Quantitative Detection of Biomolecules in Environmental Matrices Using Acoustic Wave Micropillar Biosensors

Calvin Ng and Nerissa Molejon

Environmental biosensing presents unique challenges due to the complexity of sample matrices, requiring highly robust biosensors. In this study, we introduce a portable acoustic biosensor designed for the quantitative detection of biomolecules in aqueous environmental samples. The biosensor was tested for the detection of SARS-CoV-2 viral loads in wastewater and demonstrated potential for identifying Vibrio parahaemolyticus in aquaculture systems. Bioprobes targeting nucleocapsid gene 1 (N1) in SARS-CoV-2 and tdh1 gene in V. parahaemolyticus were designed and immobilized on the biosensor's micropillar surface. Validation using plasmid-spiked aqueous matrices showed high sensitivity, with detection ranges of 100-3000 gene copies/mL in molecular water and 500-1000 gene copies/mL in wastewater for SARS-CoV-2, and 100-5000 gene copies/mL in molecular water for V. parahaemolyticus. The biosensor exhibited strong specificity and required minimal sample preparation, offering an advantage over conventional qPCR methods. These findings demonstrate the potential of this biosensor as a rapid, efficient tool for environmental monitoring and public health applications. The biosensor's flexible design allows for detection of other pathogens by replacement of target-specific capture bioprobes.

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Abstract

Environmental biosensing presents unique challenges due to the complexity of sample matrices, requiring highly robust biosensors. In this study, we introduce a portable acoustic biosensor designed for the quantitative detection of biomolecules in aqueous environmental samples. The biosensor was tested for the detection of SARS-CoV-2 viral loads in wastewater and demonstrated potential for identifying *Vibrio parahaemolyticus* in aquaculture systems. Bioreceptors targeting nucleocapsid gene 1 (N1) in SARS-CoV-2 and tdh1 gene in *V. parahaemolyticus* were designed and immobilized on the biosensor's micropillar surface. Validation using plasmid-spiked aqueous matrices showed high sensitivity, with detection ranges of 100-3000 gene copies/mL in deionized (DI) water and 500-1000 gene copies/mL in wastewater for SARS-CoV-2 N1, and 100-5000 gene copies/mL in molecular water for *V. parahaemolyticus* tdh1. The biosensor exhibited strong specificity and required minimal sample preparation, offering an advantage over conventional qPCR methods. These findings demonstrate the potential of this biosensor as a rapid, efficient tool for environmental monitoring and public health applications. The biosensor's flexible design allows for detection of other pathogens by replacement of target-specific bioreceptors.
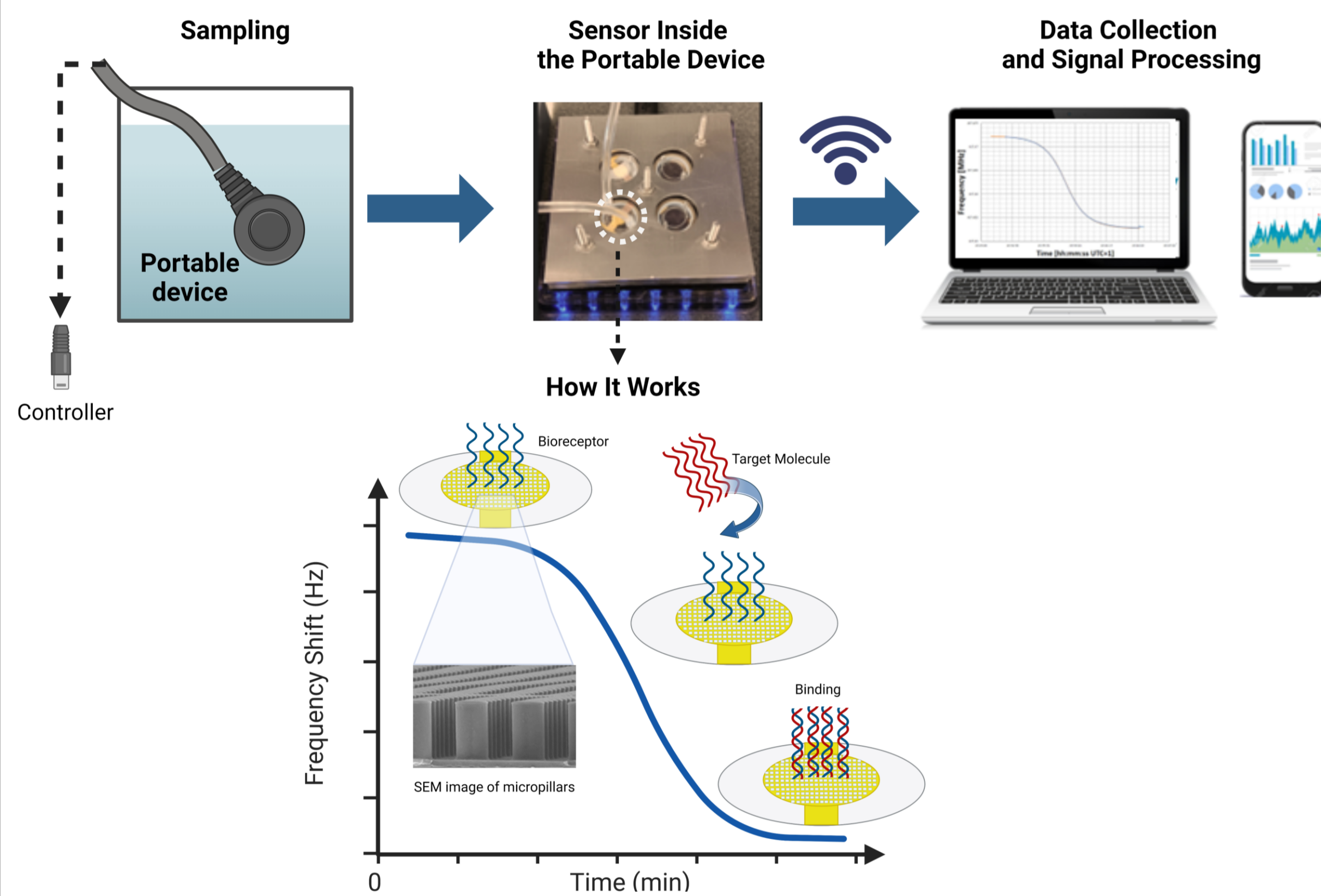
Introduction

