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Application of Optical and Acoustic Methods for Detection of Bacterial Pathogens Using DNA Aptamers as Receptors †

Ivan Piovarci [1,*], Judit Süle [2], Mihailia Angelopoulou [3], Panagiota Petrou [3], Leda Bousiakou [4], Sotiris Kakabakos [3] and Tibor Hianik [1]

[1] Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynska dolina F1, 842 48 Bratislava, Slovakia; e-mail@e-mail.com
[2] Hungarian Dairy Research Institute Ltd., Lucsony utca 24, 9200 Mosonmagyaróvár, Hungary; e-mail@e-mail.com
[3] National Centre for Scientific Research "Demokritos", Patr. Gregoriou E & 27 Neapoleos Str., 15341 Agia Paraskevi, Greece; e-mail@e-mail.com (M.A.); e-mail@e-mail.com (P.P.); e-mail@e-mail.com (S.K.)
[4] IMD Laboratories Co, R&D Section, Lefkippos Technology Park, NCSR Demokritos, Agia Paraskeyi, PO Box 60037, 15130 Athens, Greece; e-mail@e-mail.com
* Correspondence: piovarci.i@gmail.com
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Abstract: Bacterial contamination in food is real and valid danger for human health. Therefore, we focused on the detection of *Escherichia coli* and *Listeria innocua* with optical and acoustic methods. In both methods we used specific DNA aptamers as receptors. For optical method we modified gold nanoparticles (AuNPs) with aptamers and analyzed the interaction of AuNPs with bacteria by measurement of the changes of the absorbance spectrum. We also applied white light reflectometry to measure change in thickness on a silicon chip modified with aminylated aptamer through silica chemistry. We also used quartz crystal microbalance (QCM) in multiharmonic mode. In this case the thiolated aptamers were chemisorbed at the gold layer of the quartz crystal and the changes of resonant frequency were measured following addition of bacteria. The limit of detection (LOD) of optical method using AuNPs was estimated to be 10^5 CFU/mL of *Listeria monocytogenes*. For reflectometric method we were able to detect *E. Coli* at concentrations around 2×10^4 CFU/mL. Using TSM we performed analysis of viscoelastic properties of the aptamer layers during their formation at the surface.

Keywords: *Escherichia coli*; *Listeria monocytogenes*; QCM; colorimetry; reflectometry

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1. Introduction

Bacterial contamination in food presents serious danger for human health. Every year tons of food need to be thrown out and it is estimated billions of people get sick from food poisoning, leading to deaths in some hundreds of thousand cases (mostly in children) [1]. Most of the bacterial contaminations can be traced to about 20–30 pathogenic bacteria [2,3]. In our work we focused on the detection of *Escherichia coli* and *Listeria monocytogenes* with optical and acoustic methods. In both methods we used specific DNA aptamers as receptors. Aptamers are single stranded DNA or RNA that in correct environment folds into structures specifically binding to a target with constant of dissociation, K_d, around 10 nM or lower [4,5]. For optical method it is possible to modify gold nanoparticles (AuNPs) with aptamers and to study the interaction of AuNPs with bacteria by colorimetry. Aptamers also electrostatically interact with the AuNPs without specific binding, and it is possible to increase the stability of AuNPs at presence of high ionic strength. The incubation of AuNPs with bacteria then removes the protecting aptamer and after

addition of salts resulted in AuNPs aggregation. This process can be measured by changes in absorbance spectra as a function of bacterial concentration. Another optical method used was white light reflectometry. This method measures changes in the thickness of the sensing layer deposited on a silicon chip modified with amino group-modified aptamer through silica chemistry. In this method the white light from an optical wire illuminated the silicon surface and the reflected light is taken from the same wire to a spectrometer to measure the resulting spectrum and interference pattern of the reflected light. Using mathematical model it has been possible to calculate change in apparent thickness of sensing layer on a SiO_2 surface. We also used quartz crystal microbalance (QCM) in a multiharmonic mode for detection bacteria. In this case the gold surface of the piezo crystal has been modified by thiolated aptamers. Changes in resonant frequency and dissipation served as a signal related to the interaction of bacteria with aptamers. It seems that the interaction of bacteria with the surface is not strictly due to the mass changes. Therefore, we performed also analysis of the changes of viscoelastic properties of the sensing layer.

