A Label-Free QCM Biosensor Based on Target-Triggered Release of Cargo Molecules in Gold Nanocages Capped with Aptamers for Thrombin Detection

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Introduction

- Thrombin is an enzyme for blood coagulation and a biomarker of chronic inflammatory diseases, vascular diseases, coagulation abnormalities, cancers, atherosclerosis, etc.[1-3]
- Excessive thrombin will cause venous thrombosis, and low amount of thrombin will increase bleeding risks.[4]
- The concentration of thrombin during coagulation varies from pM to μM, and thus the limit of detection and detection range need to cover the pM- μM range.[5, 6]
- It is critical that thrombin detection methods with sufficiently low detection limit and wide dynamic range are developed.

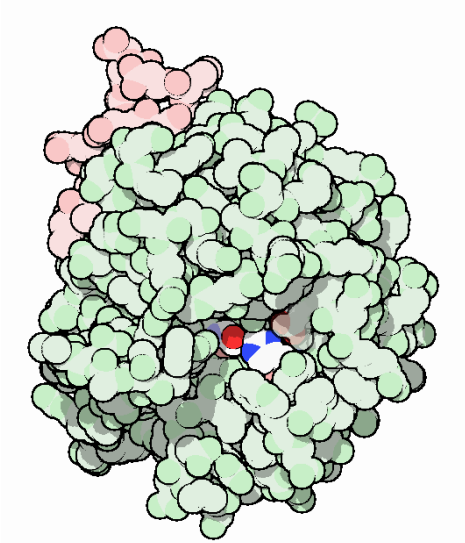

Fig. 1. Thrombin with the active site colored.

*Reprinted with permission from PDB-101 (PDB101.rcsb.org). [7]

1. Mol. Asp. Med 2008, 29 (4), 203-254.

2. Biosens. Bioelectron. **2013,** *40* (1), 24-31.

3. J. Cell. Biochem. **2019,** *120* (4), 4757-4765.

4. J. Am. Heart Assoc. **2016,** *5* (8), e003553.

5. Sens. Actuators B **2018,** *267*, 412-418.

6. Anal. Chim. Acta **2014,** *837*, 1-15.

7. PLoS Biol, 13(5), e1002140.

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Introduction

- Thrombin detection methods include enzyme-linked sandwich assays, stips, optical sensors, electrochemical platforms, and piezoelectric methods.

Tab. 1. Comparison of thrombin detection to other sensing platforms.

Sensing platform	Linear range	Detection limit	Detection time*	Reference
Enzyme linked assay	0.5-34 pM	2 pM	1.5 h	ELISA Kit (ab270210)
	0-300 fM	25 fM	6 h	Park et al., 2014
	0.3-100 nM	0.15 nM	2 h	Wang et al., 2017
Lateral flow assay	-	1.5 nM	10 min	Liu et al., 2019
Resonance light scattering	10-500 pM, 1-60 nM	0.71 pM	1 h	Liu et al., 2018
	0.5-75 nM	8.6 nM	1 h	Chen et al., 2017
Fluorescence	50 pM-5 nM	1.0 pM	2 h	Li et al., 2019
Chemiluminesce	1-25 pM	0.55 pM	2.5 h	Wang et al., 2018
Electrochemistry	1 pM-10 pM, 10 pM- 1 µM	10 pM	1.5 h	Chen et al., 2019
	1 pM-10 nM	0.2 pM	1.5 h	Yang et al., 2020
QCM	1.0-100 nM	1 nM	2 h	Bayramoglu et al., 2019
	0.5–12.5 nM	0.1 nM	1 h	Chen et al., 2010
	8.6 pM-86 nM	0.8 pM	1.5 h	this study

*Some of the detection times were estimated based on the experimental description in the corresponding references.

Introduction

- Thrombin detection methods include enzyme-linked sandwich assays, stips, optical sensors, electrochemical platforms, and piezoelectric methods.

Tab. s1. An example of theoretical signal caused by thrombin.

item	value	unit
initial frequency	7.99	MHz
volume added	200	μL
thrombin concentration	8.6	pM
thombin amount	1.72×10^{-15}	mol
molecular weight of thrombin	65045	g/mol
total mass of the AuNCs	1.12×10^{-15}	g
electrode diameter	0.511	cm
surface area	0.205	cm^2
frequency change	0.1	Hz

$$\Delta f = -2.3 \times 10^6 f_0^2 \Delta M/A$$

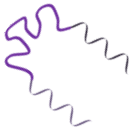
Δf is the change in frequency;
f_0 is the initial resonance frequency of the crystal;
ΔM is the change in the mass;
and A is the surface area tested in cm^2.