Figure 1. Schematic diagram of biosensor deployment, which includes sampling, target detection, and data processing. The sensor is a quartz crystal microbalance (QCM) nanoimprinted with micropillars to create a two-degree-of-freedom resonant system, making it eight times more sensitive than a conventional QCM. The bioreceptor-target binding is detected by the sensor through a frequency shift caused by the mass change.

Objective

Investigate an application of an acoustic sensor as a low cost, ultra-sensitive, and field-deployable biosensor for the detection of biomolecules in environmental matrices.

Methodology

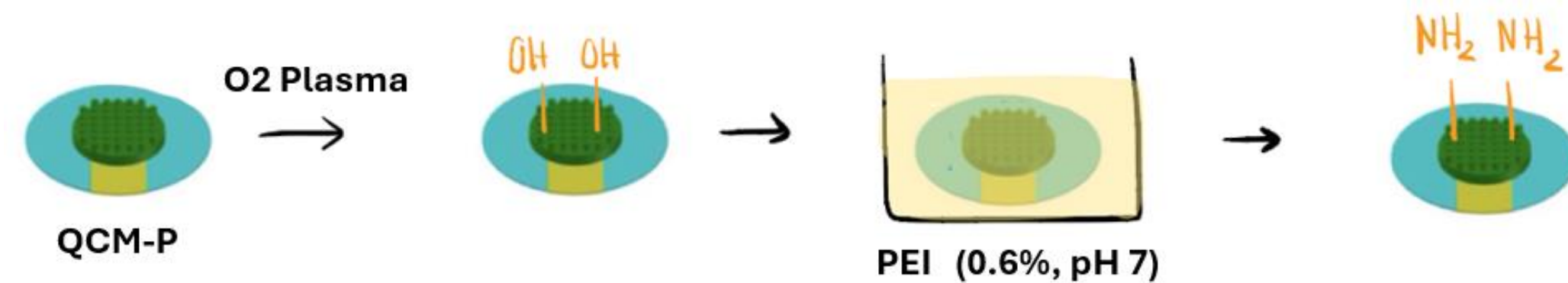### 1. Sensor Fabrication

Figure 2. Poly(methyl methacrylate) (PMMA) micropillars were nanoimprinted onto quartz crystal microbalance (QCM-P) followed by O_2 plasma treatment and immersion in polyethylenimine (PEI) (Su et al., 2018).
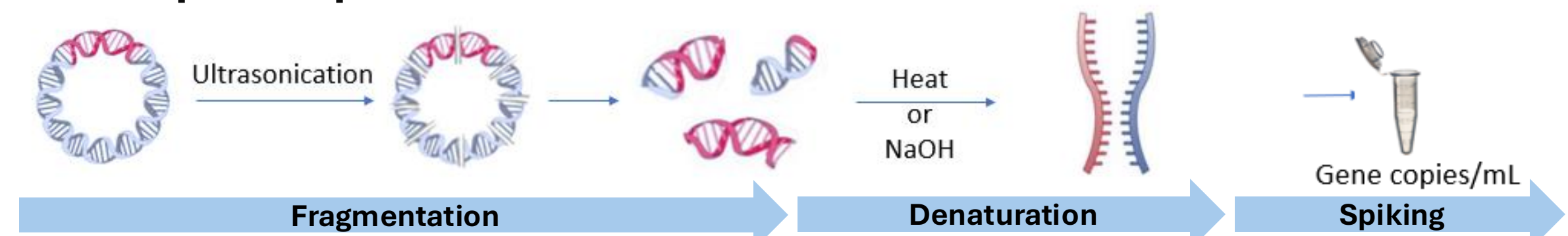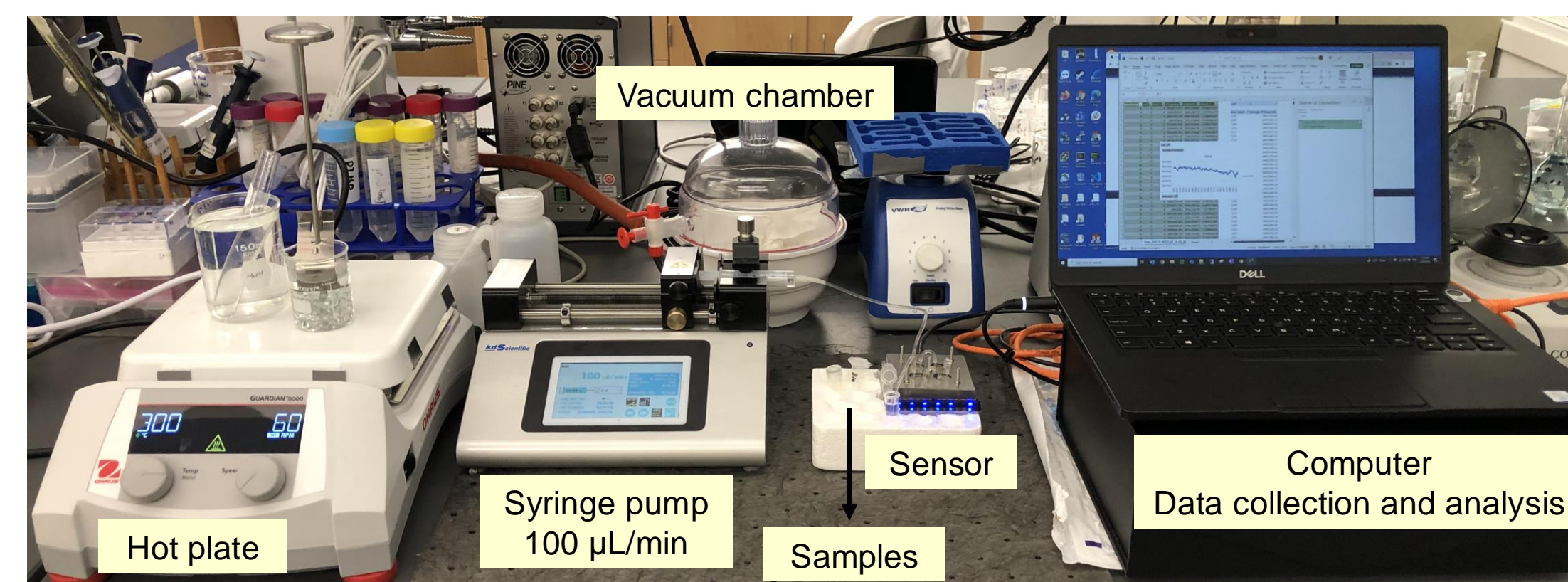
2. Sample Preparation