2. Materials and Methods

2.1. Chemicals and Materials

$HAuCl_4$, sodium citrate, phosphate buffer saline (PBS) tablets, TRIS-HCl, NaOH, BS3 linker, bovine serum albumin (BSA), (3-Aminopropyl)triethoxysilane (APTES), tris(2-carboxyethyl)phosphine (TCEP), NH_3, H_2O_2, H_2SO_4, acetone, isopropyl alcohol were from Sigma Aldrich (Darmstadt, Germany), DNA aptamers for *Listeria monocytogenes* and *E.coli* has been purchased from Generi Biotech, Ltd. (Hradec Králové, Czech Republic).

2.2. Preparation of Gold Nanoparticles and Colorimetric Method

The gold nanoparticles (AuNPs) were prepared by Turkevich method. Briefly of 100 mL of 0.25 mM $HAuCl_4$ has been boiled by putting it on a hotplate set to 99.9 °C for around 20 min. under continuous mixing with magnetic stirrer. The top of the flask has been covered with Petri dish in order to protect the solution from external contaminants and to reduce loss of solvent due to evaporation. After the solution was brought to boil, 3.4 mL of 39 mM trisodium citrate was added, so that the resultant molar ratio was 1:5 of $HAuCl_4$ to trisodium citrate. The solution first lost its yellow color and then darkened to violet color. This solution was continuing in boiling under mixing for around 15–20 min after which the solution continually changed its color to red. We then removed the solution from the hot plate and left it to cool to room temperature. The AuNPs were stored in fridge at 4 °C. This method allowing to obtain AuNPs with a diameter of approximately 5 nm.

The colorimetric experiments were performed by means of Thermo Scientific Genesys UV/vis spectrophotometer (Waltham, MA, USA). *Listeria monocytogenes* was prepared in Hungarian Dairy Research Institute (Mosonmagyarovar, Hungary) using standard microbiology methods. *Escherichia coli* O157:H7 has been prepared in National Centre for Scientific Research "Demokritos" (Agia Paraskevi, Greece) in Petri dishes on agar using standard microbiology method. Concentrations of both bacteria was determined by plate counting method.

2.3. Preparation of Reflectometric Surface and Method of Detection

The surface of silicone SiO_2 chip has been cleaned with acetone and isopropyl alcohol for 10 min during sonication. We then used acidic piranha for 20 min (1:1, H_2SO_4:H_2O_2) and then cleaned the surface with distilled water and dry under nitrogen. The surface has been then incubated in 2% solution of APTES for 20 min. After that it has been dried with nitrogen and put into the oven at 120 °C for 20 min. Then we added 80 μL of 10 μM aptamer with 8 μL of 600 μM BS3 linker for one hour. After this step the surface has been cleaned with distilled water and leave to incubate in TRIS buffer overnight. For blocking of the naked surface, it has been immersed into 1% BSA solution for one hour. The samples were measured in flow by a system of visible-near infrared light source (ThetaMetrisis

S.A., Athens, Greece) with miniaturized USBE controlled spectrometer (Maya 2000 Pro; Ocean Insight, Orlando, FL, USA) and a reflection probe (AVANTES Inc., Broomfield, CO, USA) consisting of seven optical fibers.

2.4. Preparation of QCM Surface and Method of Detection

The surface has been cleaned using basic piranha (1:1:7, NH_3:H_2O_2:H_2O). We then applied sodium citrate buffer (250 mM, pH 3) in the flow. After stabilization the aptamers in a concentration of 10 μM in a citrate buffer were added. We detected the samples in flow by using an injection pump. The QCM crystals (Total Frequency Control, Storrington, UK) were fitted in a quartz flow cell. The signal from the cell was taken with SARK-110 (Seeed Studios, Shenzhen, China) antenna analyser.

3. Results and Discussion

First, we explored colorimetry for detection of *Listeria monocytogenes.* We then determined lowest NaCl concentration (200 mM) that causes the aggregation of AuNPs. This is demonstrated in Figure 1.

