Nanorobotic Sensors as a Novel Platform for Nucleic Acid Biomarker Detection in Blood

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Background: The ability to detect nucleic acid-based biomarkers from circulating blood is paramount for numerous conditions like cancer where early diagnosis is critical for better patient prognosis. Current nucleic acid detecting techniques are costly, time consuming, require multiple pre-assay sample preparation steps, specialised equipment and complex data interpretation software. **Aim:** Here, we utilize Nanovery nucleic acid biomarker detection kits as diagnostic devices to detect spiked biomarkers in serum, complex media and Nanovery Buffer®. **Experimental procedures:** The nanorobot is assembled based on 4 interchangeable modules namely detector, translator, amplifier and responder. We utilize artificial intelligence to efficiently design our nucleic acid-based detectors against target miRNAs and mutations in mRNA and ctDNA. The remainder 3 modules are tailored to the sample characteristics to ensure maximum sensitivity and selectivity. **Conclusion:** Our Nanorobots can be used across different sample types, from complex media to serum, for the detection of nucleic acid biomarkers.

Introduction

Motivation: Cancer remains one of the leading causes of morbidity and mortality worldwide accounting for nearly 10 million deaths and about 19 million new cases registered in 2020 alone (GLOBOCAN 2020).

Despite the current efforts in the drug development front, routine screening of high-risk individuals (as per NHS recommendation) remains the best disease management strategy as treatment is most effective in early-stage cancers.

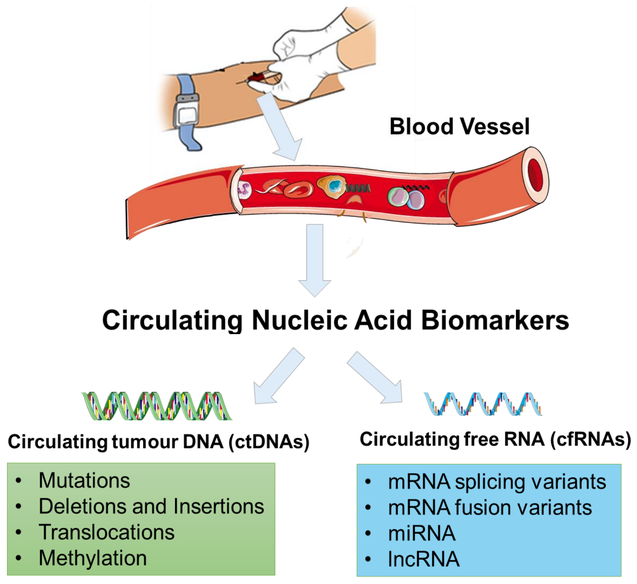

Fig 1. *Overview of liquid biopsy and potential nucleic acid biomarkers.*

Why: The analysis of different types of nucleic acid biomarkers including circulating tumour DNA (ctDNA), mRNA and miRNAs are emerging as breakthrough non-invasive clinical tools (i.e., a liquid biopsy) with the potential for molecular level cancer screening. However, traditional sequencing based diagnostic solutions like PCR and next generation sequencing have a limiting utility for rapid and cost-effective screening due to their complex workflow, high cost (£5,500/test) and time consuming (1-2 weeks) nature.

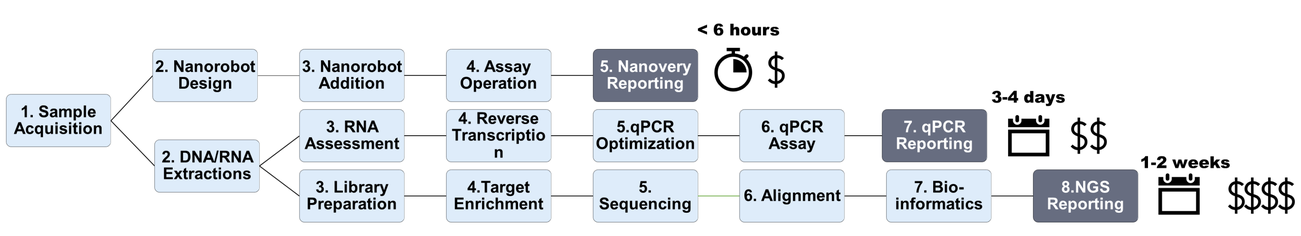

Fig 2. *Comparison of Nanovery Nanorobot workflow to next generation sequencing (NGS) and polymerase chain reaction (PCR).*

