Novel screening platform for highly multiplexed biomarker analysis

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ABSTRACT | To date, most diagnostic tests rely upon the detection of a limited number of biomarkers. This often results in a lack of specificity to provide enough clinically useful detail on the pathology. To overcome this challenge, we developed a new strategy, which combines nanopore sequencing[1,2] with barcoded nucleic acid probes, engineered to recognise a panel of biological targets (miRNAs, proteins, and small molecules). Our workflow is rapid, from sample preparation to results in 1 hour. The established method is easily adaptable, as the number and type of targets can be greatly expanded. Preliminary results indicate a significant advantage over existing techniques, which makes us believe that this approach could have an extensive impact on diagnostics and facilitate the transition to personalised medicine[3].

CONCEPT

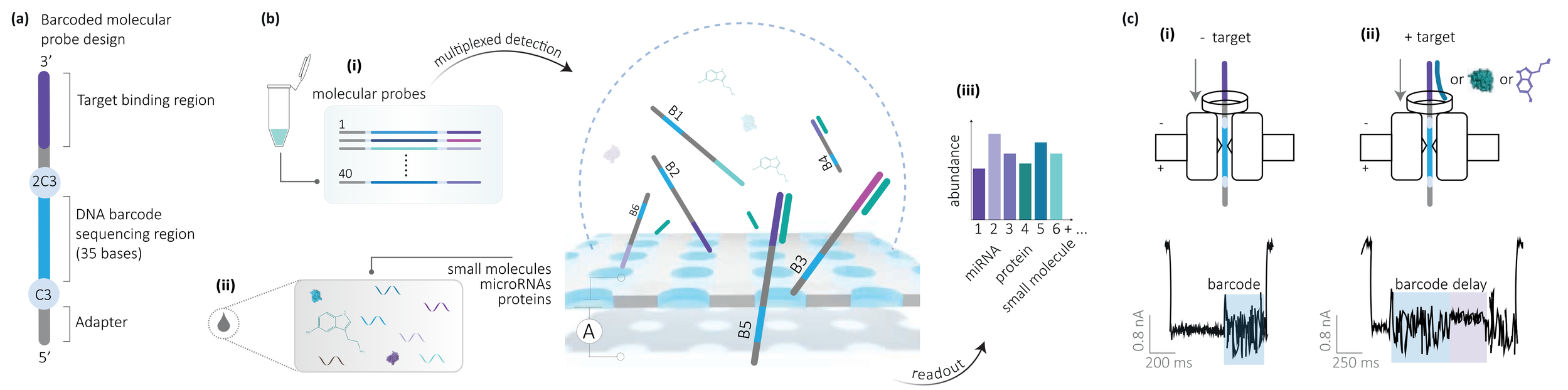

Fig. 1 Concept of multiplexed biomarker detection. (a) Barcoded molecular probe design[4]. The barcoded molecular probe consists of mainly three parts: 1) a Y-adapter (ONT), 2) the DNA barcode sequencing region (also called barcode), and 3) the target binding region (complementary miRNA sequence or aptamer). **(b) Workflow. (i)** Barcoded molecular probes were incubated with synthetic targets or **(ii)** serum samples. Nanopore sequencing of the barcode was performed to classify the barcoded molecular probe, and **(iii)** the presence of targets was determined by delay analysis. **(c) Delay analysis. (i) Without target.** Analysis of events without target result in current traces without delay. **(ii) With target.** Analysis of events with analyte-bound result in current traces with delay, due to the unzipping of the double-strand or unraveling of the aptamer.