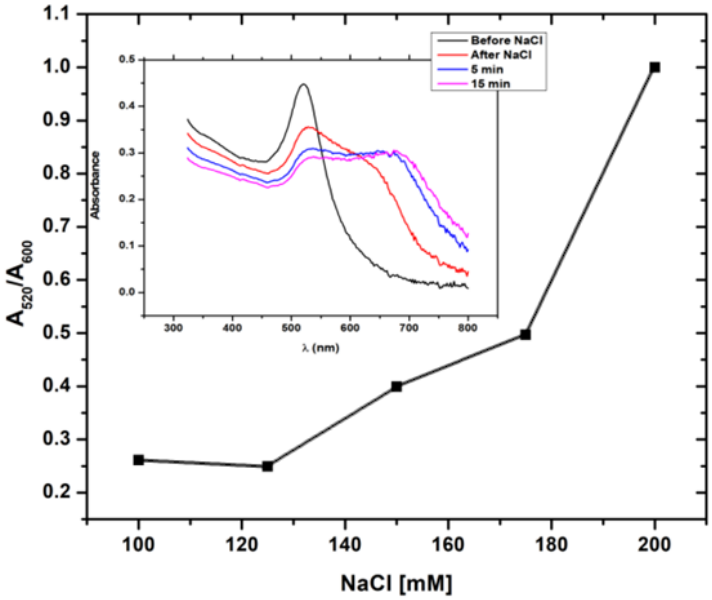

Figure 1. Changes in absorbance of non-modified AuNPs after addition of different concentrations of NaCl. The absorption spectra of naked AuNPs, those at presence of 200 mM NaCl and at times 5 and 15 min (see the legend) are shown in the inset..

In the next series of the experiments, we incubated AuNPs for 15 min with different concentrations of DNA aptamers. The interaction of AuNPs with the aptamers protected them from aggregation in the presence of 200 mM NaCl. The spectrum of such AuNPs changed little with the use of aptamer concentration of 10 μM. In the next experiments, we incubated 10 μM aptamer with different concentrations of *Listeria monocytogenes* for 40 min at 37 °C. The sample has been then centrifuged for 10 min at 14,500 rpm. Bacteria binds to the aptamers in sample and with increased concentration of bacteria there is less aptamers in the supernatant after centrifugation. The supernatant has been incubated with AuNPs for 15 min and the absorbance spectrum has been measured immediately after adding 200 mM NaCl and 10 min after the addition of NaCl. Figure 2 shows the calibration curve consisting of change in absorbance (proportion of absorbance at 520 nm and 700 nm as peaks for non-aggregated and aggregated AuNPs, respectively) vs. concentration of bacteria. It can be seen that the absorbance starts to change at about 10^4–10^5 (CFU)/mL This method is capable of relatively fast and easy detection of bacteria with

relatively high LOD. However, it is likely that the sensitivity of detection can be improved by optimization of the conditions, such as salt concentration, application of different salts or AuNPs with another size.

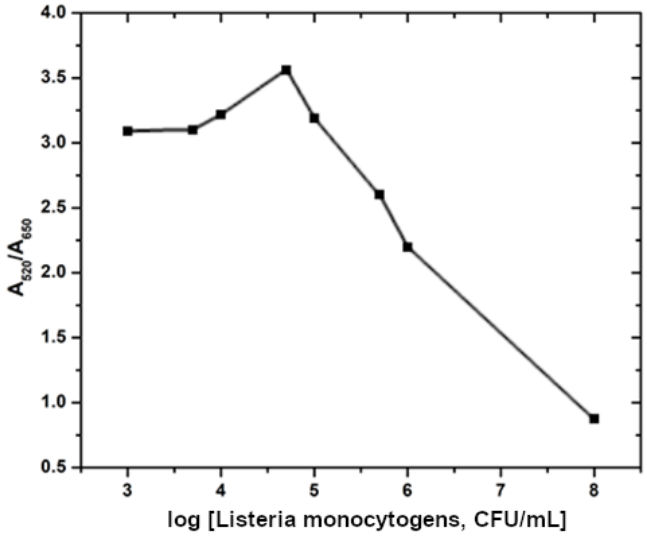

Figure 2. Calibration curve of change in A520/A650 vs. concentration of *Listeria monocytogenes* in log. scale.

