SUPPORTING INFORMATION

Sample-to-answer acoustic detection of DNA in complex samples

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Experimental

Materials

H_2O_2, H_2SO_4, Tris(hydroxymethyl)aminomethane hydrochloride (TRIS HCl), Phosphate buffered saline; 10 mM phosphate buffer; 138 mM NaCl; 2.7 mM KCl (PBS, P4417), PLL(225) and fetal bovine serum (FBS) were purchased from Sigma-Aldrich/Merck KGaA (Darmstadt, Germany). PLL(25)-g-PEG(2) and PLL(25)-g-PEG(5) were purchased from Nanocs Inc. (PG2K-PLY and PG5K-PLY, New York, U.S.A.). QCM gold sensors were purchased from Biolin Scientific (QSX301, Stockholm, Sweden). Nucleospin Gel and PCR clean-up kit (Macherey-Nagel, Germany). DNA primers 100μM (Metabion, Germany). λPstI ladder (Minotech, Greece). *Salmonella* Typhymurium cells have been kindly provided by Institut Pasteur (Paris, France). UHT milk was used as a model real complex sample (milk consists of 3.5% fat, 3.5% proteins, 5% lactose (carbohydrate) and 10^4 to 10^5 somatic cells per mL). Luria-Bertani (LB) a nutrient-rich microbial broth that contains peptides, amino acids, water-soluble vitamins, and carbohydrates was prepared by mixing 10g/L Tryptone, 5 g/L Yeast Extract and 5 g/L NaCl.

Methods

Experimental setup of acoustic measurements

Gold sensors were cleaned with piranha solution prepared *in situ*, adding 4 drops H_2SO_4 (95-97%) and 2 drops H_2O_2 (30%) on gold surface (*Caution: Piranha solution reacts violently with all organics and should be handled with care*). Then the surface was rinsed with H_2O and dried under a stream of nitrogen gas. In the present work all the experiments were carried out in buffer solution. Resonance frequency (ΔF) and energy dissipation (ΔD) changes were measured by Q-Sense E4 (Biolin Scientific, Stockholm, Sweden) at operating frequency of 5 MHz and its overtones, with continuous flow rate of 50 μL/min at 25 °C. PLL (25)-g-PEG (2) and PLL (25)-g-PEG (5), as well as PLL (225) films were formed on the clean gold-coated QCM surface by applying a solution of 0.1 mg/mL in PBS or Tris buffer on the device surface; PLL films were formed by applying a solution of 0.01 % (w/v) in Tris or PBS. All results reported in this study regard the 7th harmonic overtone i.e. 35 MHz; the reported frequency changes are not divided by the overtone number.

QCM-D/Ellipsometry measurements

In-situ monitoring of the film formation at the gold electrode/liquid interface was performed by using a combined QCM-D/Ellipsometry instrument comprising an M-2000V Variable Angle Spectroscopic Ellipsometer (Woollam, USA) with a spectral range of 370-1000 nm and a Q-Sense E1 (Biolin Scientific) acoustic biosensor. Measurements were performed under a constant flow rate of 50 μL/min at 25 °C. Briefly, the gold surface was equilibrated with Tris buffer; then PLL (25)-g-PEG (2) and PLL (25)-g-PEG (5) polymer films were formed by applying a solution of 0.1 mg/mL in Tris buffer over the gold surface. Finally, the surface was rinsed with Tris buffer. After the formation of the PLL (25)-g-PEG (2) layer on the gold surface, a λPstI DNA 'ladder' (250 bp - 11.5 kbp) was

introduced by applying a solution of 5 μg/mL in Tris buffer until saturation. In-situ QCM-D/Ellipsometry measurements were performed on at least three different samples to evaluate both the "dry" and "wet" mass of the polymer layer.

The water content of the formed film is given as: $H\,\% = (m^{wet} - m^{dry}) / m^{wet}$ x100 where m^{wet} is the mass obtained from a Voigt-type modeling[1] of the QCM-D recorded data while m^{dry} is obtained from the simultaneously recorded ellipsometry data.
The term m^{QCM} (adsorbed mass/unit area) is given by $m^{QCM} = \rho_{film} * d_{film}$ where d_{film} is the acoustic thickness. For the simulation we assumed often employed reasonable film densities ρ_{film} equal to 1.05, 1.10 and 1.15 g/mL and the fitting process produced the corresponding d_{film} values; while ρ_{film} and d_{film} are inversely related to each other, their product is rather steady and can be reliably used in further calculations.