Figure 3. Preparation of ssDNA from fragmented and denatured plasmid.

Methodology

3. Experimental Laboratory Set-Up

- Bioreceptors were designed to be complementary to the target sequence and were bound to the sensor membrane.
- Water samples spiked with the target sequence were loaded to the biosensor at 100 μL/min for 6 minutes until stabilization was achieved.
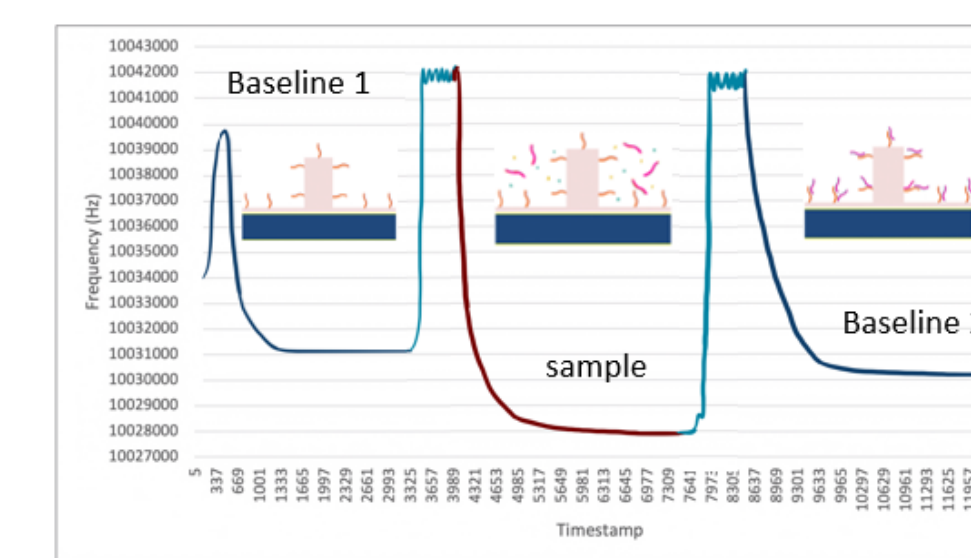
Figure 4. Experimental laboratory set-up (top) and data collection (bottom right).