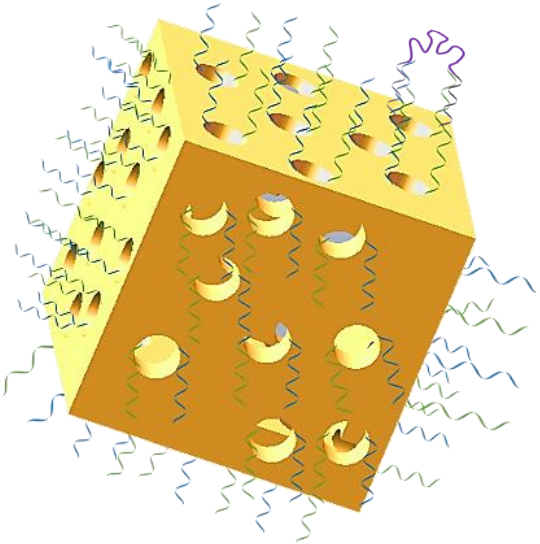
Objectives

- To use various molecules loaded gold nanocages (AuNCs) capped with aptamers as biosensing material for signal amplification and improved sensitivity;
- To simplify the detection process and shorten the detection time of thrombin by the target triggered controlled-release mechanism of the loaded AuNCs;
- To achieve simple, rapid, and sensitive detection of thrombin.

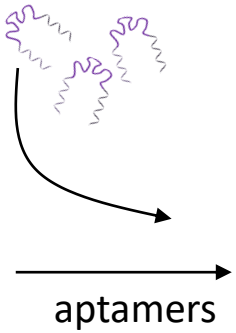
Materials and methods

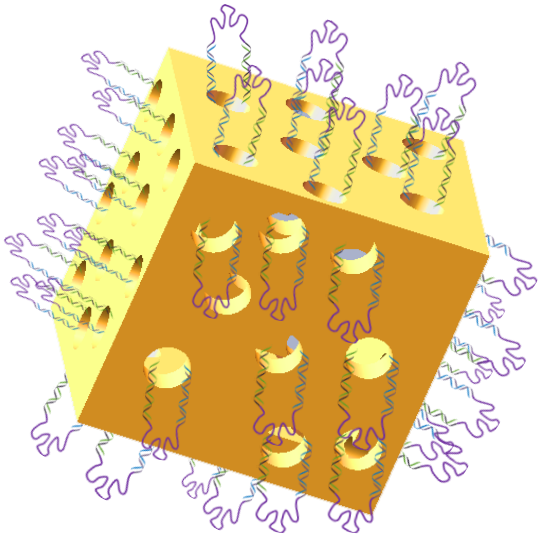
- Thrombin aptamer (TTT TT**G GTT GGT GTG GTT GG**C CCC)
- DNA probe 1 (SH-GGG GG)
- DNA probe 2 (AAA AAA-SH)





