



HAL
open science

Rapid and sensitive identification of uropathogenic Escherichia coli using a surface-enhanced-Raman-scattering-based biochip

Cristina-Cassiana Andrei, Anne Moraillon, Stephanie Lau, Nordin Félidj, Nao Yamakawa, Julie Bouckaert, Eric Larquet, Rabah Boukherroub, Francois Ozanam, Sabine Szunerits, et al.

► To cite this version:

Cristina-Cassiana Andrei, Anne Moraillon, Stephanie Lau, Nordin Félidj, Nao Yamakawa, et al.. Rapid and sensitive identification of uropathogenic Escherichia coli using a surface-enhanced-Raman-scattering-based biochip. *Talanta*, 2020, 219, pp.121174. 10.1016/j.talanta.2020.121174. hal-03013203

HAL Id: hal-03013203

<https://hal.science/hal-03013203>

Submitted on 7 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Rapid and sensitive identification of uropathogenic *Escherichia coli* using a Surface-Enhanced-Raman-Scattering-based biochip

Cristina-Cassiana Andrei,[†] Anne Moraillon,[†] Stephanie Lau,[§] Nordin Felidj,[§] Nao Yamakawa,^{||} Julie Bouckaert,^{||} Eric Larquet,[†] Rabah Boukherroub,[‡] François Ozanam,[†] Sabine Szunerits,^{*,‡} Anne Chantal Gouget-Laemmel^{*†}

[†] Laboratoire de Physique de la Matière Condensée, Ecole Polytechnique, CNRS, IP Paris, 91128 Palaiseau, France

[§] Université de Paris, ITODYS, CNRS, UMR 7086, 15 rue J-A de Baïf, F-75013 Paris, France

^{||} Unité de Glycobiologie Structurale et Fonctionnelle (UGSF), UMR 8576 of the CNRS and the Univ. Lille, 50 avenue de Halley, 59658 Villeneuve d'Ascq, France

[‡] Univ. Lille, CNRS, Centrale Lille, Univ. Polytechnique Hauts-de-France, UMR 8520-IEMN, F-59000 Lille, France

Abstract

Rapid, selective and sensitive sensing of bacteria remains challenging. We report on a highly sensitive and reproducible surface-enhanced Raman spectroscopy (SERS)-based sensing approach for the detection of uropathogenic *Escherichia coli* (*E. coli*) bacteria in urine. The assay is based on the specific capture of the bacteria followed by interaction with cetyltrimethylammonium bromide (CTAB)-stabilised gold nanorods (Au NRS) as SERS markers. High sensitivity up to 10 CFU mL⁻¹ is achieved by optimizing the capture interface based on hydrogenated amorphous silicon a-Si:H thin films. The integration of CH₃O-PEG₇₅₀ onto a-Si:H gives the sensing interface an efficient anti-fouling character, while covalent linkage of antibodies directed against the major type-1 fimbrial pilin FimA of the human pathogen *E. coli* results in the specific trapping of fimbriated *E. coli* onto the SERS substrate and their spectral fingerprint identification.

* To whom correspondence should be send to: sabine.szunerits@univ-lille.fr (SS); anne-chantal.gouget@polytechnique.edu (AG)

Keywords: biochips, surface-enhanced Raman spectroscopy (SERS), gold nanorods (Au NRs), *Escherichia coli*, Principle Component Analysis (PCA), hydrogenated amorphous silicon (a-Si:H)

1. Introduction

Urinary tract infections (UTIs) are extremely common and highly recurrent, notably for women. [1-2] *Escherichia coli* (*E. coli*) is the predominant uropathogen (50–90%) in community-acquired infections using a multitude of virulence factors, which enable the bacteria to establish UTI. While time consuming (18–48 h for pathogen identification), urine culture remains the golden standard to identify UTI pathogens. Even though Gram-staining, flow cytometry and urine dipstick testing allow the rapid exclusion of negative samples, information obtained from these screening methods is often insufficient for making decisions regarding antibiotic treatment. [3] To overcome the limitations of culture-dependent analysis, immunological detection methods such as various enzyme-linked immunosorbent assays (ELISA) have been established. While these assays are fast and sensitive, binding and detection are separated in time in plate-based ELISA testing. This limitation may be overcome using different biosensor architectures where bacteria binding to antibodies and other bacteria-specific ligands directly trigger a detectable signal, which is proportional to the concentration of the bacteria. [4-7] In contrast to biosensors which are mainly specific towards a given pathogen and might suffer from cross-reactivity problems, there are a few examples of sensing arrays using several ligands to detect multiple pathogens simultaneously (e.g., *Salmonella enterica* Enteritidis, *Streptococcus pneumoniae* and *Escherichia coli* O157:H7) and offer better discrimination capabilities. [8-11] Such multiplexing sensors (i.e. biochips) represent a significant advance for the detection of other pathogens responsible for UTI (i.e., *Klebsiella*, *Enterobacter*, *Pseudomonas aeruginosa*...) together with a better reliability of the detection by minimizing false negative or positive responses.