Results
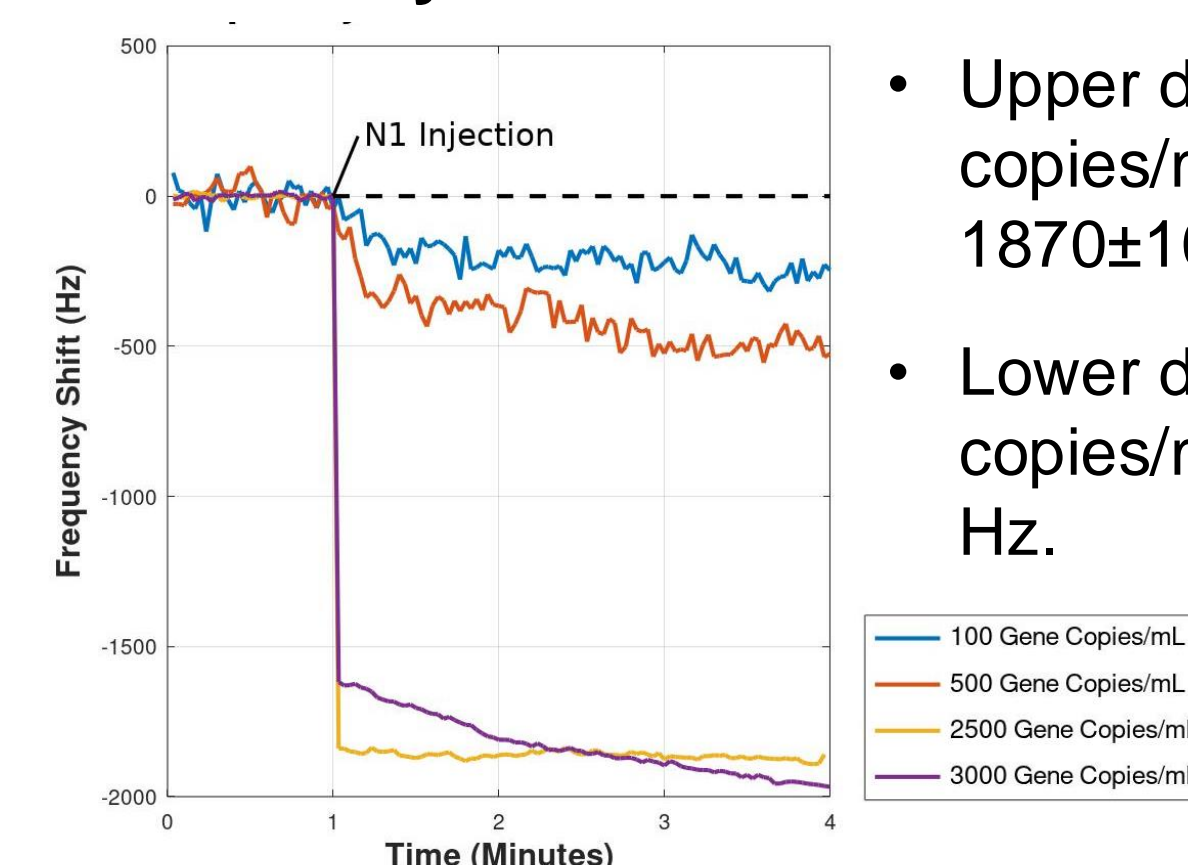
1. Sensitivity test on N1 in DI water

- Upper detection limit was 3000 gene copies/mL, with sensor readings of 1870±100 Hz.
- Lower detection limit was 100 gene copies/mL, with sensor readings of 216±76 Hz.

Figure 5. Frequency response of the sensor to N1 target in DI water at different concentrations.