empty AuNC:
pores open





empty AuNC:
pores closed

Materials and methods

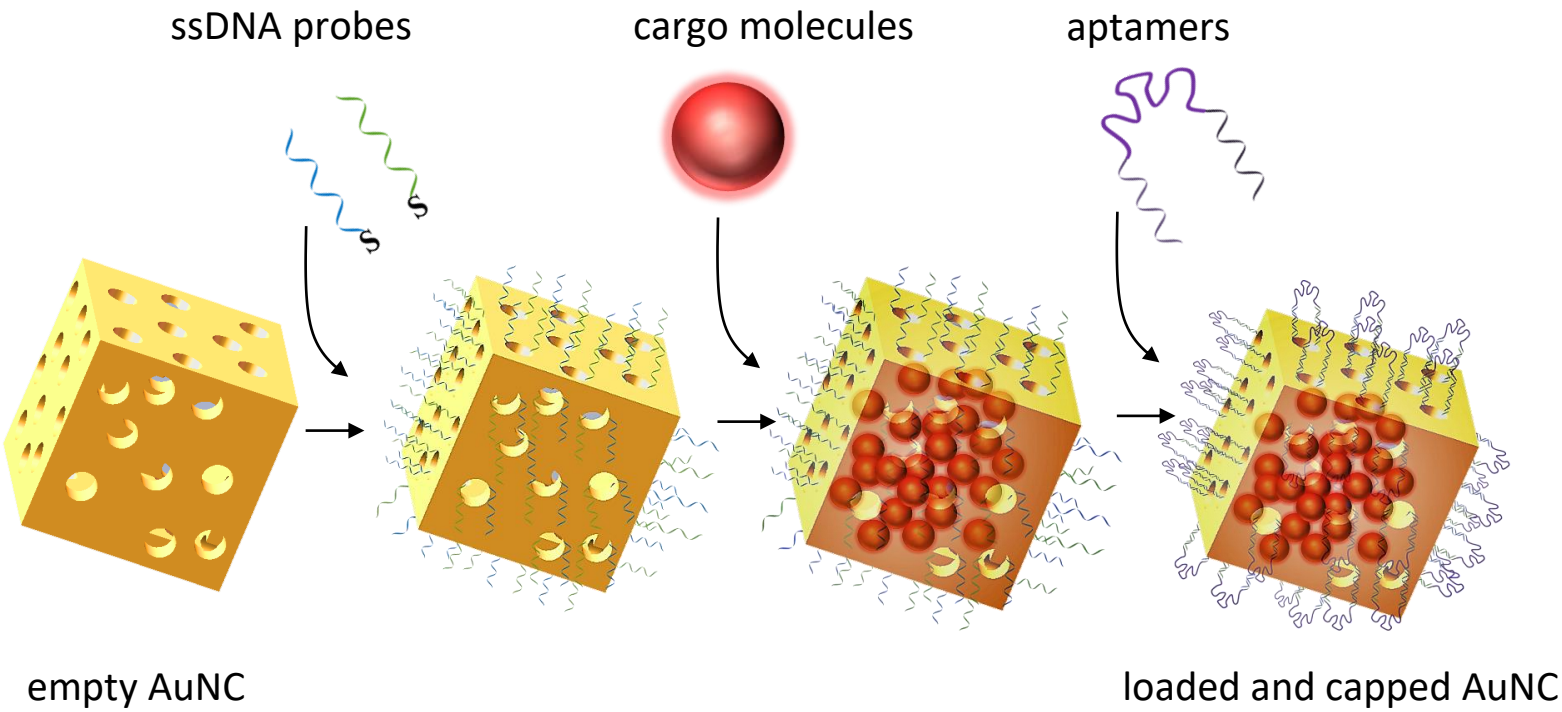


Fig. 2. The process of loading cargo molecules into AuNCs.

Materials and methods

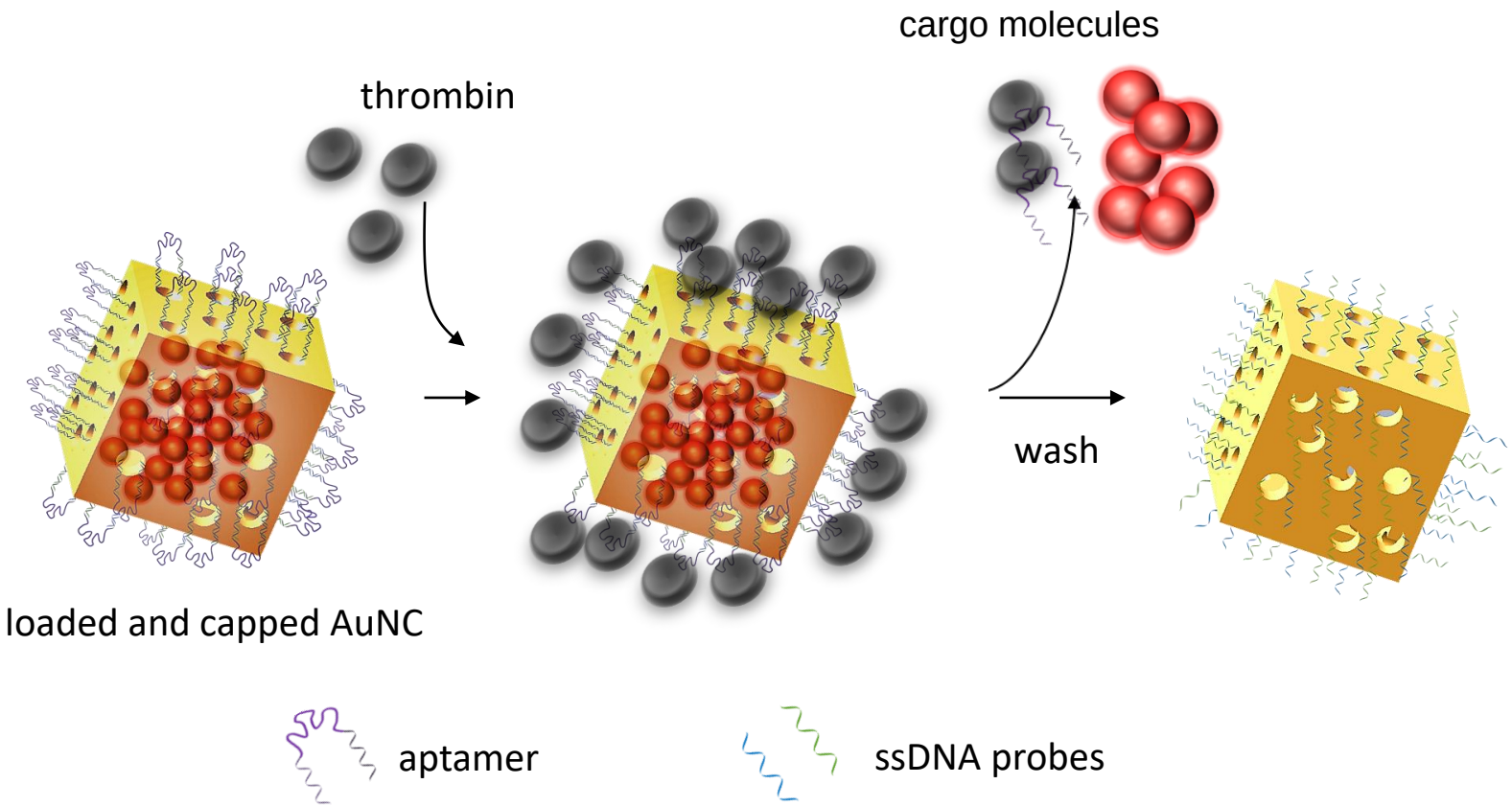


Fig. 3. The thrombin-triggered release of cargo molecules.