The term $m^{Ellips.}$ is obtained through the de Feijter equation[2] as

$$m^{Ellips.} = h_{film} * (n_f - n_s) / dn/dC$$

where h_{film} is the optical thickness, n_f and n_s the refractive indices of the polymer film and the solvent while dn/dC is the refractive index increment of the polymer solution; dn/dC is obtained here by interpolation between the known values of 0.181 and 0.135 mL/g for lysine and PEG using a median grafting ratio value ≈ 3.5 for the particular polymers. In the fitting procedure n_f had to be assigned certain values since as it is often the case for very thin films (h_{film} << wavelength/ $2\pi n_f$) the thickness and refractive index could not be simultaneously determined. Specifically, we assumed the values n_f = 1.38-1.42 and $n_s \approx 1.33$. As is the case with the Voigt modeling the $m^{Ellips.}$ quantity can be reliably obtained. Then, from the independently determined $m^{Ellips.}$ and m^{QCM} the ("combined") thickness of the film Z_f is obtained through

$$Z_f = m^{QCM} / \rho_{solvent} + m^{Ellips.} * (\rho_{polymer} - \rho_{solvent}) / \rho_{polymer} * \rho_{solvent} \qquad \text{Eq. (1)}$$

where $\rho_{polymer} \approx 1.20$ for PLL-g-PEG and 1.80 g/mL for DNA and $\rho_{solvent} \approx 1.0$ g/mL.
CompleteEase® (Woollam) software was used to fit the ellipsometric data (Ψ and Δ) in a two-layer model: 'layer1' using the B-spline algorithm to fit for the gold surface and 'layer2' using a Cauchy layer to fit all the polymer films; in the model, parameter A was given the values 1.38, 1.40 and 1.42 as mentioned above, C was set equal to zero while B & h_f were the fitting parameters.

DNA amplification from whole *Salmonella* cells
DNA amplicons were produced from 1 μL of *Salmonella* Typhimurium cells (provided by Pasteur Institute, Paris, France) added in various concentrations in the PCR reactions using the Hotstart polymerase kit (KAPA Biosystems Inc., Wilmington, MA, USA) and following the manufacturer instructions. 10 pmoles of each of the forward and reverse primers were included in each amplification reaction. The reactions were conducted with a PeqStar 2x (Peqlab Biotechnologie GmbH, Erlangen, Germany) thermocycler at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 sec, 62.5 °C for 10 sec and 72 °C for 10 sec. The final step was at 72 °C for 1 min. The primers used for the 635bp DNA were: Forward: 5'-GACACCTCAAAAGCAGCGT-3', Reverse: 5'-AGACGGCGATACCCAGCGG-3' and for the 195bp fragment were: Forward: 5'-GGATCACTAAGCTGTGGATTACCTATTATC-3', Reverse: 5'- CTGTTATTTCCTGCGTGGATATTTCTTTAG-3'.

DNA amplification from whole *Salmonella* cells in milk samples
UHT whole milk was diluted 10 times (according to EU regulation) in LB growth medium and then spiked with *Salmonella* cells to a final concentration of 10^3 CFU/μL. 1 μL of the complex sample was added in the PCR mix (25 μL in total) as described in the previous section with the exception of the addition of 1.5 μL of $MgCl_2$ (25 mM) that was required to compensate for the PCR inhibitory effect of high calcium present in the milk sample. DNA amplicons were produced by following the cycling protocol described in the previous section. Negative control

PCR reactions with 1 µL of the complex sample without bacterial cells were also performed in parallel. 5 µL of the PCR reactions were loaded in 2% agarose gels (running at 120V for 30 min) for verification of the presence of *Salmonella* amplicons (Fig. S1).