One important analytical technique of broader interest for pathogen detection is Surface-enhanced Raman scattering (SERS) spectroscopy due to its fingerprint-identification capability and excellent sensitivity in aqueous media. [12-14] This vibrational technique relies on the Raman signal enhancement of targets in close vicinity of a metallic surface, owing to the strong electromagnetic field generated by the localized surface plasmons. Numerous SERS-active substrates, mainly based on noble metal nanostructures, have been developed for the bacteria detection due to their tuneable optical properties in the visible range together with their ease of synthesis and biofunctionalization with probes for a specific pathogen detection. [15-18] Special efforts have been dedicated to combine SERS with other functionalities such as bacteria filtration or concentration on dedicated platforms, using e.g., additional matrixes, microfluidics or dielectrophoresis. [19-21] However, to best of our knowledge, their

implementation in a multiplexing array was only demonstrated by a few pioneer works of C. Haischl [22-24] and A. Grow *et al.* [25] The main reason is the difficulty to generate various types of ligands (i.e., DNA aptamers, antibodies, glycans ...) specific to various types of bacteria (Gram-positive or negative) and a generic chemistry for their grafting. Moreover, the reproducible SERS response of bacteria remains challenging, mainly due to the uncontrolled distribution of nanoparticles on the bacteria surface or from inhomogeneity of the SERS substrate, the batch-to-batch variation of bacteria due to growth conditions or the different experimental Raman parameters. [15, 26] In order to clearly characterize the bacterial fingerprint and distinguish different microorganisms [27], the use of chemometrics is essential for efficient discrimination. [13,28] However, the successful combination of microarray multiplex analysis with SERS discrimination capability remains a challenge for achieving fast and reliable analysis of bacteria.

In this context, we focus on the specific and sensitive identification of *E. coli* strains containing type-1 fimbriae by designing a SERS biochip with bound anti-fimbrial *E. coli* antibodies, as displayed in **Figure 1**. Indeed, uropathogenic *E. coli* strains express surface-adhesive organelles known as type-1 pili. [29] Recently, anti-fimbrial antibodies against the major pilin protein FimA were generated and used as surface probes for the selective magnetic isolation or electrochemical detection of *E. coli* UTI type-1-fimbria wild-type strains. [30, 31] We demonstrate here that the covalent integration of anti-fimbrial antibodies onto acid-terminated oligoethylene glycol (OEG₁₂) molecular layers grafted on amorphous hydrogenated silicon (a-Si:H) thin films allows for specific trapping of fimbriated *E. coli* onto the surface. SERS detection was achieved upon interaction of positively-charged gold nanorods (Au NRs) with the negatively-charged cell membrane of *E. coli* trapped on the surface. The SERS fingerprints proved to be reproducible due to the homogeneous coverage of the bacterial membrane with Au NRs, a feature often not well-addressed in the literature. Our strategy differs from others as it is based on trapping the bacteria on well-defined areas at the surface of the biosensor and post-coating the bacteria with Au NRs for SERS mapping and fingerprint identification.

Figure 1. *E. coli* selective trapping on hydrogenated amorphous silicon a-Si:H surfaces modified with anti-fimbrial antibodies against the major pilin protein FimA. Capture bacteria are analyzed using their interaction with positively-charged gold nanorods (Au

NRs) used as SERS markers: (A) Surface architecture for the selective trapping of FimA-expressing *E. coli* and post-incubation with positively-charged Au-NRs for SERS sensing. (B) anti-fimbriae modified array, optical images of spots after interaction with *E. coli* and SERS signature of the trapped bacteria.

2. Experimental Part

2.1. Materials

Hydrogen peroxide (H₂O₂, 30%), sulfuric acid (H₂SO₄, 96%), and hydrofluoric acid (HF) were of RSE grade and supplied by Carbo Erba. Undecylenic acid (99%, Acros Organics), carboxypoly(ethylene glycol) amine (HOOC-PEG₁₂-NH₂, Fischer Scientific, chloroauric acid (HAuCl₄, Alfa Aesar) and hexadecyltrimethylammonium bromide CTAB (98%, TCI) were used as received. N-hydroxysuccinimide (NHS), N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC), sodium dodecyl sulphate (SDS), tween 20, sarkosylate, methoxypoly(ethyleneglycol)-amine 750 (CH₃O-PEG₇₅₀-NH₂), silver nitrate, L-ascorbic acid, dextran sulfate, ammonium sulfate, sodium phosphate, ethylenediaminetetraacetic acid EDTA, sodium citrate, glycine, magnesium chloride, calcium chloride and sodium chloride were supplied by Sigma Aldrich. Ultrapure water from a Milli-Q system (18 MΩ cm⁻¹) was used in all experiments. PBS 1X, 0.2X and 0.1X were obtained by dilution in Milli-Q water of PBS 10X purchased from Ambion. All glassware used for the AuNRs synthesis was cleaned using freshly prepared aqua regia (HCl: HNO₃ in a 3:1 ratio by volume), followed by rinsing with Milli-Q water.