Materials and methods

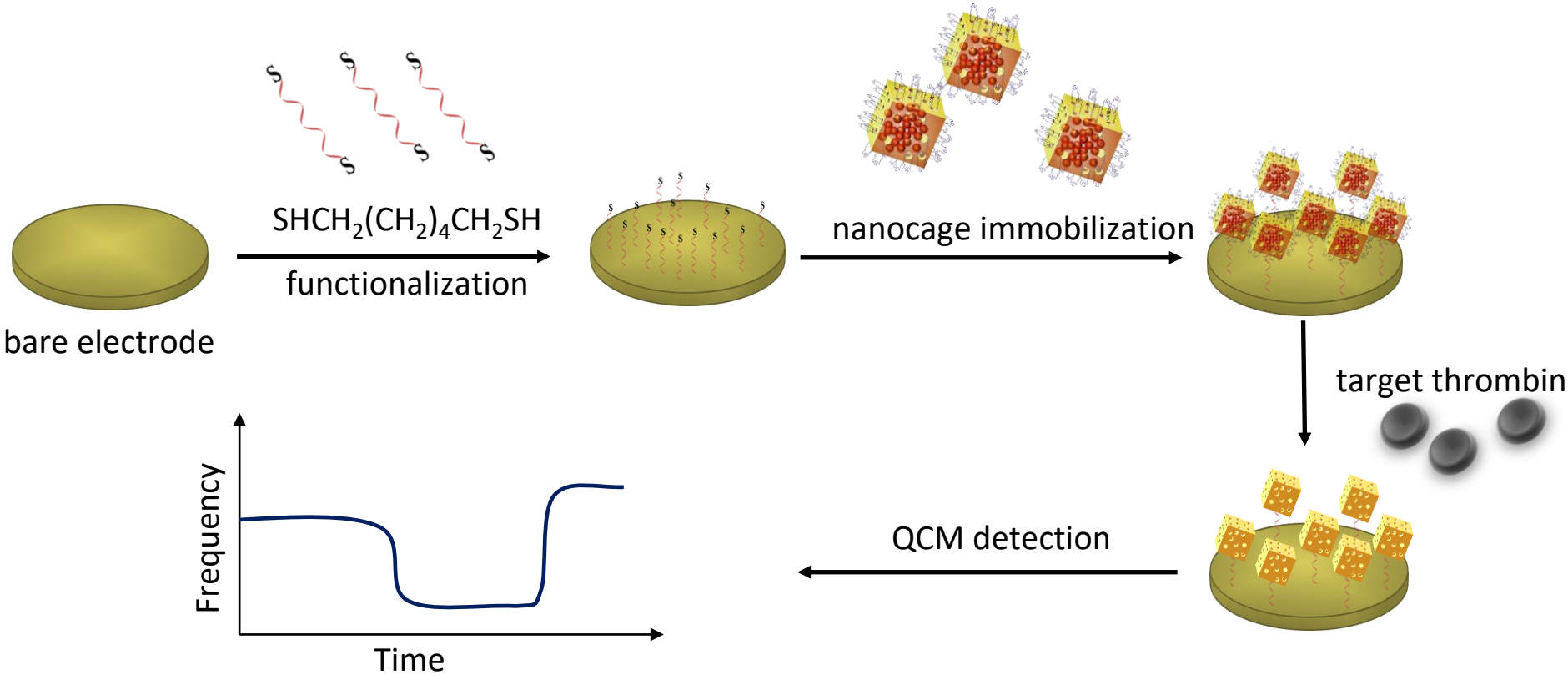


Fig. 4. Illustration of the QCM biosensor based on target-triggered release of cargo molecules inside AuNCs capped by aptamers for sensitive detection of thrombin.