In the white light reflectometry method, we modified the silicon chip with an amino group-modified aptamers using APTES and a BS3 diester linker. Subsequently, we measured reflection of white light from the surface of the silicon chip. After adding different concentrations of bacteria, we were able to monitor the change in the reflected spectrum and, using a mathematical model, we linked these changes to the variations in layer thickness. Typical kinetics of the changes of the thickness of naked silicon surface and those modified by aptamers (the naked SiO_2 surface has been blocked by BSA) at presence of *E. coli* is shown on Figure 3. It can be seen that there is an optical signal from bacteria for both surfaces, however the kinetics for blocked surface were faster. After adding different concentrations of *E. coli* to the surface, we observed a 0.01–1 nm shift in the thickness depending on the concentration of bacteria starting from 2×10^2 CFU/mL (Figure 4).

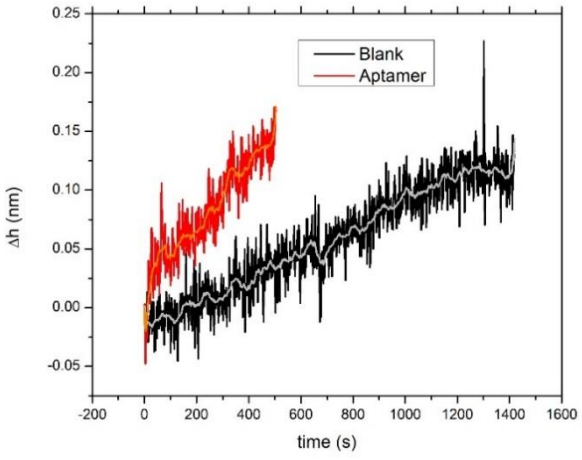

Figure 3. The kinetics of the changes of the thickness (Δh) obtained from reflectometry signal for SiO_2 surface with and without aptamers.

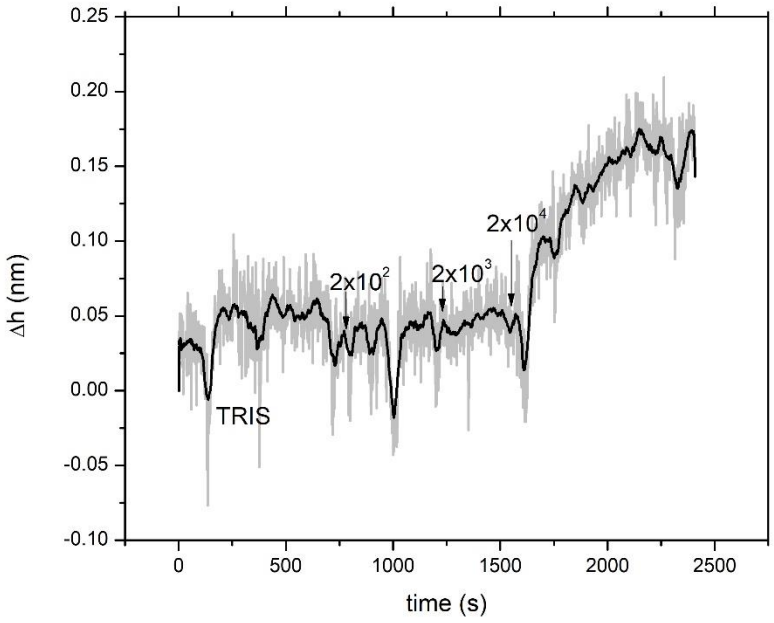

Figure 4. The kinetics of the changes of the thickness (Δh) obtained from reflectometric signal following addition of *E. coli* to a surface modified by DNA aptamers. The naked SiO_2 surface was blocked by BSA.. Addition of bacteria is shown by arrows.

We also applied QCM method for *E. coli* detection at the surface of piezo crystal modified by DNA aptamers. For thiolated aptamers, we observed that the surface binding was rather weak and it was necessary to lower the pH leading to about a 10 Hz decrease in the resonant frequency in the flow following addition of thiolated aptamer (Figure 5). The resulting change of frequency after addition of around 10^4 CFU/mL was hard to distinguish from the noise and drift of the QCM system. Viscoelastic analysis using Voinova-Voigt viscoelastic model [6] show that the estimated height of this aptamer is about 5 nm at layer formation and the resulting height is below 1 nm (Figure 6). Equations used for Voinova model are presented next.