2.2. Synthesis of gold nanorods (Au NRs)

Gold NRs were synthesized using a protocol described by Nikoobakht and El-Sayed [32] as follows. The seed solution for gold NR growth was prepared by mixing 5 mL of aqueous HAuCl₄ solution (0.5 mM) with 5 mL of aqueous CTAB solution (0.2 M). Fresh NaBH₄ (0.6 mL, 0.01 M) was injected to the Au(III)-CTAB solution under vigorous stirring during 2 min at 25°C. To prepare the growth solution, 0.10 mL of aqueous AgNO₃ (4 mM) was quickly added to the CTAB solution (5 mL, 0.20 M) at 30 °C. To this solution, 5 mL of 1 mM HAuCl₄ was quickly added and after gentle mixing of the solution, 70 μL of aqueous ascorbic acid (78.8 mM) was added and the color of the solution changed from dark yellow to colorless. Finally, 12 μL of the seed solution was added to the growth solution at 30 °C and

gently mixed for 2 h. The color of the solution gradually changed within 10-20 min. The final dark-blue colloidal solution was purified by repeated centrifugations (2×) at 6000 rpm for 20 min.

2.3. Biosensor construction

2.3.1. Deposition of amorphous silicon (a-Si) thin films on glass

Microscope glass slides were copiously rinsed with water then with TFD4-type detergent (Franklab), before being immersed in absolute ethanol for 15 min. After vigorously rinsing with deionized water, the slides were further immersed into piranha solution (1/3 H₂O₂/H₂SO₄; **caution:** very corrosive) for 15 min. After a final rinse with ultrapure Milli-Q water, the clean slides were dried under nitrogen flow.

Hydrogenated amorphous silicon thin layer (3 nm) was deposited onto the glass slides using plasma-enhanced chemical vapor deposition (PECVD) in a low-power regime as described by Solomon *et al.* [33] The deposition was performed using the following parameters: 40 mTorr pressure, 250°C, 0.06 Wcm⁻² power density and 33 sccm gas flow rate (SiH₄).

2.3.2. Surface modification of glass/a-Si:H

Glass/a-Si:H slides were treated using HF vapor for 15 s to generate hydrogen-silicon bonds and then placed at room temperature into a Schlenk tube containing undecylenic acid previously deoxygenated and heated at 100°C during 30 min. The tube was subsequently introduced into a UV chamber and exposed to 312 nm irradiation for 3 h. The slides were then rinsed for 30 min with hot acetic acid (75°C, two times), and finally with PBS 1X/0.1% SDS for 15 min, followed by 5 min in PBS 0.2X, 5 min in PBS 0.1X and 2 min in Milli-Q water. The slides were dried under nitrogen flow and stored under vacuum.

The acid-functionalized surfaces were subsequently immersed in 10 mL of a mixture of 10 mM EDC and 10 mM NHS for 90 min at 15°C. [34] The as-obtained substrates were copiously rinsed with Milli-Q water and dried under nitrogen flow and then immersed into 20 mM HOOC-PEG₁₂-NH₂ solution during 3 h at room temperature. After a final rinse in Milli-Q water, the substrates were dried under nitrogen flow and stored under vacuum at room temperature until next use.

The PEGylated surface was activated using EDC/NHS mixture as described above. A pin spotter (Biorobotics Microgrid II) was used to locally deposit droplets of a few nL of antibody solutions by contact on the freshly activated surface. The antibodies were used at various concentrations (0.57 mg/mL, 1.43 mg/mL for purified antibodies and 20× dilution for serum antibodies) diluted in different spotting buffers (SB) as follows: 150 mM phosphate, 0.005%

tween 20, 0.001% sarkosyl (SB1); 150 mM phosphate, 0.005% tween20 (SB2); 150 mM phosphate, 0.01% sarkosyl (SB3), 10 mM phosphate, 10 mM NaCl, 10 mM sucrose (SB4). After spotting, the slides were stored in a desiccator overnight at controlled humidity (~75% at 15°C). The slides were then blocked for 1 h using PEG750-NH₂ (50 mM in PBS 1X). After a final rinse using PBS 1X and water Milli-Q, the sample (dried under a nitrogen stream) was stored under vacuum at room temperature.