Results and discussion

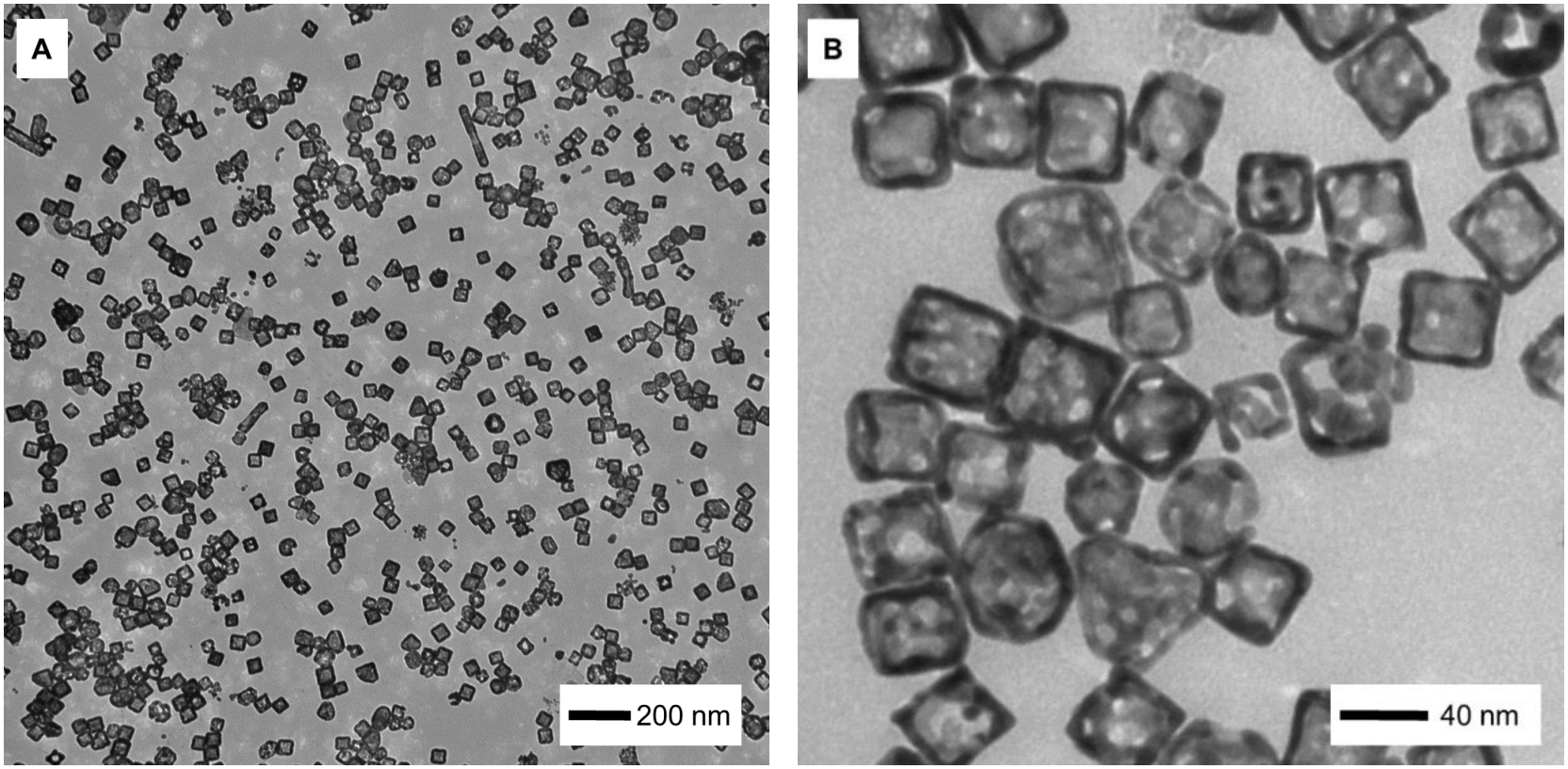
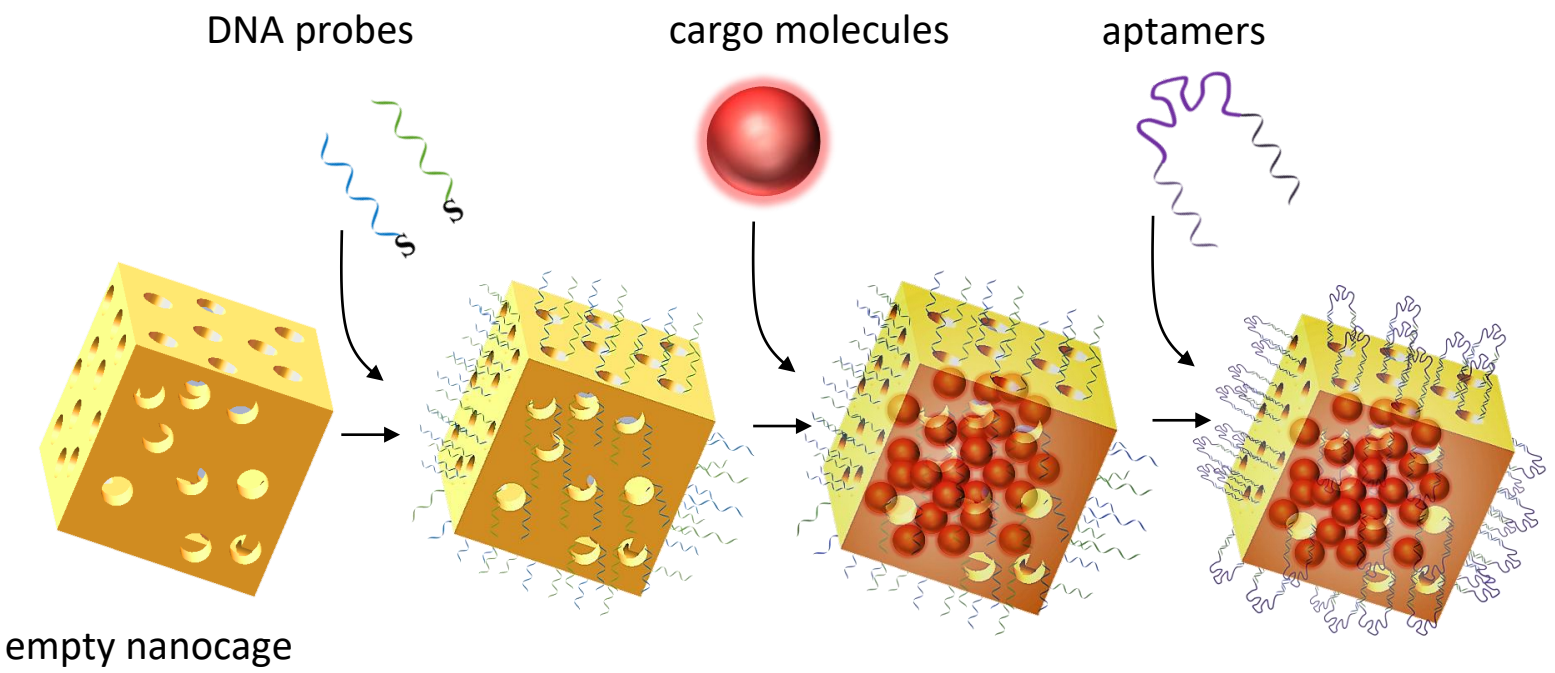


