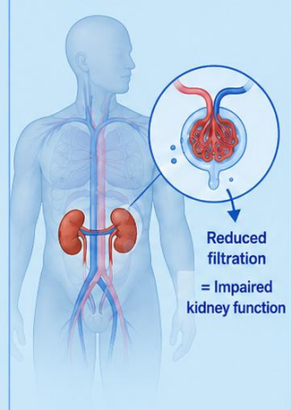
Solid-State Nanopore-Based Detection of Acute Kidney Injury-Related RNA Biomarkers

Jay Shen[1], Joshua B. Edel[1], Aleksandar Ivanov[1]
[1]Department of Chemistry, Imperial College London, London, United Kingdom

I. Introduction

1 What is AKI?

Acute kidney injury (AKI) is a rapid decline in kidney function occurring over hours to days.



2 Clinical importance

Common in hospital and ICU patients

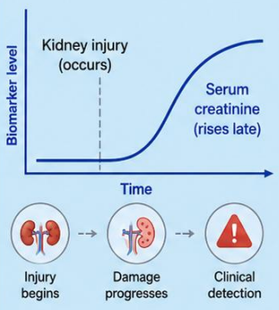
Associated with high morbidity and mortality

Early detection is critical for timely intervention

AKI increases risk of prolonged hospitalization, dialysis, and death.

3 Current diagnostic limitation

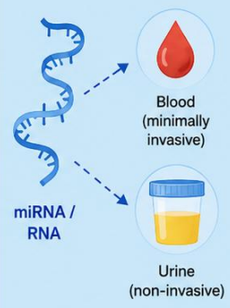
Conventional markers (e.g. serum creatinine) often detect AKI only after kidney damage has progressed.



Delayed detection limits the window for effective treatment.

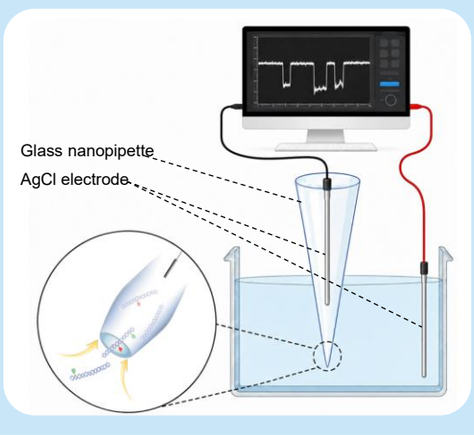
4 Why RNA biomarkers?

AKI-related RNA biomarkers may enable earlier and more specific detection than conventional markers.