2.3.3. Trapping of bacteria using a fluidic cell and SERS labeling

By placing the biosensor inside a flow cell with an internal volume of 200 μ L, suspensions of bacteria were passed over the sensor at a flow rate of 0.25 mL.min⁻¹. Cycles consisting of 5 min flow followed by 15 min static contact were repeated until bacteria were observed on spots through the glass window of the cell by using a microscope (setup see **Figure S11**). 150 μ L of the as-synthesized Au NRs were deposited on the biochips which had reacted with bacteria and placed on a rotating plateau. After 15 min interaction, the excess of Au NRs was rinsed off using Milli-Q water and the surface was dried using a nitrogen stream. SERS mapping was then performed.

2.4. Biological part

2.4.1. Bacterial growth

E. coli JM101TR strain was kindly provided by the biochemistry laboratory (BIOC) at Ecole Polytechnique. [35-36] Katushka fluorescent *E. coli* was obtained from the transformation of *E. coli* K12 MG1655 (ATCC 700926) with the pDONR221-nadB-cat recombinant plasmid, as described before. [37] *E. coli* AAEC185 are K12 strains transformed with the pUT2002 plasmid, which carries the *fim* operon with a deletion of the *fimH* gene encoding the FimH adhesion [38] and /or with pMMB66 low-copy number plasmid with the LacI repressor and tac promoter, that controls the expression of the cloned wild-type *fimH* gene [39] respectively.

Both *E. coli* Katushka and *E. coli* JM101TR strains were grown in 2 \times YT medium at 37°C with shaking until the OD₆₀₀ reached 0.5-1. Part of the culture (10 mL) were washed with PBS 1X or Milli-Q water (20 mL), re-suspended in 1mL of PBS 1X or Milli-Q water (1 mL) and diluted to required concentration. *E. coli* AAEC185 was grown in LB medium with antibiotic selection (25 μ g/mL chloramphenicol for pUT2002 and 100 μ g/mL ampicillin for the pMMB66 transformant) while shaking at 37°C overnight. Part of *E. coli* AAEC185 (pMMB66) was washed with PBS 1X or Milli-Q water and diluted to required concentration

as described above. In case of *E. coli* AAEC185 (pUT2002), part of the first overnight culture was inoculated in fresh LB media (varying volumes but keeping 100× dilution) in a flask with a broad liquid air interface, with 1 μM of IPTG added to the medium to induce the expression of the type-1 fimbriae and incubated without shaking for about 48 h. The obtained culture was washed with PBS 1X or Milli-Q water and diluted to required concentration as described above.

2.4.2. Type-1 fimbriae extraction

E. coli K12 MG1655 and *E. coli* LF82 were used as the source for the extraction and purification of type-1 fimbriae, whereas *E. coli* AAEC185(pUT2002) [40] was used to extract type-1 fimbriae lacking the two-domain adhesin FimH. The latter preparation was further used to generate antibodies against type-1 fimbriae, independent of the presence of FimH adhesin.

Bacterial cultures were washed repeatedly by centrifugation, for exchange of the medium with PBS. Fimbriae and flagellae were sheared from the bacterial surface by homogenization using an Ultra-Turrax blender, 3 times for 40 s at 2000 rpm, interrupted by breaks of 30 s with incubation on ice. Upon clearing from the cell debris by centrifugation at 10000 rpm for 10 min at 10 °C, the sheared fimbriae were recuperated from the supernatant. Precipitation of the sheared organelles was induced using 100 mM MgCl₂ and incubation for 1 h on ice, followed by centrifugation at 10000 rpm at 4°C during 60 min. The pellet was resolubilized in 5 mM Tris-HCl at pH 8.

2.4.3. Type-1 fimbriae purification

A purification step was applied to separate the major pilin protein FimA from the band between 50 and 75 kDa, corresponding to flagellin. The samples (4, 5 and 6), resulting from the extraction, were loaded on SDS-PAGE without trifluoroacetic acid (TFA) and the pure type-1 fimbriae were recovered from the entry well of the stacking gel (3.6% polyacrylamide), similar to what was described earlier [41] (**Figure SI2A**). Next, the type-1 fimbriae were quantified using a densitometer on the bands of FimA in SDS_PAGE (**Figure SI2B**), prior to immunization of rat with FimA for antibody generation.

2.4.4. Generation of anti-FimA polyclonal antibody

The purified FimA pUT2002 was used for immunization of a Whistar rat (Eurogentec, Belgium) for antibody generation. Polyclonal sera from rat SYR474 (non-purified) showed active up to 1000× dilution using Elisa (**Figure SI3A**).