Fig. 5. (A) TEM image of AuNCs; (B) Zoom-in TEM image of the 45 nm AuNCs with 5 nm pores.

Results and discussion



Tab. 2. The step-by-step size monitoring of the AuNCs immobilization process.

Immobilization steps	TEM size (nm)	Hydrodynamic diameter (nm)
AuNCs	45.8 ± 3.5	87.6 ± 0.6
AuNCs-ssDNA	51.1 ± 8.7	117.5 ± 1.7
AuNCs-ssDNA-aptamers	56.2 ± 5.5	122.7 ± 2.0

Results and discussion

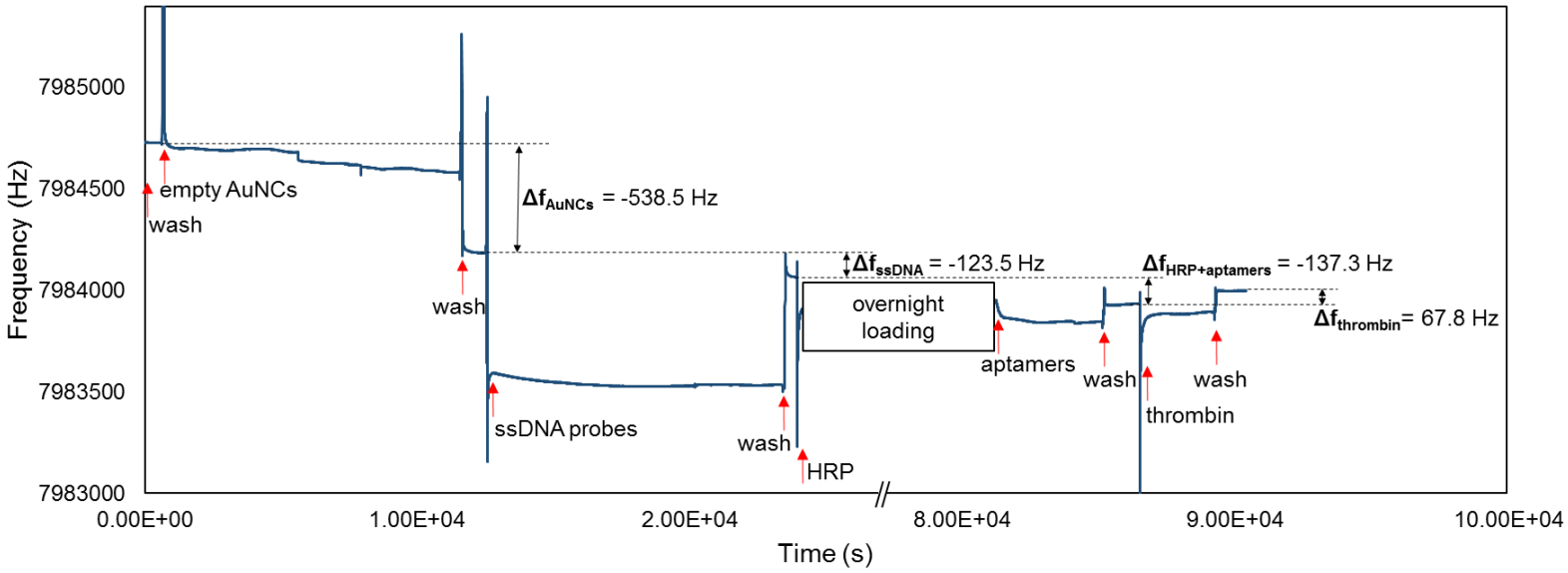
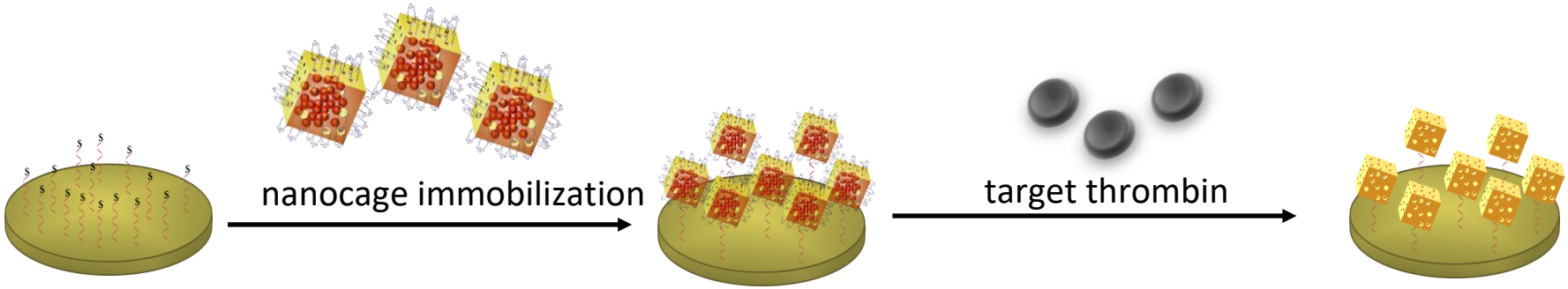


