

Supplementary Material

Staphylococcus Aureus Detection in Milk Using a Thickness Shear Mode Acoustic Aptasensor with an Antifouling Probe Linker

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S1. FTIR Analysis of DTT_{COOH}

Analysis of DTT_{COOH} by Fourier transform infrared spectroscopy (FTIR) was performed using a Prestige-21 FTIR spectrometer (Shimadzu, Kyoto, Japan). The measurement was carried out in transmittance mode using CaF₂ cell (Specac, Orpington, UK). DTT_{COOH} was solubilized in ethanol at a concentration of 4 mM. Subsequently, drops of the sample were poured onto the cell and the measurement was conducted before and after solvent evaporation.

The FTIR spectra for DTT_{COOH} is shown in Figure S1. C-H and S-H bond stretches are expected from 3000-2700 cm⁻¹. However, DTT_{COOH} absorbs infrared broadly due to numerous O-H bonds.

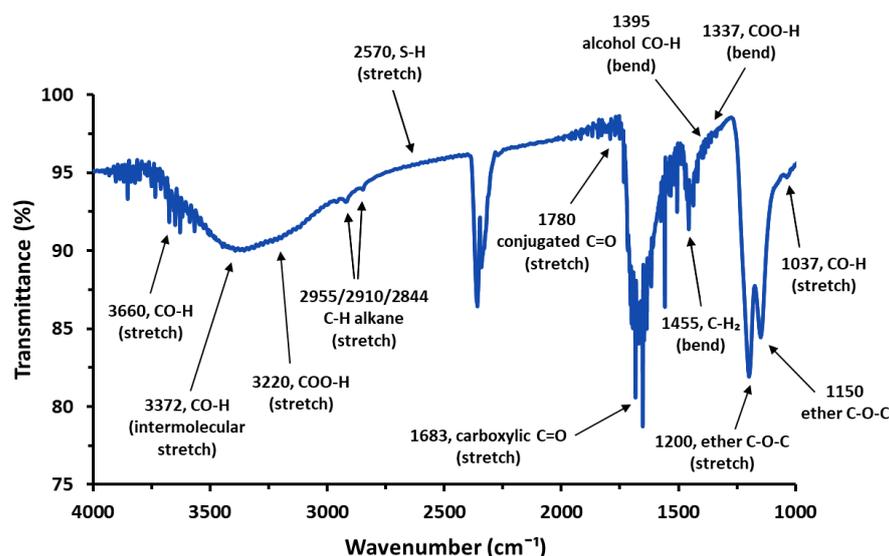


Figure S1. FTIR transmittance spectrum of DTT_{COOH} following solvent evaporation.

To reduce the broad absorption of O-H stretches, further analysis was carried out in solution (Figure S2), allowing for the solvent spectrum to be subtracted.

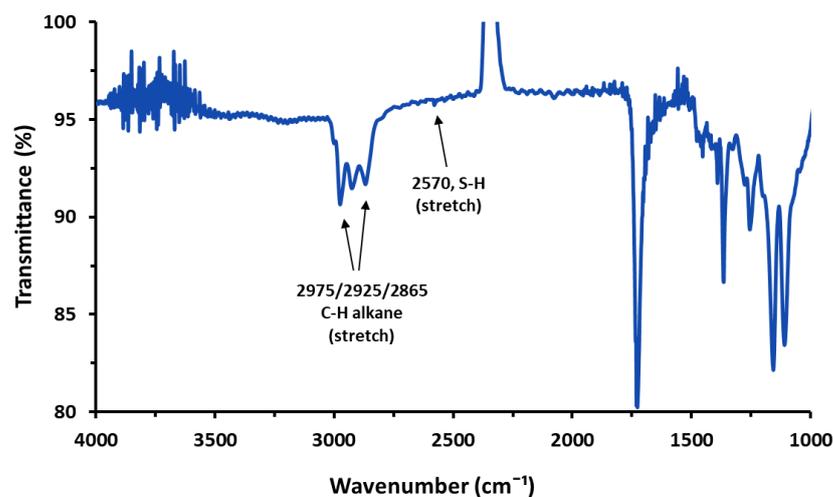


Figure S2. FTIR spectrum of DTT_{COOH} in a solution of ethanol. C-H and S-H bond stretches are more noticeable in the region of 3000-2700 cm⁻¹.

S2. Secondary Structure of DNA Aptamer

We applied OligoAnalyzer Tool™ (Integrated DNA Technologies, Inc., Coralville, IO, USA) program for analysis of possible secondary structure of the aptamer (5' NH₂-TCC CTA CGG CGC TAA CCT CCC AAC CGC TCC ACC CTG CCT CCG CCT CGC CAC CGT GCT ACA AC-3'). According to this program there exists 9 variations of the secondary structure. Two of them that are characterized by lowest Gibbs energy, and consequently most stable: - 12.2 kJ/mol (A) and -11.8 kJ/mol (B), respectively, are presented on Figure S3.