What: Nanovery overcomes the previous wide-spread implementation limitations as our point-of-care diagnostic technology is 10X cheaper (£500/test) and 100X faster (1hr). Our solution is based on pioneering DNA nanotechnology/DNA computing techniques to create nanorobots that distinguish between mutated and wild type nucleic acids in blood. Our nanorobots rapidly produce a fluorescent signal upon recognizing a biomarker thus enabling prompt clinical response. Furthermore, we leverage from multifaceted AI techniques which allow us to flexibly re-design our nanorobots as cancer mutates thus enabling a truly personalized patient care. Our technology is highly specific, enables screening, monitoring and direct treatment decisions.

Materials and Methods

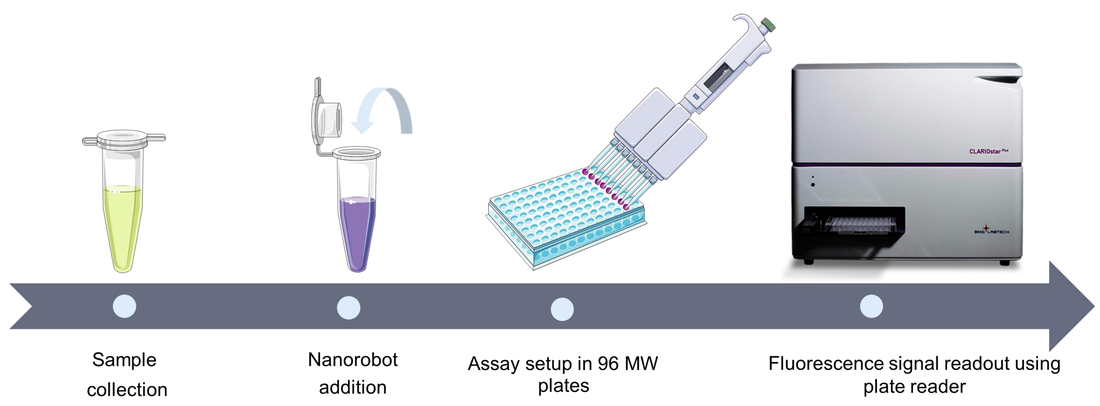

Fig 3 *Single sample analysis with Nanovery workflow.*

Study Design: To demonstrate that our technology can perform in a variety of complex environments synthetic biomarker mimics were spiked in serum, complex media and Nanovery Buffer®. The biomarkers tested were miR21, miR122 and AR-V7. These biomarkers are implicated in several types of cancer, including prostate and liver. Subsequently, our nanorobots were added to the above-mentioned samples and their performance was measured by monitoring the biomarker-induced fluorescence increase as a function of time.

Nanorobot Assembly: Our DNA nanorobots are designed with the aid of molecular simulations & AI for optimal performance. The current prototypes employ a modular design with 4 interchangeable modules namely detector, translator, amplifier and responder, for a customizable performance.

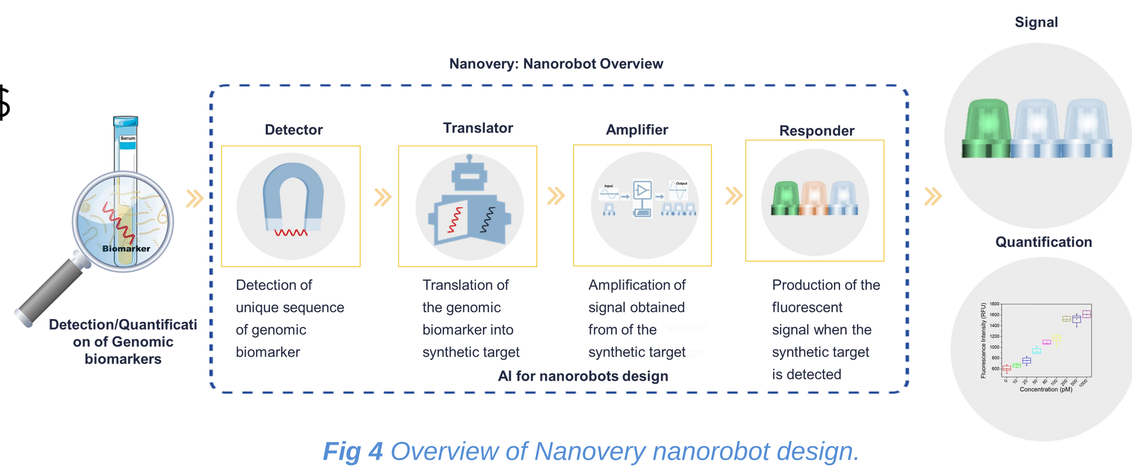

Fig 4 *Overview of Nanovery nanorobot design.*

Measurement: For easy implementation, our assays are designed for the standard 96 MW plate format and conventional plate reader set up for measuring relative fluorescence units or Förster resonance energy transfer (FRET) events.