Fig. 6. Stepwise monitoring of empty AuNCs, ssDNA probes immobilized AuNCs, HRP loaded and aptamer capped AuNCs, and a typical thrombin detection.

Tab. 3. Negative control: comparison between empty and loaded AuNCs as biosensing elements

biosensing element	empty AuNCs	loaded AuNCs
signal (Hz)	1	68

Results and discussion



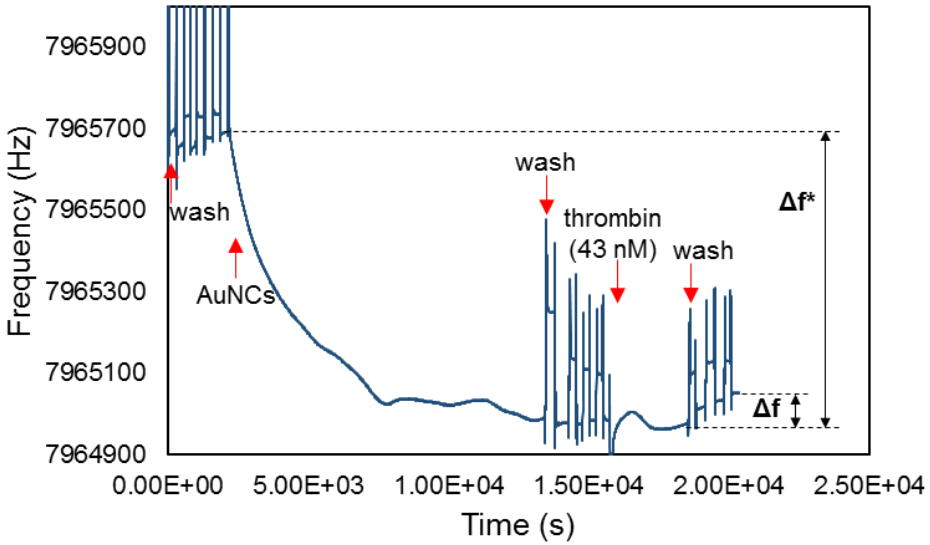


Fig. 7. A typical sensorgram of the QCM aptasensor, showing frequency changes of AuNC immobilization (ΔF*) and target detection (ΔF).