MULTIPLEXED DETECTION OF miRNAs, PROTEINS, AND SMALL MOLECULES

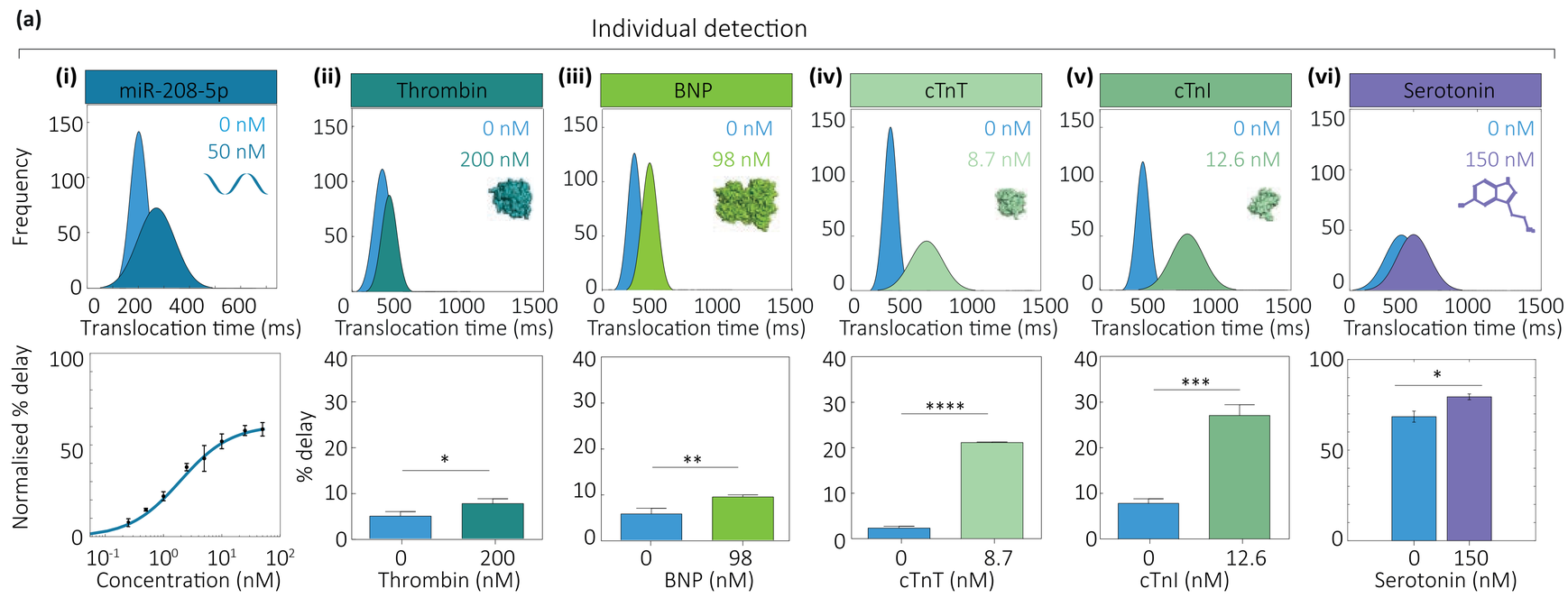

Fig. 2 Demultiplexing of miRNAs, proteins, and small molecules. (a) Individual detection of biomarkers. Specific barcoded molecular probes (30 nM) +/- corresponding **(i)** miRNA-208-5p, **(ii)** Thrombin, **(iii)** BNP, **(iv)** cTnT, **(v)** cTnI, and **(vi)** serotonin. Significance was determined by Student's t-test (*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001, N=3, n=1024-3395). **(b) Multiplexed detection of biomarkers.** Low concentrations (10 nM miR-208-5p, 150 nM serotonin, 300 nM thrombin) and high concentration (50 nM miR-208-5p, 750 nM serotonin, 1500 nM thrombin) were compared to the normalised control (no biomarker present). Significance was determined by ANOVA (*p≤0.05, **p≤0.01, ***p≤0.001, **** p≤0.0001, N=3, n=1253-8197).

MULTIPLEXED QUANTIFICATION OF 40 miRNAs

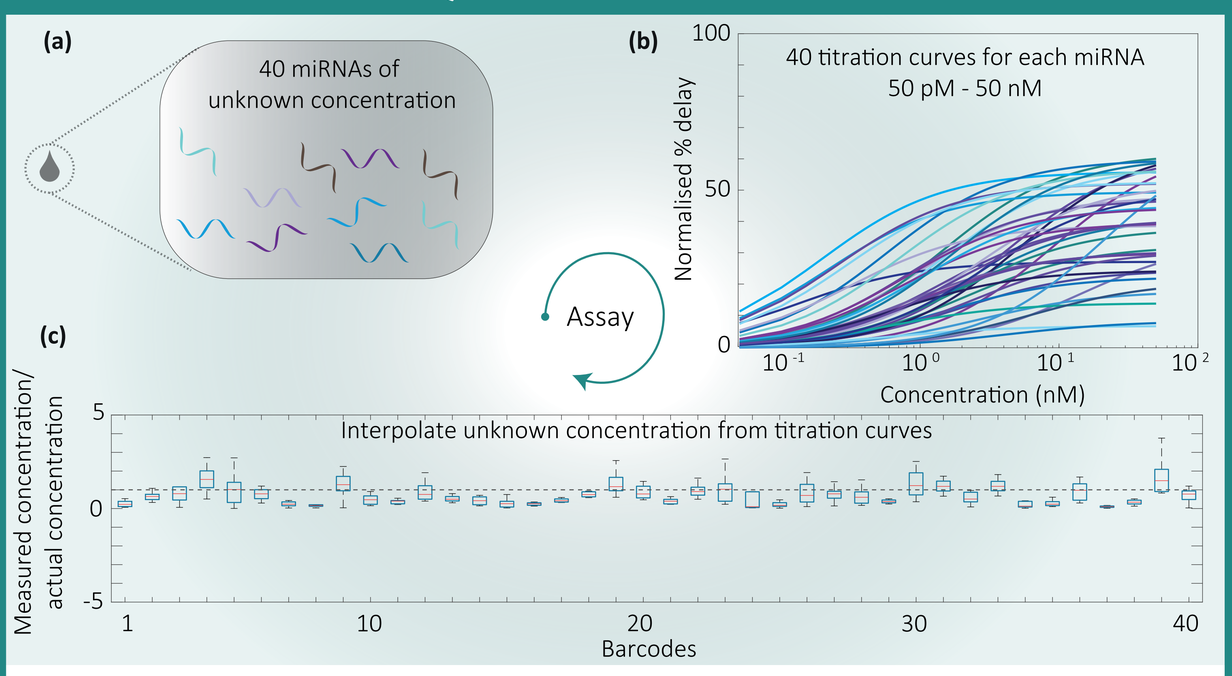

Fig. 3: Quantification of 40 miRNAs. (a) Unknown concentration of 40 miRNAs was quantified using **(b) Concentration-/percentage delay curves** of 40 individual barcoded molecular probes derived from a multiplexed experiment (N=5, n=26798-308954). **(c) Single-blinded prediction** of miRNA concentration showing the value for (precited/actual concentration) (N=4, n=6189-71031).

