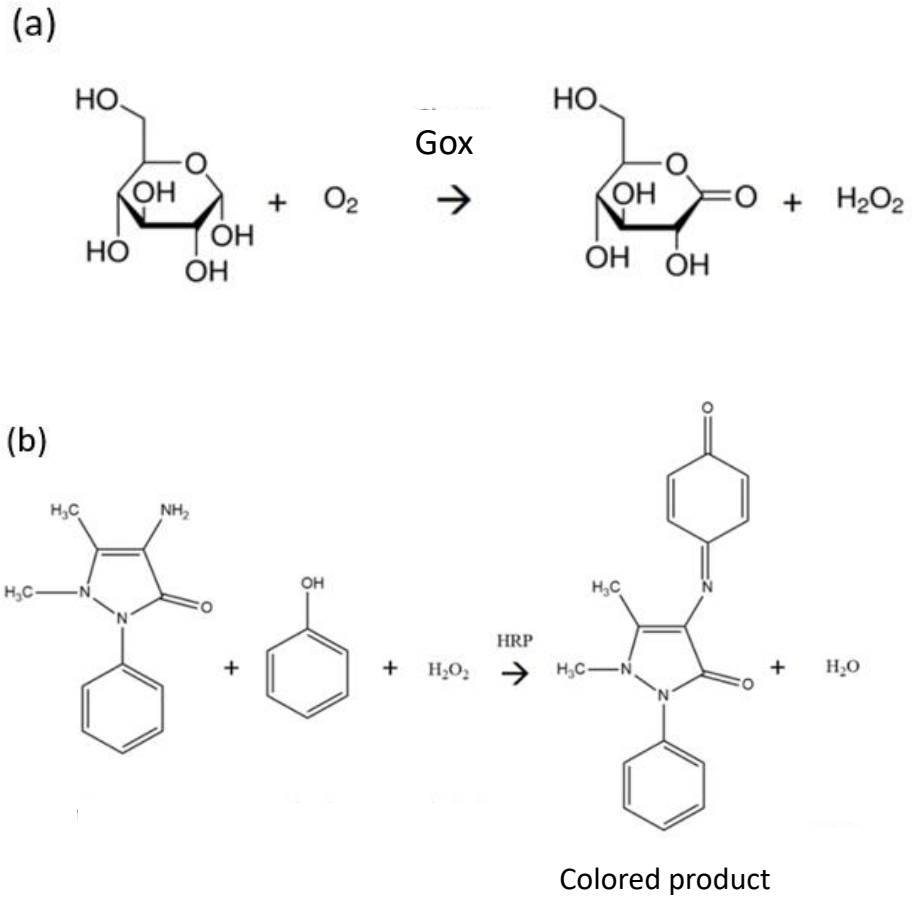
2. Hybridization genoassay onto magnetic beads

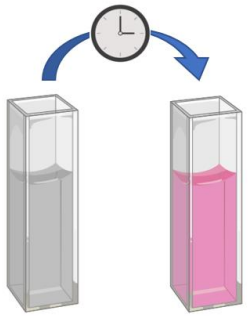




Trinder method

- Enzymatic method for the determination of glucose
- Main enzyme: glucose oxidase, Gox
- Enzyme for colorimetric determination: horseradish peroxidase, HRP
- Spectrophotometric recorded, maximun absorbance at 505 nm