Example AKI-related targets
miR-7-5p • miR-382

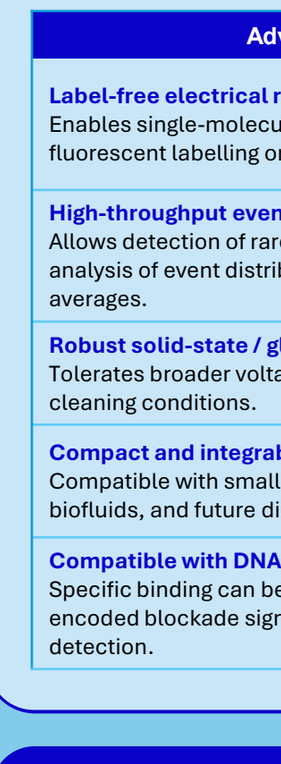
II. Solid-State Nanopore Platform

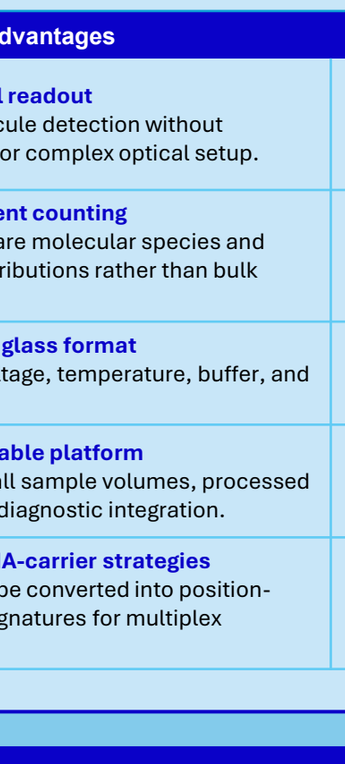


Solid-state nanopore sensing is an electrical single-molecule detection method. **A nanometre-scale pore**, formed in a robust solid material such as silicon nitride, silicon dioxide, or a pulled glass nanopipette, separates two electrolyte reservoirs.
When a voltage is applied across the pore, ions pass through the constriction and generate a stable baseline current. The translocation of a biomolecule transiently blocks ion flow, producing a **current blockade event**. Key event features, including blockade **depth, dwell time**, and event shape, can be extracted from the current trace to identify molecular populations and binding states.

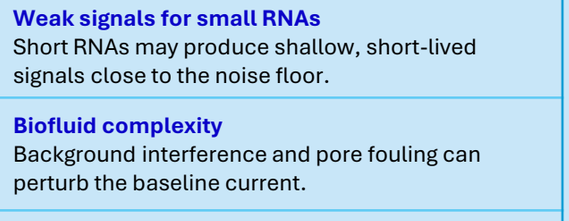
Advantages	Limitations
Label-free electrical readout Enables single-molecule detection without fluorescent labelling or complex optical setup.	**Higher electrical noise and variability** Solid-state nanopores show greater device-to-device variation and noise compared with biological nanopores.
High-throughput event counting Allows detection of rare molecular species and analysis of event distributions rather than bulk averages.	**Limited intrinsic selectivity** Analytes with similar size or charge can generate similar current blockade signals.
Robust solid-state / glass format Tolerates broader voltage, temperature, buffer, and cleaning conditions.	**Weak signals for small RNAs** Short RNAs may produce shallow, short-lived signals close to the noise floor.
Compact and integrable platform Compatible with small sample volumes, processed biofluids, and future diagnostic integration.	**Biofluid complexity** Background interference and pore fouling can perturb the baseline current.
Compatible with DNA-carrier strategies Specific binding can be converted into position-encoded blockade signatures for multiplex detection.	**Requires engineered recognition / classification** Advanced molecular design and event analysis are needed to distinguish unbound, singly bound, and multiply bound carrier states.