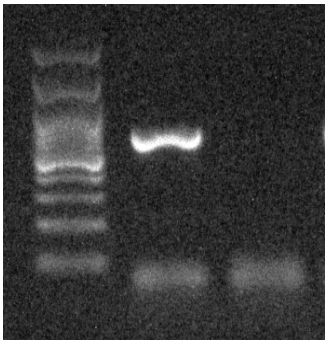

Fig. S1: Gel electrophoresis image of a positive (lane 2) and a negative (lane 3) PCR reaction showing the presence of amplified 635bp *Salmonella* DNA amplicons. Lane 1: DNA ladder.

Acoustic detection of DNA amplicons from whole *Salmonella* cells in milk samples

Acoustic measurements with unpurified PCR reactions with whole cells in the presence of milk and LB were performed as described before. Fig. S2 shows a real-time acoustic measurement of a positive and a negative PCR reaction. Five positive and five negative samples were measured and the results are shown in Fig. S3.

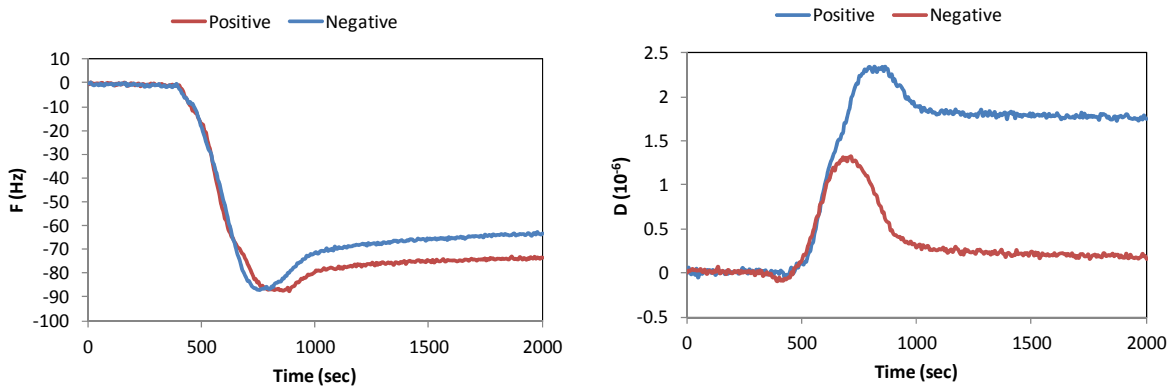

Fig. S2: Comparison of changes in frequency (F) and dissipation (D) of a positive and a negative PCR reaction monitored in real-time with QCM-E4.

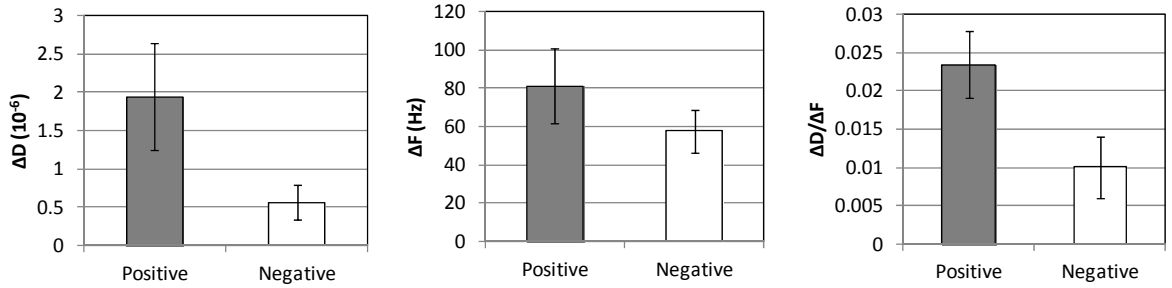

Fig. S3: Comparison of changes in frequency (F), dissipation (D) and acoustic ratio (ΔD/ΔF) between positive and negative PCR reactions (measurements were repeated 5 times).

References

1. M. V. Voinova, M. Rodahl, M. Jonson, B. Kasemo, *Physica Scripta*, 1999, **59**, 391-396.
2. J. A. De Feijter, J. Benjamins and F. A. Veer, *Biopolymers*, 1978, **17**, 1759-1772.