2.4.5. Purification of anti-FimA polyclonal antibody

Purification of polyclonal anti-FimA pUT2002 was carried out on Protein G column according to the provided protocol (HiFliQ Protein G FPLC Column from Generon). Prior to injection on Protein G column, delipidation and filtration steps were performed. For delipidation, the serum was treated with a solution of 10% dextran sulphate and 1 M calcium chloride (for 1 mL of serum, 1 mL of calcium chloride and 0.14 mL of dextran sulphate were added). After 15 min interaction, the obtained precipitate was discarded and the supernatant was washed three times with the binding buffer and concentrated (binding buffer 20 mM sodium phosphate/0.8 M ammonium sulphate pH 7.4). The as-obtained stock was injected on Protein G column for purification (flow rate 1 mL/min). Two elution buffers were used: 0.1 M sodium citrate pH 5.46 (elution peak 1) and 0.2 M glycine pH 2.5 (elution peak 2) (**Figure S3B**) and analyzed for purity on SDS-PAGE (**Figure S3C**).

2.4.6. Reactivity of anti-FimA polyclonal antibody versus FimA

To analyze their reactivity, both antibody fractions were flown at 20 $\mu\text{L}\cdot\text{min}^{-1}$ in HBS-EP buffer (10 mM Hepes pH 7.2, 150 mM NaCl, 5 mM EDTA and 0.005% Tween-20), over the pure FimA (pUT2002), that had been immobilized at a rate of 87 RU (response units) on a CM5 Biacore sensor chip using the standard EDC-NHS coupling procedure (**Figure S13D**).

2.5. Instrumentation

UV/Vis absorption spectra were recorded using a Cary300 UV-Vis spectrophotometer. The wavelength range was 200-800 nm.

Surface-enhanced Raman spectroscopic (SERS) measurements were performed using Horiba/Jobin Yvon LabRam HR instrument equipped with a laser emitting at 633 nm. The spectra of *E. coli* strains were recorded in the 200-2200 cm^{-1} spectral range using different incident laser powers (from 0.1 to 2.5 mW) in order to avoid the photo-degradation of the samples and spectral data acquisition times from 10 to 30s. The 521 cm^{-1} band of the silicon wafer was used for frequency calibration. After analysis with Labspec6 (baseline subtraction and normalization) SERS data was treated using Origin Pro for Principal component analysis (PCA). The full spectral range was taken into account (200-2200 cm^{-1}).

Scanning electron microscopy (SEM) images of bacteria deposited on silicon substrates were recorded using a SEM Hitachi 4800 microscope operating from 3 to 5 kV.

Transmission electron microscopy (TEM) images of *E. coli* K12 MG1655, *E. coli* AAEC185(pUT2002) and *E. coli* LF82 were recorded on samples grown statically overnight

at 37 °C in LB. An aliquot of *E. coli* AAEC185(pUT2002) was adsorbed to a carbon-coated FormVar film on 400-mesh copper grids (Plano GmbH, Germany) for 1 min. The grids were blotted, washed twice in droplets of Milli-Q water and negatively stained using 1% (w/v) uranyl acetate. The grids were allowed to dry before the samples were observed/studied with a FEI Morgagni™ 268(D) microscope operated at 120 kV.

3. Results and discussion

3.1. Isolation and characterization of anti-fimbrial antibodies

It is well known that uropathogenic *E. coli* strains express surface-adhesive organelles known as type-1 pili [29] (**Figure 2A**). These type-1 pili consist of a 7 nm-thick rod joined to a thin 3-nm tip fibrillum containing the adhesin, FimH, which mediates binding to mannose glycoproteins, critical in establishment of *E. coli* UTI infections. [42] The major pilus rod possesses between 500 to 3000 FimA subunits along its full-length. In this work, *E. coli* K12 MG1655 and *E. coli* LF82 [43] were used as alternative type-1 fimbriae sources of FimA, whereas *E. coli* AAEC185(pUT2002) was used to extract type-1 fimbriae lacking the two-domain adhesin FimH. Indeed, using TEM (**Figure 2B**) it was verified that *E. coli* AAEC185(pUT2002) expresses type-1 fimbriae, despite the absence of the FimH gene.

Figure 2. *E. coli* type 1-pili: (A) Schematic representation of *E. coli* type-1 fimbriae with its FimA pilin subunits and their recognition by the polyclonal anti-FimA antibodies. (B) Transmission electron microscopic (TEM) imaging of *E. coli* AAEC185 (pUT2002) shows the presence of fimbriae used to generate the anti-FimA polyclonal antibodies used in this work.

The extracted anti-fimbrial antibodies were formed in two steps: (i) FimA encoded on the pUT2002 plasmid carries the K12 FimA sequence. [38] As can be seen from lanes 9 and 10 in **Figure SI2A**, the presence of trifluoroacetic acid (TFA) in the Laemmli loading buffer is required to dissociate type-1 fimbriae into FimA pilin; (ii) Gel exclusion of type-1 fimbriae had to be used thus in a second purification step to separate the major pilin protein FimA from the band between 50 and 75 kDa, corresponding to flagellin FimC (**Figure SI2B**).