III. Carrier Design



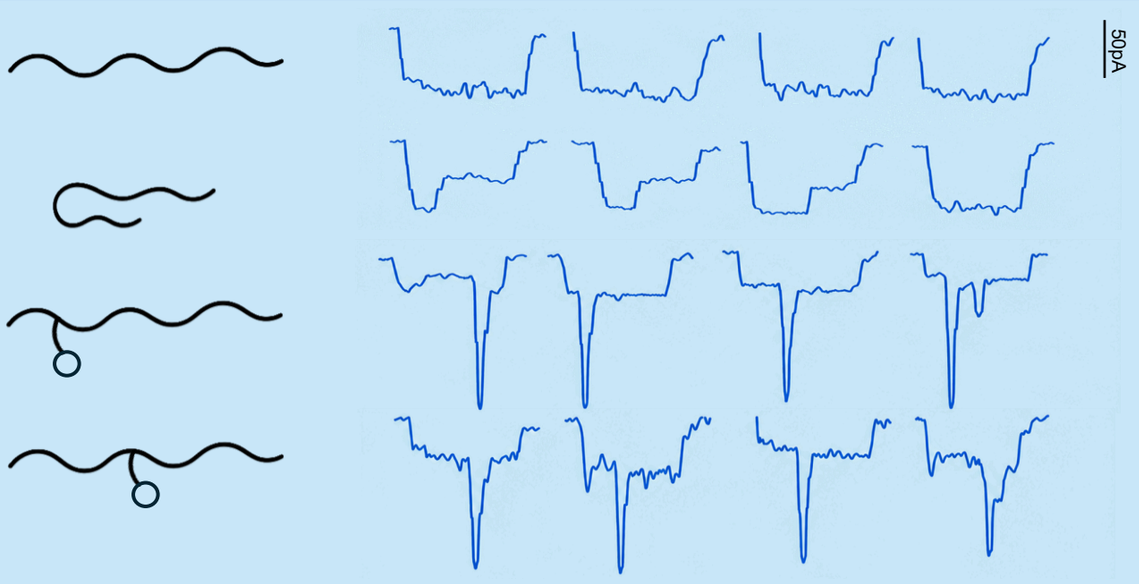


A 9.1 kbp λ-DNA-derived carrier was used as a long, well-defined scaffold for dual-miRNA recognition. Two spatially separated probe sites were engineered along the carrier backbone, with each site carrying a sequence-specific DNA probe for either miR-7-5p or miR-382. Upon target hybridisation, a local probe–miRNA duplex is formed. The assay uses a sandwich structure in which the DNA probe provides sequence selectivity, the target miRNA bridges the recognition complex, and a biotin–streptavidin reporter introduces a bulky structural tag. This reporter amplifies the local size of the bound RNA complex, making short miRNA targets more detectable by nanopore sensing.

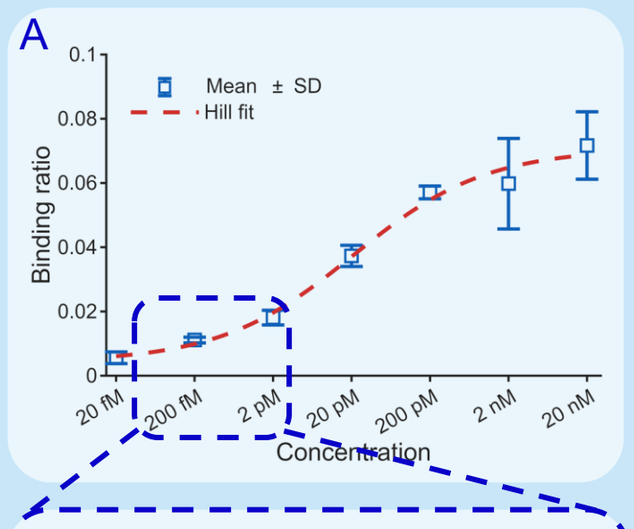


P0.2	enzyme	Nt.BsmAI	5'-AAGCGAGATGG/G **GAGAC** AGGGCTGCATGA-3' 3'-TTCGCTCTACCC **CTCTG** TCCCGACGTACT-5'
	analyte	hsa-miR-7-5p	UGGAAGACUAGUGAUUUUGUUGUU
P0.5	enzyme	Nb.BtsI	5'-ATCGATACCGGC **GCAGTG** TTCTGCGGAAAC-3' 3'-TAGCTATGGCCG **CGTCAC**/ AAGACGCCTTTG-5'
	analyte	hsa-miR-382-5p	GAAGUUGUUCGUGGUGGAUUCG

IV. Typical Events



V. Result



A. Concentration-dependent binding response.
Binding ratio increased with target concentration and was fitted using the Hill equation.
B. Limit of detection (LOD).
The LOD was estimated from the intersection between the Hill fit and the 3σ threshold, giving a value of 82.4 fM.
C. Voltage dependence of sensing signals.
Increasing voltage decreased dwell time while increasing capture rate.
D. Carrier selectivity test.
The carrier showed a stronger response to miR-7.5p than to the control and miR-382, indicating selective target recognition.

Voltage dependence

Voltage-dependent measurements were used to determine the operating condition for subsequent nanopore experiments. Increasing the voltage from 200 to 400 mV increased the capture rate but shortened the dwell time, indicating a trade-off between detection efficiency and event resolution. Therefore, 300 mV was selected for later measurements, as it provided a sufficiently high capture rate while maintaining clear and distinguishable traces.