$$\omega = 2\pi f_0 n$$

$$\delta = \sqrt{\frac{2\eta_l}{\rho_l \omega}}$$

$$\Delta f = -\frac{1}{2\pi\rho_q h_q}\left(\frac{\eta_l}{\delta} + h_f\rho_f\omega - 2h_f\left(\frac{\eta_l}{\delta}\right)^2\frac{\eta_f\omega^2}{\mu_f^2+\omega^2\eta_f^2}\right)$$

$$\Delta D = \frac{1}{2\pi f_0\rho_q h_q}\left(\frac{\eta_l}{\delta} + h_f\rho_f\omega + 2h_f\left(\frac{\eta_l}{\delta}\right)^2\frac{u_f\omega}{\mu_f^2+\omega^2\eta_f^2}\right)$$

Where q l and f subscripts are designations for quartz, liquid and film respectively.

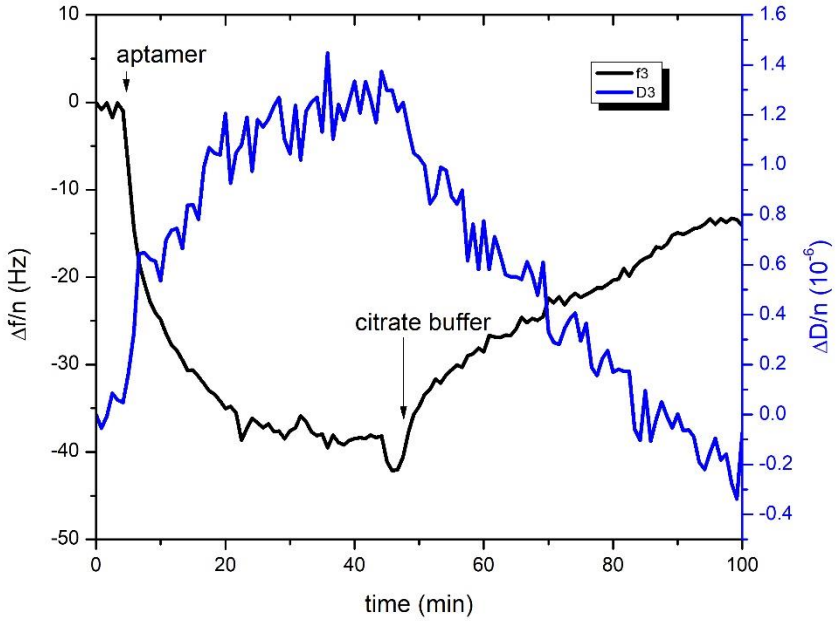

Figure 5. The frequency and dissipation change obtained from TSM experiment for 3rd overtone. The change of frequency (black) and dissipation (blue) are divided by the overtone number.

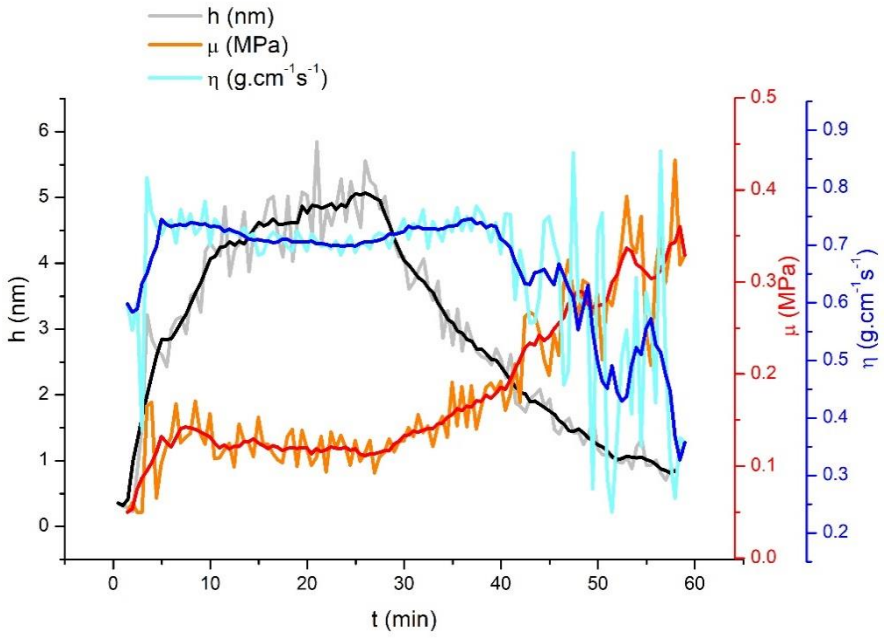

Figure 6. The kinetic changes of the thickness (h—black), viscosity (η, red) and shear modulus (μ, blue) obtained by Voinova-Voigt viscoelastic model using the data of the changes of resonant frequency and dissipation obtained from TSM experiment.