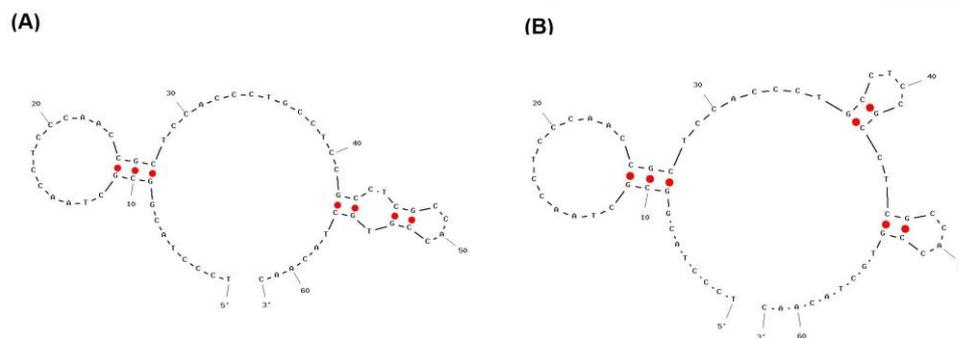


Figure S3. Possible secondary structure of DNA aptamers used in this work generated by OligoAnalyzer Tool™ that differ by Gibbs energy: - 12.2 kJ/mol (A) and -11.8 kJ/mol (B).

S3. CFU Counts of *Staphylococcus Aureus*

To quantify the amount of *S. aureus* being used in experiments optical density at 600 nm was used. To correctly determine the bacterial concentration using OD₆₀₀, CFU counts at multiple dilutions of *S. aureus* were collected and compared to the OD₆₀₀ at each dilution (Figure S4).

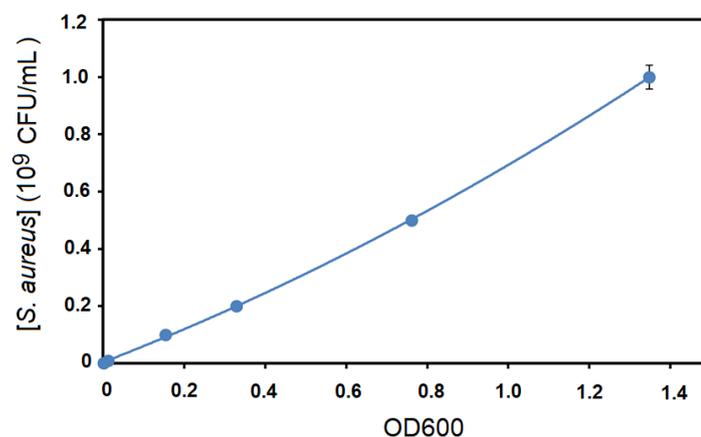


Figure S4. Concentration of *S. aureus* in PBS compared to their optical density at 600 nm (OD600).

A polynomial relationship between the OD600 measurements and CFU counts for *S. aureus* in PBS was found with a relationship of:

$$[S. aureus] \text{ (CFU/mL)} = 1 \times 10^8 x^2 + 5 \times 10^8 + 8 \times 10^6$$

where x is the OD600 value. Using this relationship the grown solutions of *S. aureus* could be diluted to the desired concentration for measurement.

S4. Experimental Setup

Figure S5 shows the scheme of experimental setup consisting of an Acryl flow cell with a volume 100 μL , syringe pump and vector analyzer.

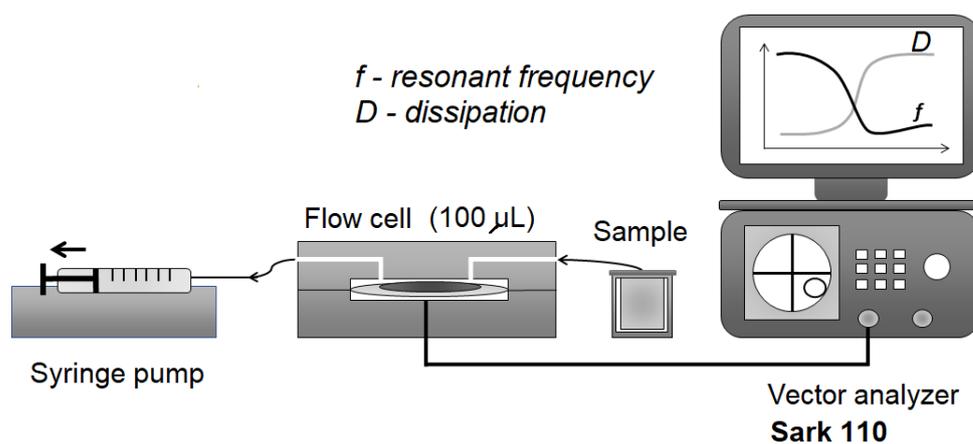


Figure S5. The scheme of experimental setup.