Sensitivity and LOD

Sensitivity measurements were performed under high-salt conditions to enhance ionic current contrast and improve event detection. LiCl was added to prolong translocation time, further improving trace clarity and event identification. The binding ratio increased with analyte concentration and followed a Hill-type response, suggesting saturable, concentration-dependent formation of the analyte–carrier–biotin complex. To balance assay efficiency and reagent consumption, the analyte concentration used in later experiments was set to 2 nM, corresponding to a 10-fold excess over the carrier concentration. The LOD was estimated using the group's standard 3σ-based method, and the result was close to the value back-calculated from the Hill fit, supporting the reliability of the sensitivity analysis.

Selectivity

The selectivity experiment was designed to compensate for the intrinsic limitation of nanopores, which cannot distinguish nucleic acid sequences by themselves. The carrier produced a significantly higher binding ratio for the target analyte miR-7.5p than for the control, while the response to miR-382 remained close to the background level. The t-test results therefore indicate that the designed carrier can selectively recognise miR-7.5p and distinguish it from a similar non-target miRNA.

VI. Key Finding

1. Carrier-assisted platform
A carrier-assisted solid-state nanopore platform improves the sequence selectivity of RNA biomarker detection by guiding the analyte–probe complex through the nanopore and reducing non-specific translocation.

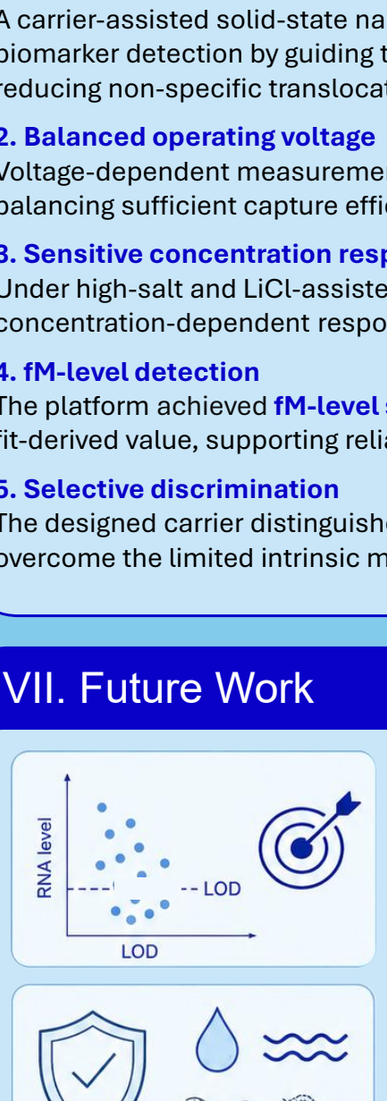
2. Balanced operating voltage
Voltage-dependent measurements identified **300 mV** as a practical operating condition, balancing sufficient capture efficiency with clear and well-resolved event traces.

3. Sensitive concentration response
Under high-salt and LiCl-assisted conditions, the analyte–carrier–biotin system showed a concentration-dependent response following a **Hill-type trend**.

4. fM-level detection
The platform achieved **fM-level sensitivity**, with an estimated LOD consistent with the Hill-fit-derived value, supporting reliable quantitative detection.

5. Selective discrimination
The designed carrier distinguished **miR-7.5p** from a similar non-target miRNA, helping overcome the limited intrinsic molecular specificity of nanopore sensing.

VII. Future Work



1. Clinical relevance

Compare the current limit of detection with reported RNA biomarker levels in AKI patient samples to assess diagnostic suitability.

2. Assay optimisation

Optimise ionic strength, LiCl concentration, voltage, incubation time, and analyte-to-carrier ratio to improve binding ratio and event quality.

3. Selectivity & matrix tolerance

Test more biological replicates, a broader non-target miRNA panel, and complex matrices such as diluted serum, plasma, and urine-like samples.

4. Carrier & microfluidics

Engineer carriers for higher capture efficiency, lower background binding, and better reproducibility; integrate microfluidics to reduce sample use and improve automation.

5. Platform engineering

Improve nanopore fabrication reproducibility, pore size and geometry control, device consistency, and anti-fouling / anti-clogging performance for robust long-term sensing.