3.2. Development of *E. coli* capturing interface

The specific detection of *E. coli* K12 is based on the selective capture of the pathogen by the surface anchored anti-FimA antibodies (**Figure 3A**). We opted for the use of a hydrogenated amorphous silicon (a-Si:H) as sensing interface as thin films can be easily deposited by plasma-enhanced chemical vapor deposition (PECVD) in low-power regime on glass substrates. Additionally, these a-Si:H thin films have shown to be ideal for the construction of stable biosensors due to the possibility of ligand attachment through robust Si-C covalent bonds. [44-45] The integration of undecylenic acid functions onto a-Si:H is based on the photochemical hydrosilylation reaction, [46] followed by the amidation of a bifunctional oligo(ethylene glycol) OEG linker, HOOC-OEG₁₂-NH₂ in a two-step process using the well-known EDC/NHS coupling activation. [47-48] The presence of at least 8 OEG units proved to be highly efficient for limiting non-specific surface interactions with proteins like lectins. To demonstrate the reliability of this surface functionalization scheme, FTIR-ATR measurements were performed after each reaction step on a 20 nm-thick a-Si:H film deposited on a silicon ATR prism. Focusing on the carbonyl region in the 1500-1900 cm⁻¹ frequency range of the spectra displayed in **Figure SI4**, characteristic peaks are recorded for the carboxylic stretching ν_{CO} mode of the acid-terminated surface (a), the triplet band of the ester-NHS function of the activated surface (b) and the two amide bands together with the carboxyl ν_{CO} band related to the termination of the OEG chains and the probable hydrolysis of unreacted ester-NHS surface (c). The OEG signature is clearly evidenced by the presence of a large band at around 2800-2999 cm⁻¹ corresponding to the stretching mode of the ethylene glycol units ν_{OCH_2} and two peaks at 1461 and 1128 cm⁻¹ related to the scissor mode δ_{OCH_2} and the stretching ν_{OCO} modes, respectively. By repeating the EDC/NHS treatment, the carboxyl functions can be easily reactivated to form the ester-NHS terminated monolayer as displayed in spectrum (d).

In order to achieve a selective harvesting of bacteria to the surface, NH₂-terminated antibodies were locally deposited using a pin-spotter on the EDC/NHS activated substrates. The remaining surface anchoring sites were blocked with hydrophilic CH₃O-PEG₇₅₀-NH₂ (MeOPEG₇₅₀), to avoid any non-specific bacteria adhesion.

Figure 3. Formation of anti-FimA antibody modified interface and interaction with fluorescent *E. coli* K12 expressing the TurboFP635 protein (Katushka): (A) Schematic

illustration of the functionalization scheme for anti-FimA antibodies covalent immobilization: (a) etching of PECVD deposited a-Si:H with HF vapor; (b) photochemical hydrosilylation of a-Si:H using undecylenic acid, (c) amide coupling reaction with NH₂-OEG₁₂-COOH, (d) integration of anti-FimA (*via* spotting), followed by blocking of the remaining COOH functions using CH₃OPEG₇₅₀; (B) Fluorescence images recorded on sensor areas modified with anti-FimA at 0.57 and 1.43 mg/mL (4 lines describing 4 replicates) in different spotting buffers (SB1, SB2, SB3, SB4) after interaction with *E. coli* Katushka (10⁸ CFU mL⁻¹) for 1 h.

The influence of buffer and anti-FimA concentration on the capture efficiency of *E. coli* (10⁸ CFU mL⁻¹) was investigated by fluorescence scanning (at 532 nm) using the fluorescent *E. coli* K12 expressing the TurboFP635 protein (Katushka) (**Figure 3B**). Two anti-FimA concentrations (0.57 and 1.43 mg mL⁻¹) and four different spotting buffers (SB) were employed containing anionic and non-ionic surfactants (SB1), a non-ionic surfactant (SB2), an anionic surfactant (SB3) and sucrose without surfactant (SB4) to keep the 3D protein structure preserved. [49] Using an anti-FimA concentration of 1.43 mg mL⁻¹ in spotting buffer 2 or 3 results in efficient and reproducible bacteria capturing. The optical microscopy images recorded before and after interaction of *E. coli* Katushka with the optimized interface (**Figure SI5A**) are in line with the fluorescence images, showing the aggregation of captured bacteria. Typically, about 700 bacteria interact with the antibodies within a 300-micrometer sized spot. No significant background fluorescence signal was detected between the sensing spots, highlighting the excellent antifouling properties of the surface architecture.

