NANOPORE-BASED SENSOR FOR SEQUENCE-SPECIFIC MICRORNA DETECTION

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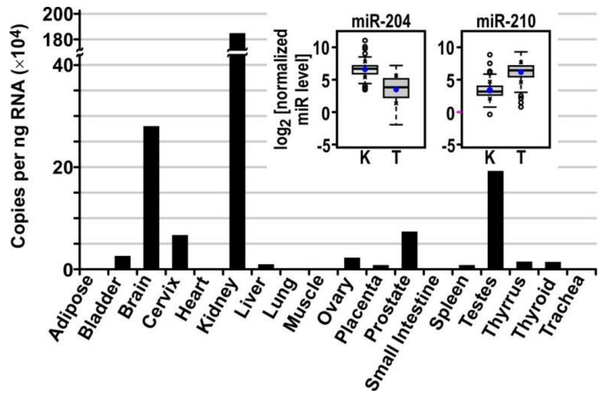
INTRODUCTION

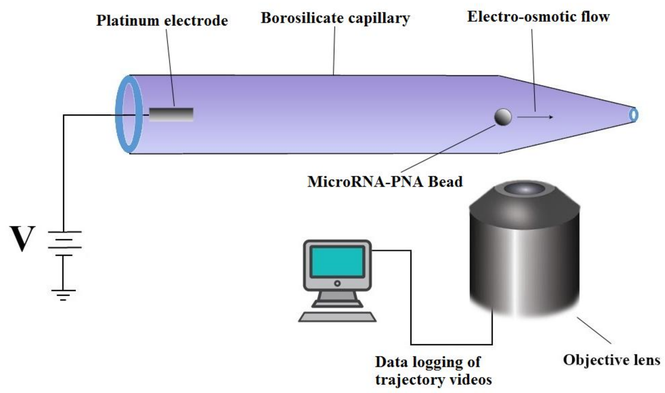
MicroRNAs (miRs) are small noncoding RNAs that play a critical role in gene regulation. Currently, qRT-PCR technology is considered as the "gold standard" for miR detection due to its high sensitivity and specificity. However, this technique requires time-consuming and expensive amplification steps. Therefore, there is a clear and pressing need for the **design and development of an accurate handheld miRs screening device that is cost-effective, easy to operate, and generates results within minutes.**

Clear Cell Renal Cell Carcinoma (CcRCC) is one kind of malignant kidney cancer. Here, we are targeting two miRs highly relevant for growth of ccRCC: (a) **miR-210**, which has pro-oncogenic activities and (b) **miR-204**, which has tumor suppressing activities.

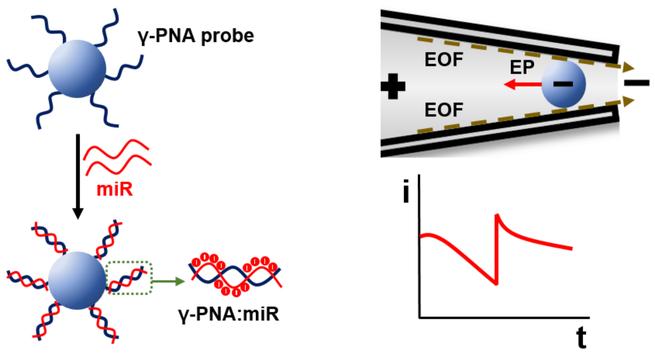


OBJECTIVES

An **electroosmotically driven nanopore-based sensor** and an assay of **γ-PNA probes** conjugated beads to detect two miRs, miR- 204 and miR-210 at fM detection limit.



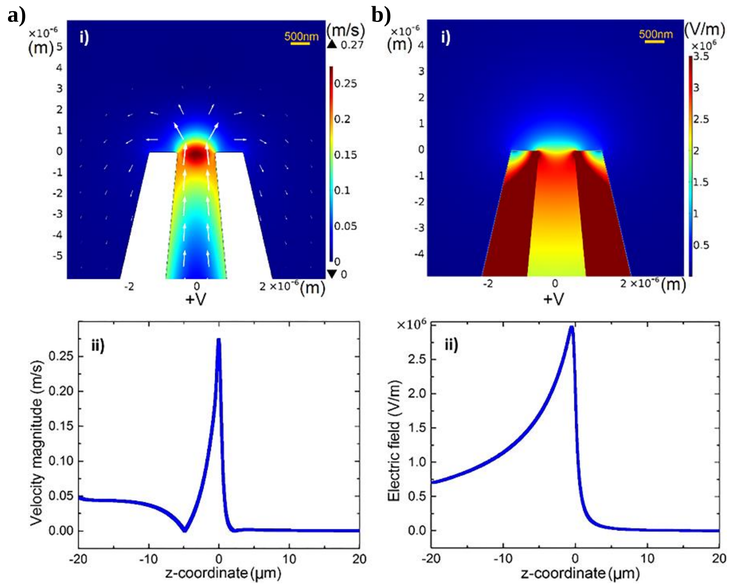
MiR-PNA-beads reach to the sensing zone under the applied DC bias and result in pore blockades with unique and easily distinguishable **serrated shape electrical signals.**



NOVELTY OF DETECTION PRINCIPLE

a) Electroosmotic flow (EOF) is induced as the driving force to transport miR-PNA-beads the sensing zone.

b) The conical shape of the pore leads to a non-uniform distribution of electrical field.