2. Sensitivity test on N1 in raw wastewater

- Expected decrease in frequency shift due to interfering substances in the wastewater matrix. Test on two concentrations showed the sensor's sensitivity to N1 detection even at complex solutions
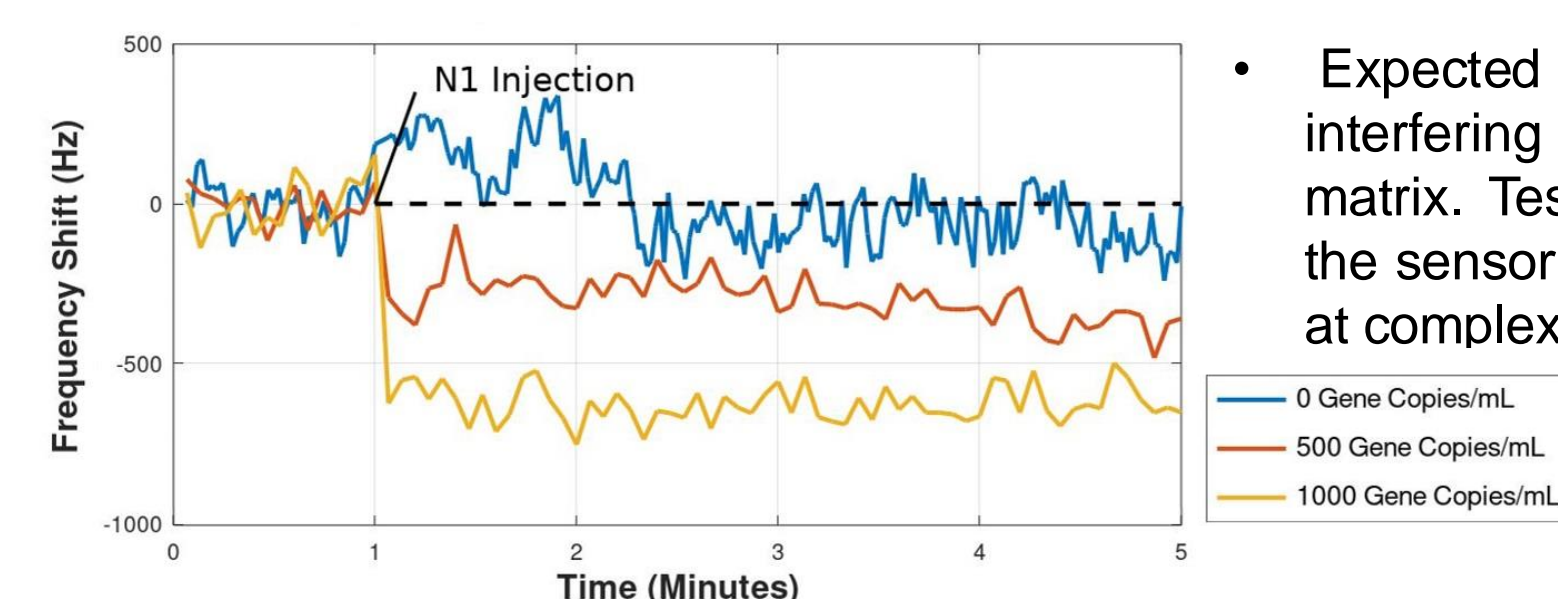
Figure 6. Frequency response of the sensor to N1 target in wastewater at different concentrations.