Optimization

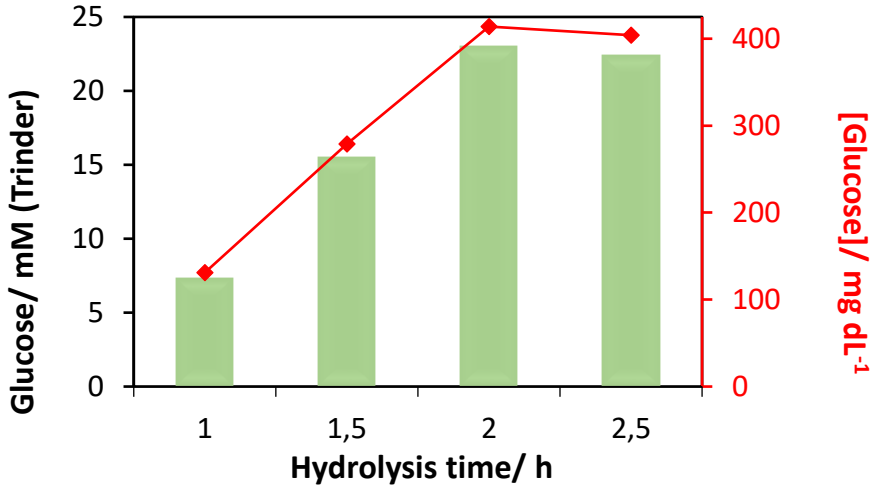
Biotin - streptavidin

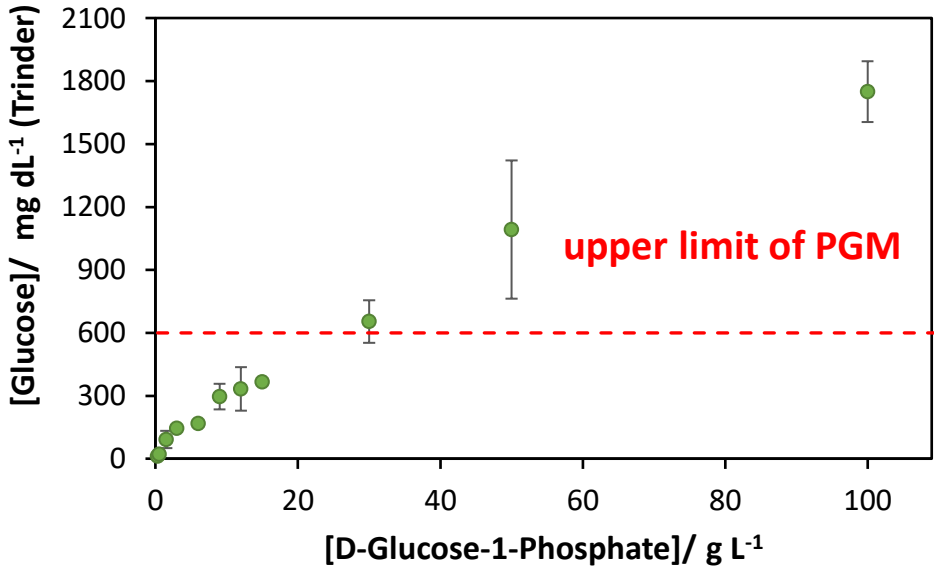
Target Poli A	50 nM
Hydrolysis buffer*	0.1 M Na_2CO_3 de pH 9.9
Shaking*	1300 rpm
Temperature*	37°C
Glucose-1-phosphate*	3 g L^{-1}
Hydrolysis time*	2.5 h
Strep-ALP	20 mg L^{-1}

Glucose-6-phosphate	DEA	129 ± 11 mg dL^{-1}
	Na_2CO_3	130 ± 5 mg dL^{-1}
Glucose-1-phosphate	DEA	163 ± 4 mg dL^{-1}
	Na_2CO_3	149 ± 3 mg dL^{-1}