RESULTS AND DISCUSSION

Sensing experiments have been conducted with **1μm diameter pore blockades by 2.36μm beads**

a) Target experiment: miR-204 hybridized with PNA_{204}-beads. ***Serrated shaped ionic current drops***

b-d) Control experiments: non-complementary RNA (NC-RNA) hybridized with PNA_{204}-beads, PNA_{204}-beads without any RNA oligomer and miR-204 hybridized with non-complementary PNA (NC-PNA)-beads. ***Right-angle shaped ionic current drops***

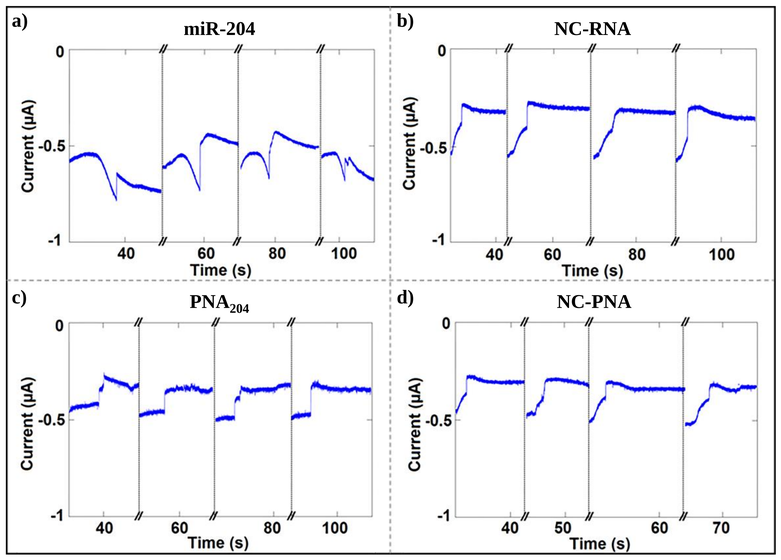


Table 1. Current blockades obtained by miR-204 detection experiments

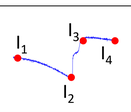
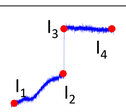
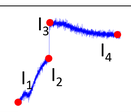
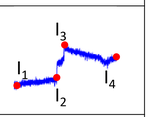
Current	miR-204	NC-RNA	PNA_{204}	NC-PNA
blockade shape				
I_1 (μA)	-0.5103	-0.5654	-0.4334	-0.4289
I_2 (μA)	-0.6885	-0.4675	-0.3252	-0.4166
I_3 (μA)	-0.4057	-0.3181	-0.2401	-0.3471
I_4 (μA)	-0.5194	-0.3155	-0.2685	-0.3448

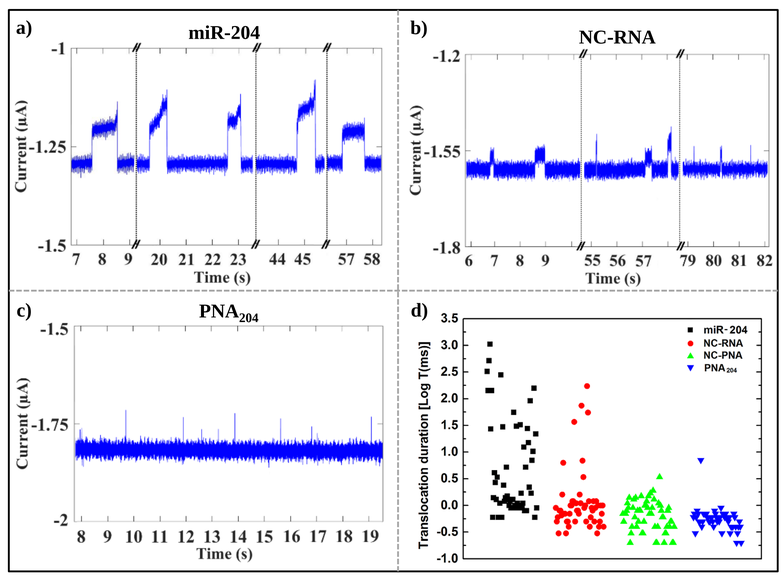
Table 2. The LOD of the system for detection of miR-204.

Concentration	miR-204		NC-RNA		NC-PNA	
						
100[nM]	22	1	5	16	0	19
1[pM]	18	0	0	23	0	22
100[fM]	11	0	0	18	0	23
10[fM]	13	0	0	33	0	23
1[fM]	11	1	0	17	0	17
0.1[fM]	2	20	0	21	0	21

To further support our results, resistive-pulse experiment has been repeated with **miR-204 at 60nM hybridized with 0.97μm beads translocating through a 1μm pore** and analyzed the duration of the resistive pulses.

Different velocity of beads carrying target miR and non-complementary RNA due to opposing electrophoretic force:

$$v_{target} < v_{non-complemetary\ RNA} < v_{PNA}$$



CONCLUSION

- A sensitive and robust nanopore-based sensing scheme has been elucidated here for small RNA detection and shown great detection sensitivity to fM level.
- The unique current blockades shapes and the long pulses dwelling times represent the miR detection by our system.
- The concentration detection limit of our sensor are 1fM for miR-204 and 10fM for miR-210.
- Detection results exhibit a high accuracy of our sensor with 97.6% in 87 experiments.
- It has a potential to be further evolved into a quantitative measurement tool for analysis of miR biomarkers in basic and clinical research.

REFERENCES

1. "VHL-Regulated MiR-204 Suppresses Tumor Growth through Inhibition of LC3B-Mediated Autophagy in Renal Clear Cell Carcinoma," O. Mikhaylova, Y. Stratton et al, Cancer Cell, 21 (4), 532–546 (2012).

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