Results and discussion

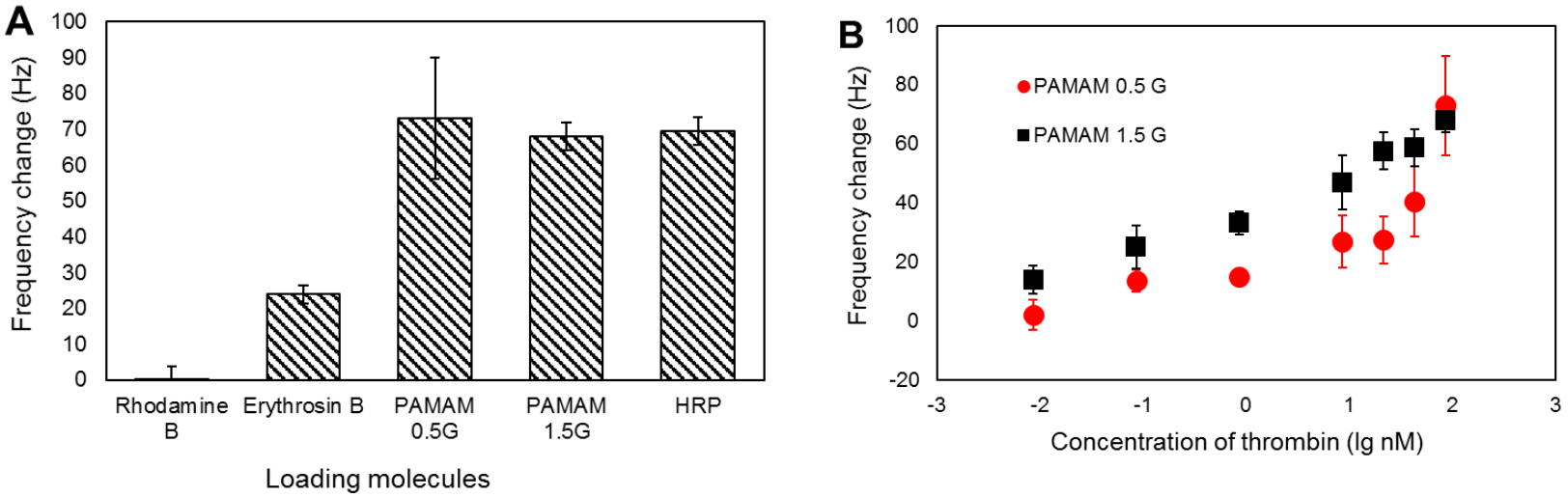


Fig 8. (A) Thrombin (86 nM) tested using different cargo molecules with the molecular weight ranging from 480 Da to 44,000 Da; (B) Comparison between PAMAM 0.5 G and PAMAM 1.5 G as cargo molecules in thrombin detection, with the concentration of thrombin ranging from 0.0086 nM to 86 nM.

Results and discussion

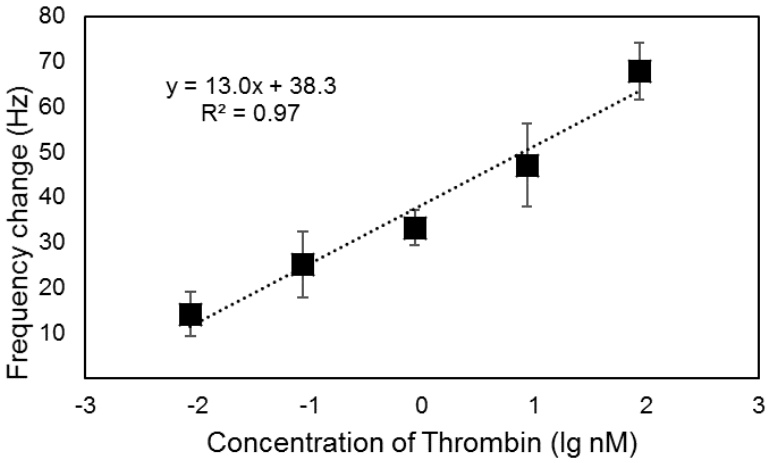


Fig. 9. Frequency change and lg thrombin concentrations from 0.0086 nM to 86 nM using PAMAM 1.5 G as cargo molecules.

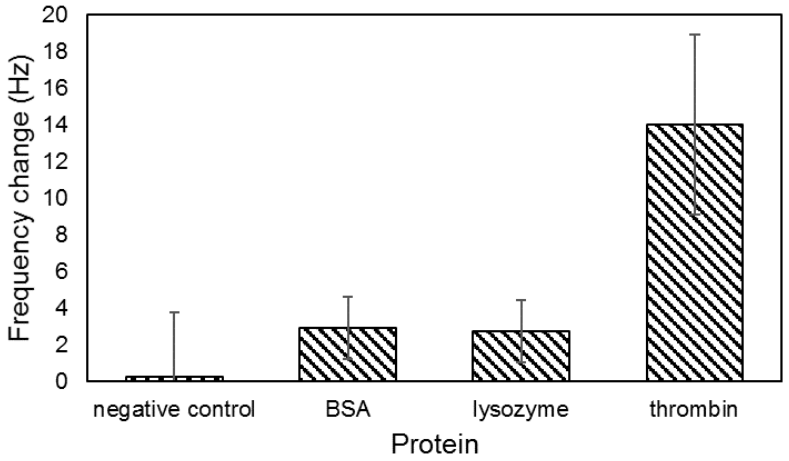


Fig. 10. Specificity test of the biosensor using BSA (1 µM) and lysozyme (1 µM), and comparison to thrombin (8.6 pM).

Conclusions

- By applying an innovative target-triggered release mechanism on a QCM platform, PAMAM loaded AuNCs capped with aptamers were developed as biosensing element for sensitive and specific response to target thrombin.
- The cargo molecules for AuNCs were selected based on their size, weight, and stability.
- The QCM biosensor had a good linear range from 0.0086 nM to 86 nM with a LOD of 0.8 pM, and the label-free platform requires simple operations and a detection time within 1.5 h.
- The biosensor had a good specificity under various interfering proteins.
- This platform has the potential to be utilized for other proteins with their related aptamers.

Acknowledgement

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Thank you!

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Lightning presentation and Q&A session
ITSC - Biosensor for Food, Agriculture and Environment
2:30pm – 3:30pm, Tuesday, July 13.