*DEA: buffer of 0.1 M of diethanolamine, pH 9.8, 1 mM $MgCl_2$

*J. Diabetes Sci. Technol. 2014, 8 (4), 855-858

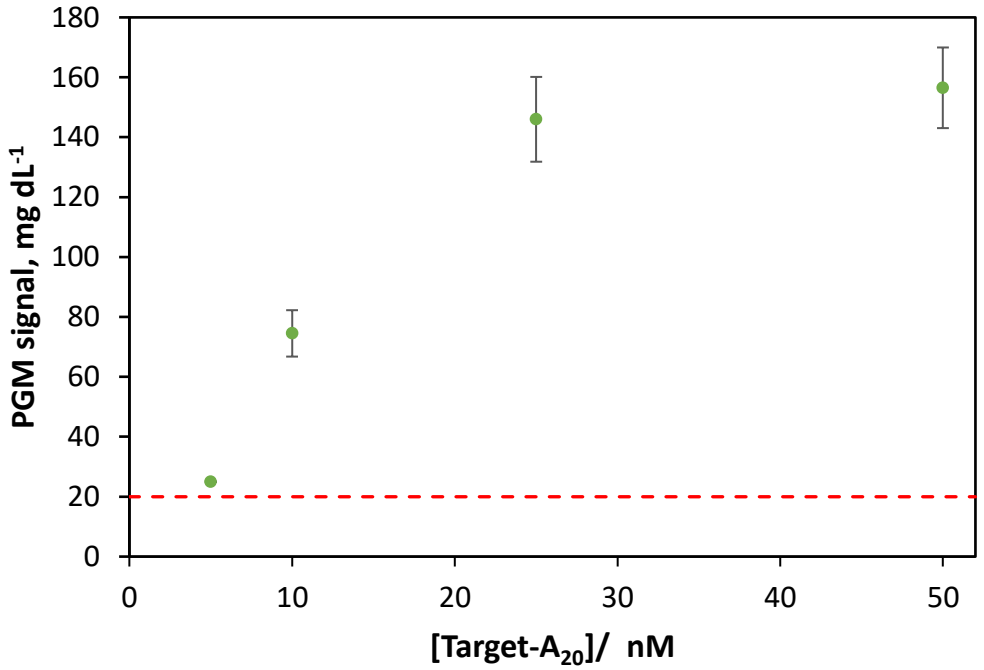




Optimization

Biotin - streptavidin

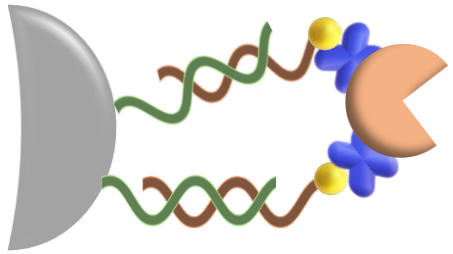
Target Poli A	50 nM
Hydrolysis buffer	DEA
Shaking*	1300 rpm
Temperature*	37°C
Glucose-1-phosphate	30 g L^{-1}
Hydrolysis time	2 h
Strep-ALP	20 mg L^{-1}



Effect of the multivalence of streptavidin?

Stoichiometry of the enzymatic conjugate 2:1

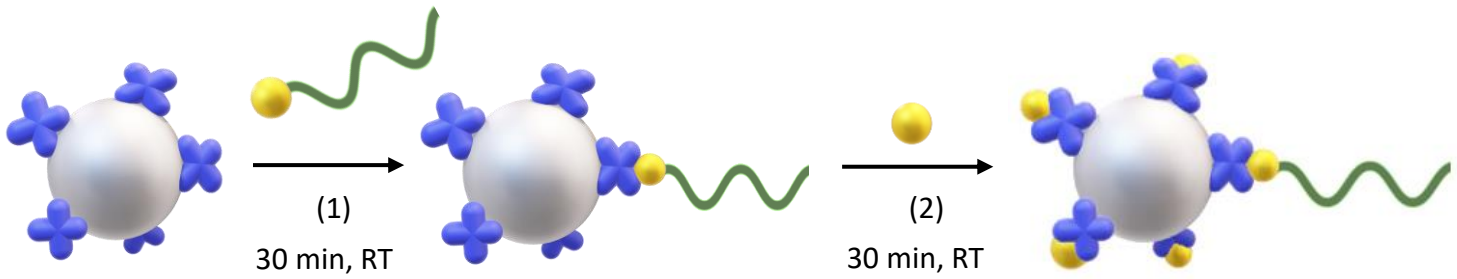
(2 streptavidin : 1 molecule of alkaline phosphate)