Results

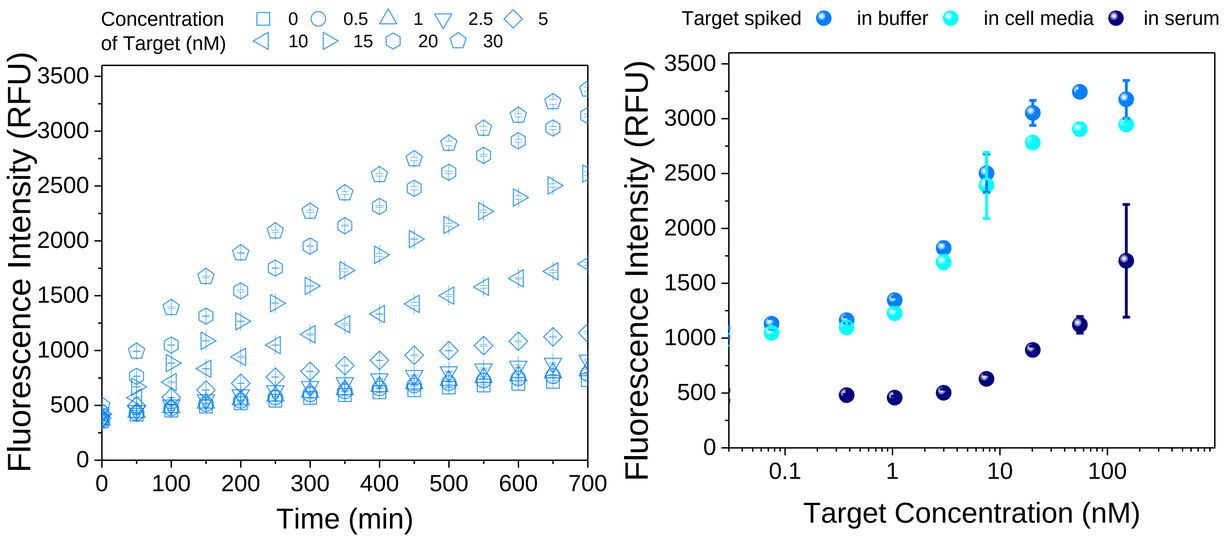

Fig 5. *Preliminary experimental results on nanorobot device comprised of detector and amplifier modules. (right) Representative fluorescence measurements for nanorobot device with varying concentrations of the synthetic nucleic acid targets as a function of time in cell media, (left) dynamic range of Nanorobot device with varied concentration of the synthetic nucleic acid target in a different media (Nanovery buffer, complex media and human serum).*

Summary

Nanorobot series	Module Pack	Amplification	Lower limit of detection (LOD)	Assay time	Media compatibility
Nano α-Bot S1	Detection+ Translation	No amplification	> nanomolar	30 min	Serum Cell extract Synthetic soup
Nano β-Bot S1	Detection+ Amplification	Catalytic	Picomolar (> 500 pM)	3 hrs	Cell media Buffer
Nano β-Bot S2	Detection+ Amplification	Entropy Driven Catalysis	Picomolar (<500 pM)	3 hrs	Drug rich media Buffer
Nano Ω-Bot S1	Detection+ Amplification + Nanomaterials (signal booster)	Catalytic	Femtomolar	3.5 hrs	Serum Drug rich media Buffer

Table 1. Overview of Nanovery's nanorobot prototype series.

At Nanovery we develop nucleic acid biomarker detection kits with different features for personalised applications. Our current prototypes are summarised in table 1. With the aid of complex AI techniques and machine learning we will provide customised and cost-effective solutions to clinical and research partners.

Acknowledgements

Newcastle University / Centre for Cancer

UKRI

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