To demonstrate the specificity of the anti-FimA sensing substrate to *E. coli* expressing type-1 fimbriae, *E. coli* pMMB66, a bacteria strain that cannot express type-1 fimbriae [38] was incubated for 1 h with the sensing substrate. No attachment was found even after interaction with high concentrations of *E. coli* pMMB66 (**Figure SI5A**).

The efficiency and the robustness of the surface chemistry were demonstrated by monitoring three successive attachment/detachment cycles of the bacteria during one week (**Figure SI5B**). A basic regeneration treatment (20 mM NaOH) shows a complete removal of the bacteria without affecting the stability and the functionality of the interface. After each regeneration cycle, the bacteria tend to form aggregates at the spot surface with area free of bacteria. Noticeably, the aggregation areas are not found at the same locations after each cycle, an expected feature since binding of bacteria on antibodies is assumed to take place randomly. It suggests that the antibodies are not denatured since they are still able to

specifically interact with the bacterial strains. However, it appears that after three regeneration cycles, the bacteria coverage on the surface is less uniform, suggesting a partial fatigue of the antibody binding capabilities upon regeneration. From a quantitative analysis with Image J program, the amount of trapped bacteria in the spot remains rather identical on the regenerated interface, indicating that the global trapping efficiency of the spot has been preserved. In addition, no unspecific bacterial adhesion outside the spot is observed, confirming the well-controlled chemistry of the sensing layer. Labeled bacteria allowed for validating the efficiency of the sensing architecture through fluorescence measurements. In the following, SERS-based detection will be integrated into the sensor concept.

3.3. SERS based sensing using CTAB-coated Au NRs as SERS markers

3.3.1. Au NRs as SERS markers

Gold nanorods were chosen as SERS markers because of their anisotropic optical properties providing a more flexible control of their resonance wavelength. They also exhibit a stronger SERS sensitivity as compared to spherical nanoparticles and long-term stability at room temperature. [50-51] SERS *E. coli* sensing was based on post-interaction of CTAB-stabilized Au NRs with surface-captured *E. coli*. The Au NRs are positively charged with an aspect ratio of 2, correlating to a length of 40 nm and a width of 20 nm as evidenced from the SEM image in **Figure 4B**. These Au NRs exhibit two plasmon bands peaking at 518 and 653 nm (**Figure 4B**). The longitudinal contribution centered at 653 nm overlaps well with the 633 nm (red laser) excitation wavelength used for SERS measurements. TEM images revealed a strong interaction of Au NRs with the membrane of *E. coli* (**Figure 4C**) most likely due to electrostatic binding interactions [52]. Different interaction durations (15 min to 3 h) were tested, as displayed in the SEM images in **Figure S6**. A large number of Au NRs are already attached to the bacterial membrane of *E. coli* after 15 min. A longer interaction time does not increase the coverage significantly. Using a short interaction time has the double advantage of considerably decreasing the analysis time as well as insuring that bacteria remain viable even upon contact with CTAB-coated particles, known for their antibacterial behavior [53]. The SERS spectrum of bacteria-free Au NRs (after drying of droplets of an Au NR solution directly deposited on glass) exhibits the CTAB characteristic bands at 757 and 1443 cm^{-1} corresponding to ν_{CN^+} and δ_{CH_2} , respectively (cf. **Figure S7**).

Figure 4. Formation of SERS active Au NRs-*E. coli*: (A) UV/Vis spectrum of Au NRS, (B) SEM image of Au NRs, TEM image: overview (C) and closer view (D) of *E. coli* after interaction with Au NRs for 3 h.

Three different *E. coli* strains, *E. coli* JM101TR, Katushka and AAEC185(pUT2002) [54] were mixed with Au NRs and then a droplet of this solution was deposited on a glass slide. This way, the SERS ability of the Au NPs for probing bacteria can be assessed independently of the capture efficiency of the bacteria by the biochip substrate. Once dried, SERS mapping was performed on $10 \times 10 \mu\text{m}^2$ areas and SERS spectra were extracted (**Figure 5**). Different signatures are obtained depending on the *E. coli* strain investigated, with good reproducibility for *E. coli* Katushka (**Figure 5A**) and pUT2002 (**Figure 5B**). Moreover, the CTAB vibrations are generally not seen except for *E. coli* JM101TR strain. In the case of *E. coli* Katushka, the SERS spectra are rich in protein signatures (amide I, II and III at 1650, 1540 and $\sim 1300 \text{ cm}^{-1}$, respectively), amino acid vibrations (at ~ 1500 and 1600 cm^{-1}) and saturated lipid vibrations (δ_{CH_2} at $\sim 1460 \text{ cm}^{-1}$). In the case of *E. coli* AAEC185(pUT2002), the signatures are richer in glycan vibrations with the presence of the stretching modes $\nu_{\text{C-C}}$ in 1,4-glycosidic link at $\sim 850 \text{ cm}^{-1}$ and $\nu_{\text{C-O-C}}$ at $1000\text{-}1130 \text{ cm}^{-1}$. Moreover, some amino acid bands are observable around 1580 cm^{-1} and the characteristic $\nu_{\text{C-C}}$ aromatic ring of phenylalanine is found at 1000 cm^{-1} . In both cases, the signatures are characteristic of the bacteria cell-wall membrane. On the contrary, for *E. coli* JM101TR (**Figure 5C**), the SERS spectra are less reproducible with main bands recorded in a large range of Raman shifts between 400 and 1600 cm^{-1} . As compared to the two other strains, *E. coli* Katushka is the only one possessing flagella, which can be distinguished by the presence of strong amide bands. The combination of SERS and Principal Component Analysis (PCA) has been shown to be able to provide the identification of bacteria at the strain level.[55] Here, even though some conspicuous differences between spectra can be recognized at the naked eye, three distinct clusters corresponding to the three strains are obtained by PCA analysis, confirming that the variability between the spectra of a given strain is statistically lower than the differences between the spectra of distinct strains (**Figure 5D**).