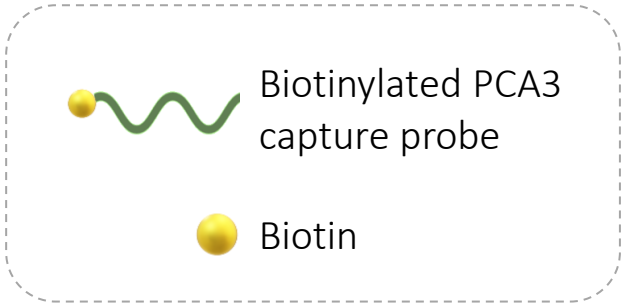


Genoassay for PCA3

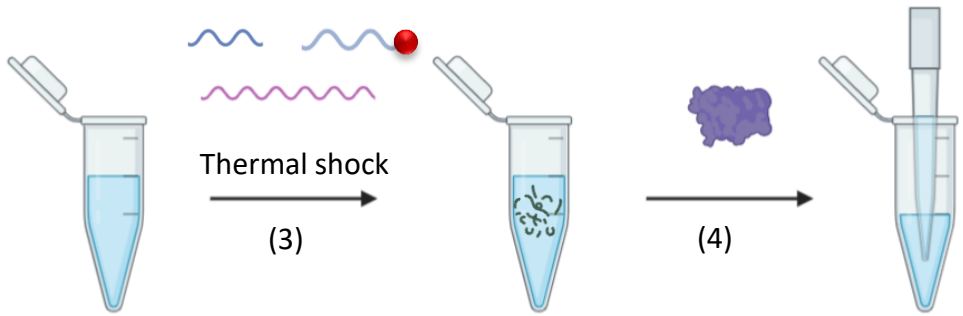
antifluorescein - Fab

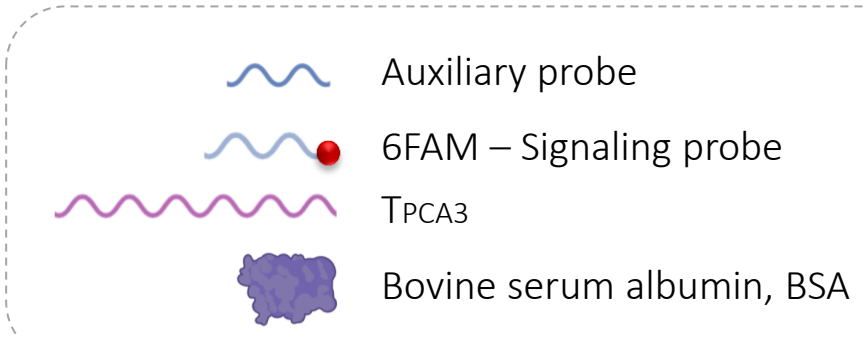
1. Magnetic beads modification: **Sensing layer**





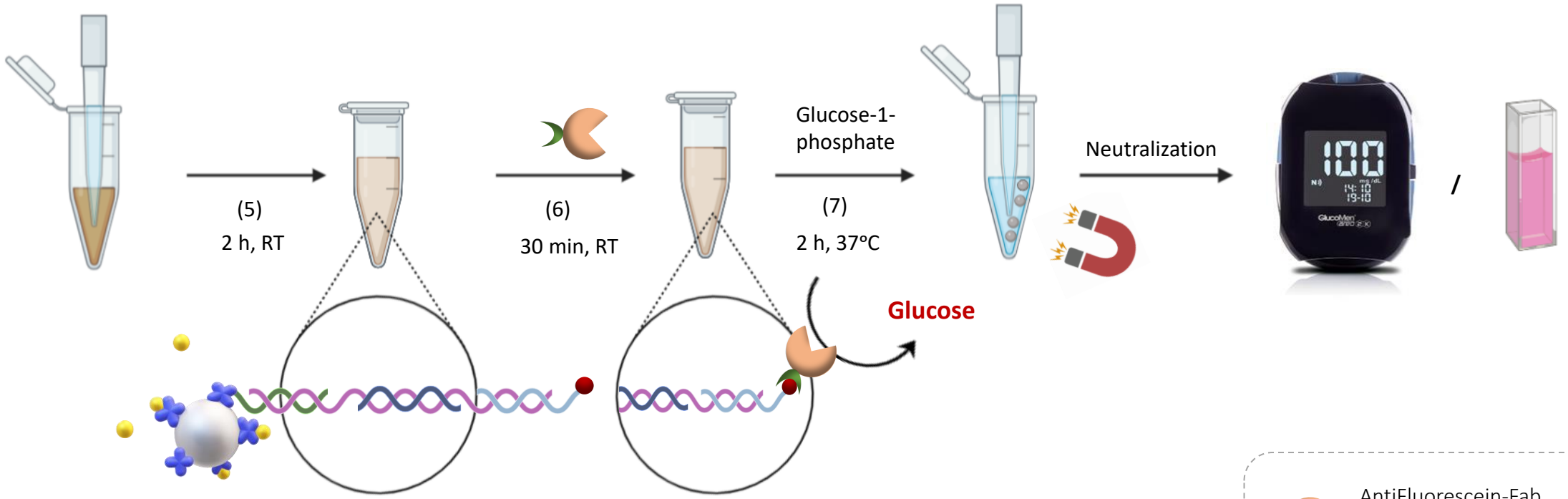
2. Sandwich-type genoassay: **Homogeneous hybridization**