The length of unfolded aptamer would be around 15 nm, indicating that the aptamer at the crystal surface is likely to be in a different conformation than the denatured random. However, low altitude can also imply insufficient coverage (as it is an average height) or an aptamer lying on the surface. These results are also supported by the change in viscosity and elasticity. Shearing modulus, μ, increased after leaching from the layer, implying

that the aptamer had a greater tendency to return, implying closer association with the layer. On the contrary, the viscosity decreased, which may mean that the aptamer is not in a tense conformation and thus rather lies on the surface of the gold electrode. To circumvent this problem, it will probably be necessary to modify the layer with small thiol molecules in sufficient proportion to the aptamer so that the aptamer is in a more advantageous position and has room to bind bacteria. Optical method based on AuNPs had highest estimated LOD. The light reflectometry method was able to measure *E. coli* in the range of 10^3 to 10^4 CFU/mL, but sensitivity as low as few 100 CFU/mL can also be reachable [7]. Acoustic method provides advantages in the possibility of probing the viscoelastic changes of the surfaces, however the bacteria can induce the changes in the frequency negatively or positively which proves a challenge to use it as a biosensor [8,9].

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References

1. Hoagland, L.; Ximenes, E.; Ku, S.; Ladisch, M. Foodborne pathogens in horticultural production systems: Ecology and mitigation. *Sci. Hortic.* **2018**, *236*, 192–206, doi:10.1016/j.scienta.2018.03.040.
2. Priyanka, B.; Patil, R.K.; Dwarakanath, S. A review on detection methods used for foodborne pathogens. *Indian J. Med. Res.* **2016**, *144*, 327, doi:10.4103/0971-5916.198677.
3. Safavieh, M.; Nahar, S.; Zourob, M.; Ahmed, M.U. Microfluidic biosensors for high throughput screening of pathogens in food. In *High Throughput Screening for Food Safety Assessment*; Elsevier: Amsterdam, The Netherlands, 2015; pp. 327–357. doi:10.1016/B978-0-85709-801-6.00015-0.
4. Siddiqui, S.; Yuan, J. Binding Characteristics Study of DNA based Aptamers for *E. coli* O157:H7. *Molecules* **2021**, *90*, 204, doi:10.3390/molecules26010204.
5. Lyu, Y.; Teng, I.T.; Zhang, L.; Guo, Y.; Cai, R.; Zhang, X.; Qiu, L.; Tan, W. Comprehensive Regression Model for Dissociation Equilibria of Cell-Specific Aptamers. *Anal. Chem.* **2018**, *90*, 10487–10493, doi:10.1021/acs.analchem.8b02484.
6. Voinova, M.V. The theory of acoustic sensors application in air quality control. *Urban Clim.* **2018**, *24*, 264–275, https://doi.org/10.1016/j.uclim.2018.04.002.
7. Angelopoulou, M.; Tzialla, K.; Voulgari, A.; Dikeoulia, M.; Raptis, I.; Kakabakos, S.E.; Petrou, P. Rapid Detection of Salmonella typhimurium in Drinking Water by a White Light Reflectance Spectroscopy Immunosensor. *Sensors* **2021**, *21*, 2683 doi:10.3390/s21082683.
8. Alexander, T.E.; Lozeau, L.D.; Camesano, T.A. QCM-D characterization of time-dependence of bacterial adhesion, *The Cell Surface*. 2019 *5* 100024 doi:10.1016/j.tcsw.2019.100024.
9. Tatarko, M.; Spagnolo, S.; Oravczová, V.; Süle, J.; Hun, M.; Hucker, A.; Hianik, T.Changes of viscoelastic properties of aptamer based sensing layers following interaction with *Listeria innocua*. *Sensors* **2021**, *21*, 5585, doi:10.3390/s21165585.