Results

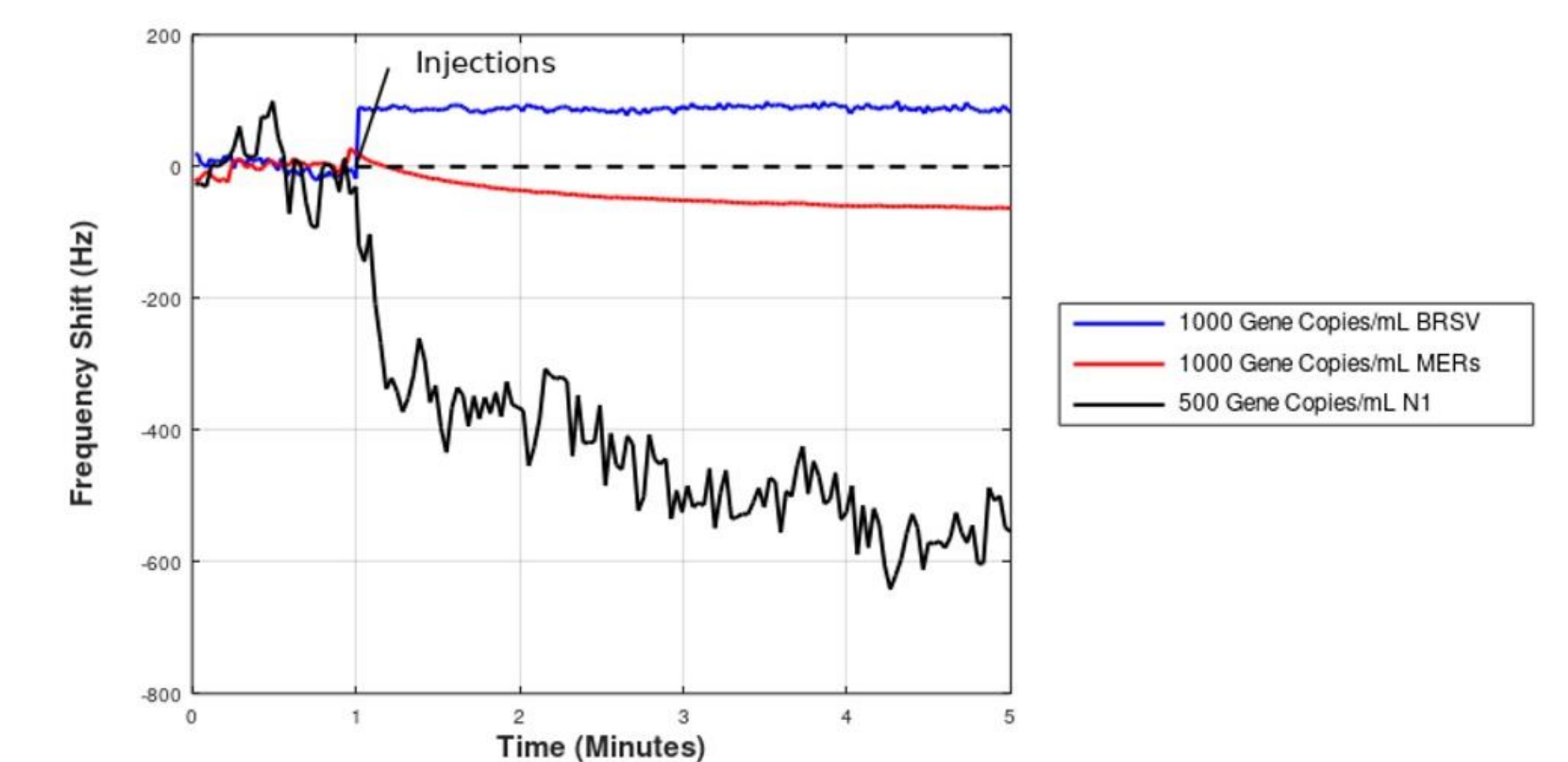### 3. Specificity test on N1 in DI water

Figure 7. Frequency response to N1 and other pathogens (BRSV and MERS).

- Significant frequency shift for N1 detection (460±127 Hz) even at lower concentration. No response was recorded for other pathogens, demonstrating high specificity.