Genoassay for PCA3

3. Sandwich-type genoassay: **Heterogeneous hybridization**

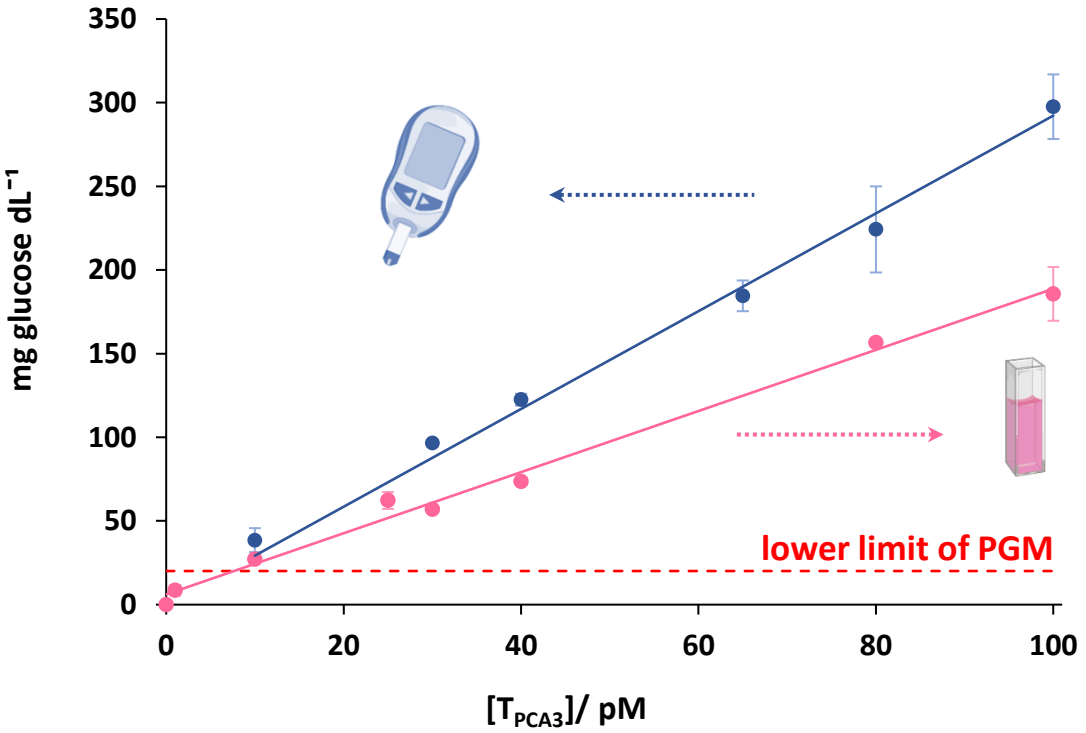


AntiFluorescein-Fab fragment alkaline phosphatase conjugate (antiF- ALP)

Results

antifluorescein - Fab

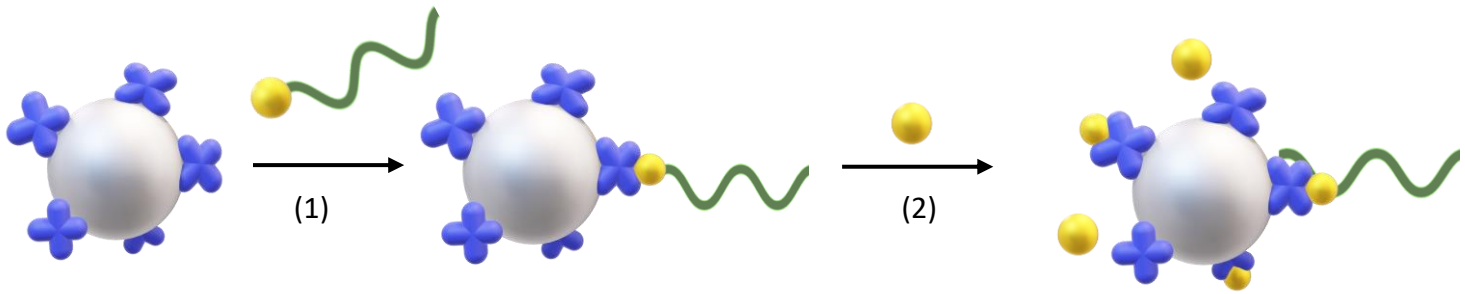
- ✓ Linear range: 10 – 100 pM
- ✓ Sensitivity (mg glucose $dL^{-1}pM^{-1}$):
  - ✓ PGM: 2.8 ± 0.1
  - ✓ Trinder method: 1.81 ± 0.05
- ✓ LOQ: 5 femtomoles in 0.5 mL of sample