CLINICAL SAMPLES

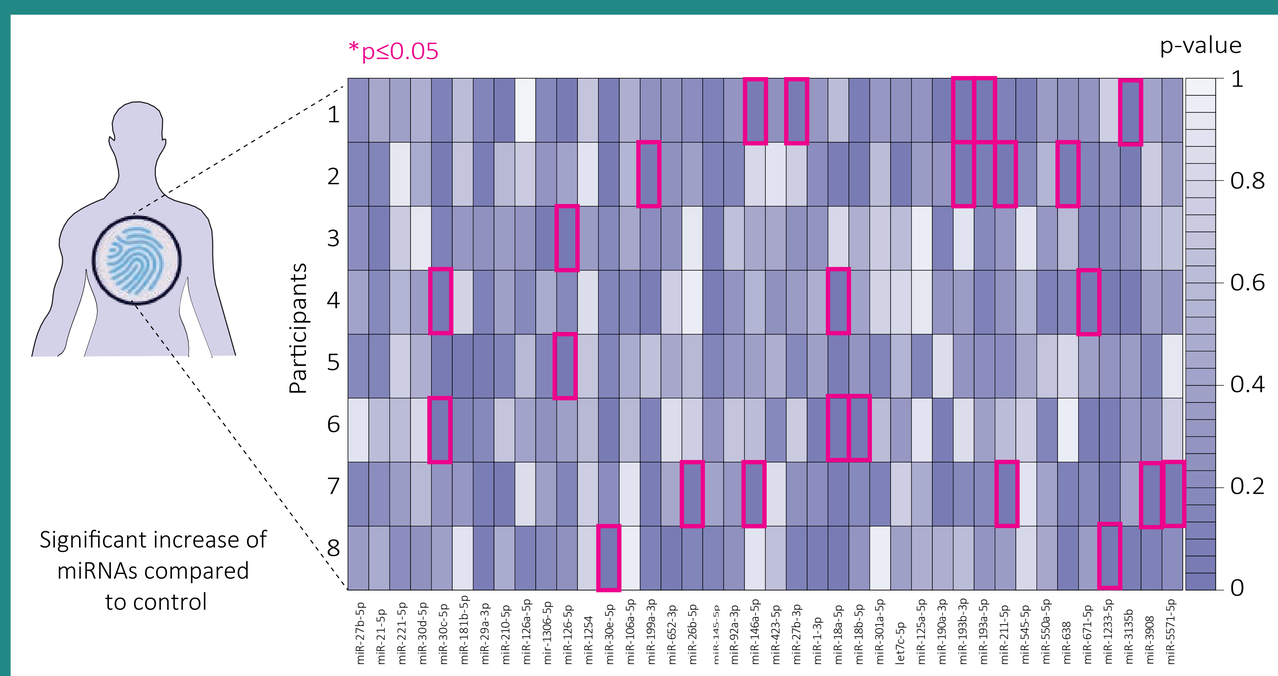

Fig. 4: Detection of 40 miRNAs directly in serum. Heatmap of p-values comparing percentage delayed events +/- serum (Student's t-test, N=3, n=17930-25776).

CONCLUSION AND FUTURE WORK

- Individual detection of 40 miRNAs, 4 proteins and 1 small molecule
- Multiplexed detection of miR-208-5p, thrombin and serotonin
- Multiplexed detection and quantification of an unknown concentration of 40 miRNAS
- Detection of miRNAs directly in serum
- Quantification of miRNAs in serum
- Detection of proteins and small molecules in serum
- Quantification of proteins and small molecules
- Transition to kit14 chemistry (ONT) to improve sensitivity
- Expansion of biomarker targets

ACKNOWLEDGEMENT AND REFERENCES

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Publication

1 Kasianowicz JJ, Brandin E, Branton D, Deamer DW. (1996) Characterization of individual polynucleotide molecules using a membrane channel. *Proc Natl Acad Sci U S A.*
2 Deamer D, Akeson M, Branton D. (2016) Three decades of nanopore sequencing. *Nat Biotechnol.*
3 Koch C. ***et al.*** (2022) Highly multiplexed detection of microRNAs, proteins and small molecules using barcoded molecular probes and nanopore sequencing, ***Nat Nanotechn.*** (under review)
4 Heron, A. Gutierrez, R. Edel, J. Ivanov, A. Koch, C. Xue, L. Reilly-O'Donnell B. (2022) 'Multiplex methods of detecting molecules using nanopores', PCT/GB2022/051268, WO2022243691A1