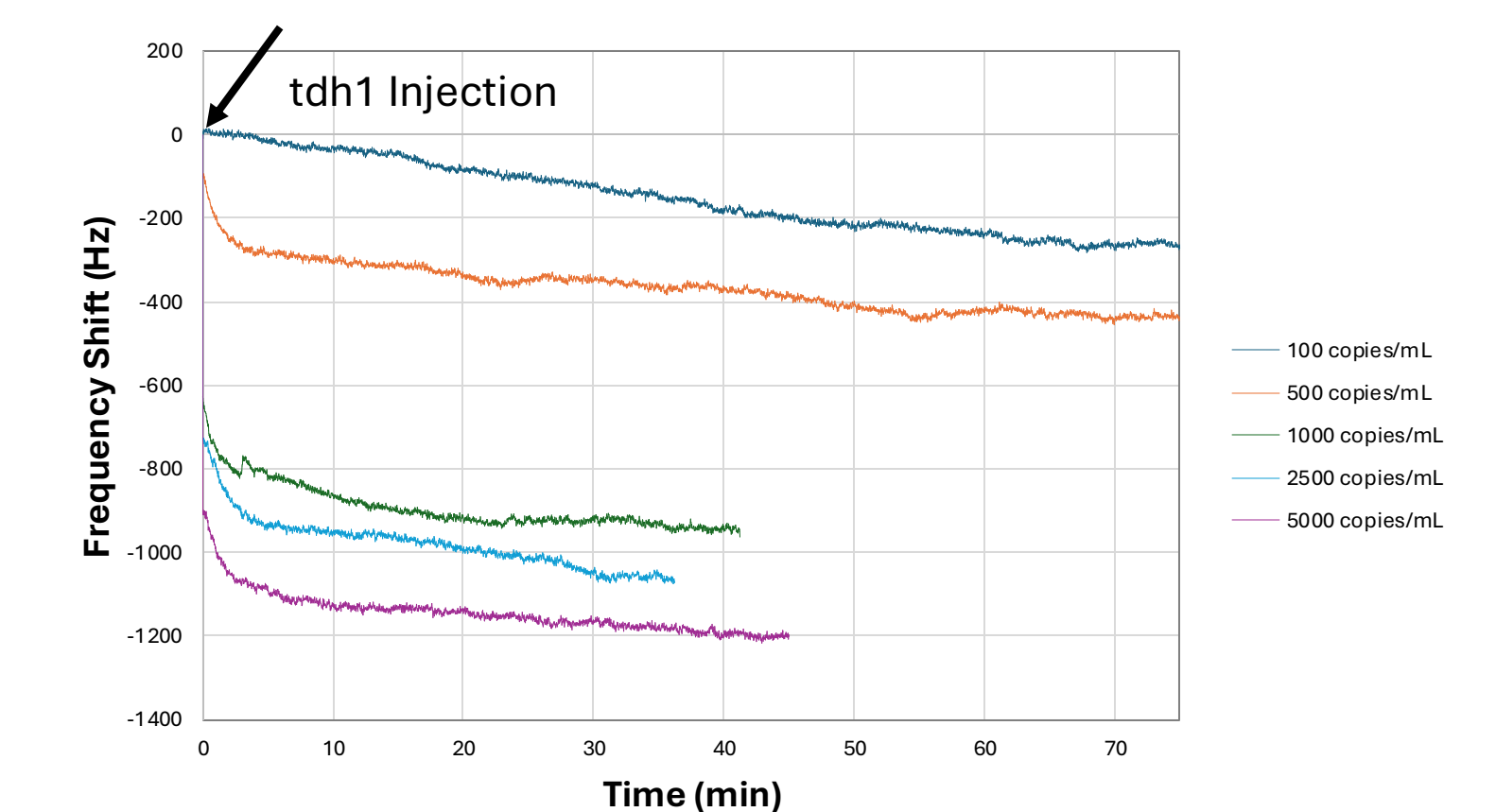### 4. Sensitivity Test on tdh1 in Molecular Water

Figure 8. Frequency response of the sensor to tdh1 in molecular water at different concentrations.

- Upper detection limit was 5000 gene copies/mL, with sensor readings of 1198.85 ± 6.19 Hz.
- Lower detection limit was 100 gene copies/mL, with sensor readings of 256.55 ± 11.21 Hz.

Conclusion

We investigated the application of an acoustic biosensor for the ultrahigh sensitivity detection of biomolecules in water. Dynamic detection range was 100-3000 and 100-5000 gene copies/mL for N1 and tdh1, respectively. Specificity test indicated the sensor's non-selectivity to non-targets.

Reference

Su J, Esmaeilzadeh H, Zhang F, et al. An ultrasensitive micropillar-based quartz crystal microbalance device for real-time measurement of protein immobilization and protein-protein interaction. *Biosensors and Bioelectronics*. 2018;99:325. doi:10.1016/j.bios.2017.07.074
Illustrations created in BioRender.com

Acknowledgement