Amplification strategy

antifluorescein - Fab

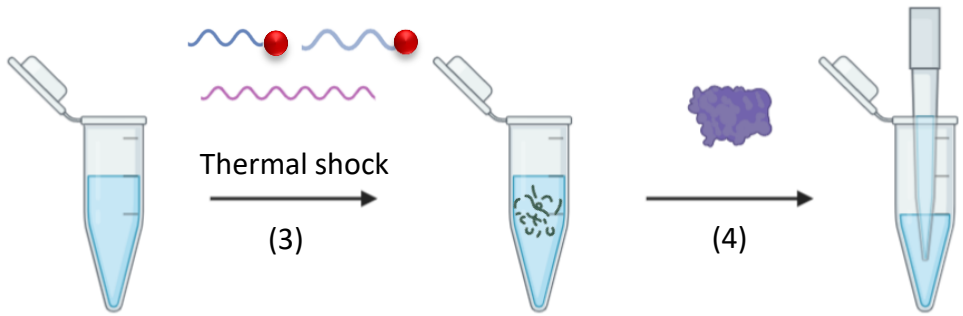
1. Magnetic beads modification: **Sensing layer**



Biotinylated PCA3 capture probe

Biotin

2. Sandwich-type genoassay: **Homogeneous hybridization**



6FAM - Auxiliary probe

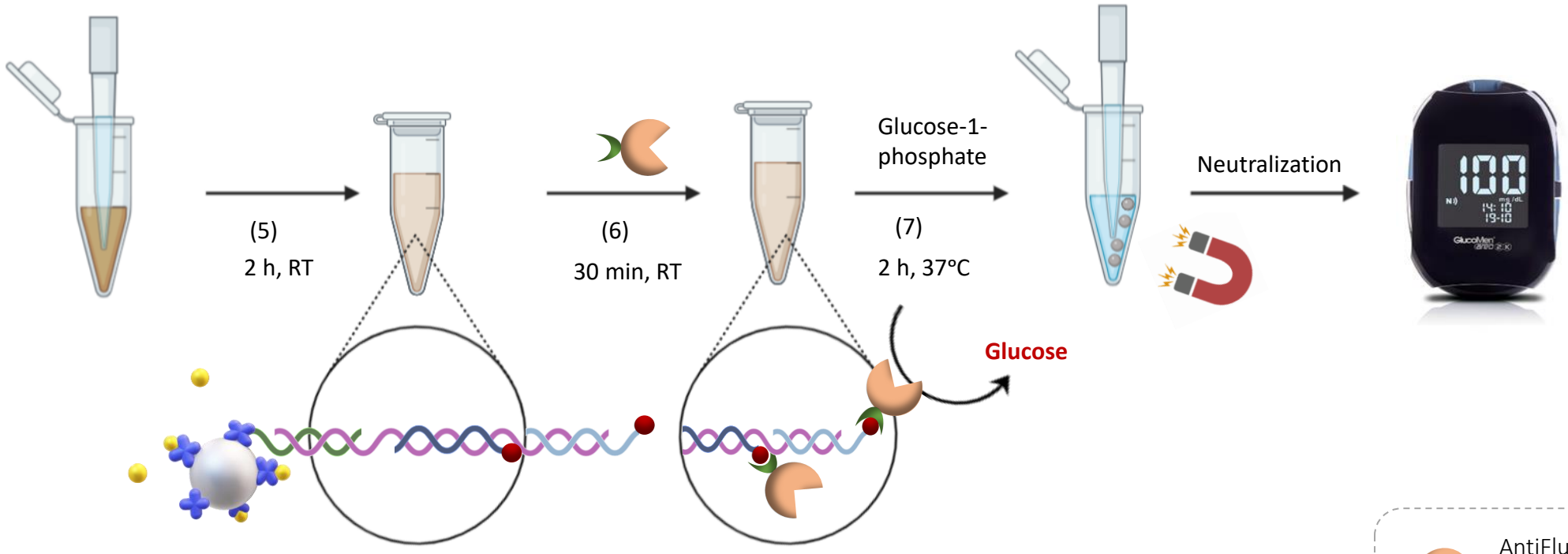
6FAM – Signaling probe

TPCA3

Bovine serum albumin, BSA

Amplification strategy

3. Sandwich-type genoassay: **Heterogeneous hybridization**

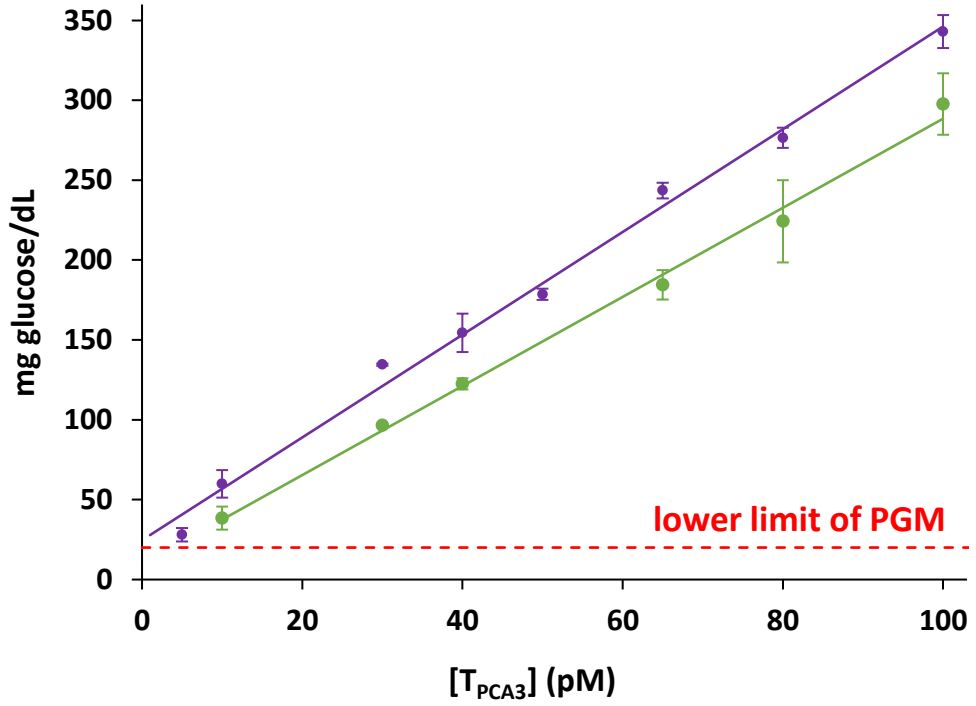


AntiFluorescein-Fab fragment alkaline phosphatase conjugate (antiF- ALP)

Results

antifluorescein - Fab

- ✓ Linear range: **5** – 100 pM
- ✓ Sensitivity (mg glucose $dL^{-1}pM^{-1}$):
  - ✓ Two signaling probes: 3.3 ± 0.2
  - ✓ One signaling probe: 2.8 ± 0.1
- ✓ LOQ: 2.5 femtomoles in 0.5 mL of sample



- ✓ Selectivity assay aginst the PSA interferent
  - ✓ 100 pM PSA interferent = <20 mg glucose dL^{-1} → Excellent selectivity
  - ✓ 100 pM PCA3 = 343 ± 35 mg glucose dL^{-1}

Conclusions

- A sandwich genoassay for quantification of PCA3 has been developed by introducing alkaline phosphatase as a tracer, through a label system on a tagged hybrid formed onto magnetic beads.

- Picomolar concentrations of PCA3 were amplified into millimolar concentrations of glucose by a monovalent label system, that can be detected with the personal glucose meter.

- The use of two signaling probes allowed the incorporation of two ALP molecules per analyte, resulting in an experimental LOQ of 5 pM.

- Excellent selectivity against a DNA homolog of PSA mRNA, also present in urine of prostate cancer patients, is achieved.

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