Figure 5. SERS spectra of three different *E. coli* strains (10^9 CFU mL^{-1}) after interaction with Au NRs as SERS marker; for each strain, the four displayed traces correspond to

spectra recorded at distinct locations of the slide: (A) *E. coli* Katushka, (B) *E. coli* AAEC185(pUT2002) and (C) *E. coli* JM101TR, (D) Principle Component Analysis of the SERS spectra of the three strains.

3.3.2. Specific sensing of *E. coli* in biochip architecture

The sensing interface was placed inside a flow cell (see **Fig. S11**). A suspension of *E. coli* K12 MG1655 (strain not genetically modified with the Katushka protein) of different concentrations (10 - 10^8 CFU mL⁻¹) was passed over the cell for 5 min at a flow rate of 0.25 mL min⁻¹, followed by 10 min of static interaction and a final rinse in PBS 1X and H₂O to eliminate the physisorbed bacteria and the medium. As shown in **Figure S18A**, there is no detectable Raman response of the bacteria or antibodies at this stage.

The surface was then incubated with the Au NRs markers for 15 min and the SERS spectra extracted from the mapping were recorded (**Figure S18B** and **Figure 6A**). As a whole, there is no SERS response of the grafted antibodies alone without interacting bacteria, suggesting no direct interaction of the gold nanorods with the antibodies. When comparing with **Figure 5A**, the bacterial fingerprint of *E. coli* is preserved in the biochip configuration. Especially, the presence of the amide bands and the deformation mode of C-H groups from the lipids is noticed. Moreover, the characteristic signatures of adenine and phenylalanine vibrations are obvious. It gives evidence that contributions from metabolites are also present in the detected spectra. When the bacterial concentration decreases, the number of trapped bacteria which are mainly agglomerated tends to diminish within the spot. Only at 10 CFU mL⁻¹, unique bacterium can be isolated. The SERS response of bacteria on a substrate is however not proportional to the bacterial concentration as it is the case in solution containing metal nanoparticles as SERS markers. [56] Therefore no quantitative information can directly be obtained. However, it is possible to recover a quantification capability, at least in a given concentration range, by combining SERS and mapping techniques, at the expense of a longer time of analysis [57] and properly modelling the trapping of bacteria on the mixed PEG-antibody surface.

Figure 6: SERS spectra of trapped *E. coli* K12 MG1655: averaged and normalized SERS spectra extracted from SERS mapping of $2 \times 2 \mu\text{m}^2$ area of surface trapped bacteria: (A) at 10^8 ,

10^4 , 10^2 and 10 CFU mL⁻¹ in PBS 1X; (B) at 10^8 and 10 CFU mL⁻¹ in artificial urine; the grey bars in the two frames highlight the spectral features shared by most of the spectra. (C) PCA of the extracted spectra of *E. coli* MG1655 at 10^8 and 10 CFU mL⁻¹ in artificial urine (data from B) compared to the spectra of *E. coli* Katushka/Au NRs and *E. coli* pUT2002/Au NRs deposited on glass (data from Figure 5A and 5B).

In order to test the capability of the sensing technique of detecting *E. coli* K12 in biological fluids, artificial urine was spiked with *E. coli* at 10^8 and 10 CFU mL⁻¹. The SERS spectra clearly evidence the signatures of the cell membrane, as indicated in **Figure 6B**. Remarkably, a positive detection is reproducibly obtained at 10 CFU mL⁻¹ within less than 3 h, this time representing the whole measurement period including *E. coli* strains incubation on the biochip and the SERS analysis. This demonstrates the potential of the biochip architecture for the specific trapping of uropathogenic bacteria at very low concentration in urine, below the clinical threshold of 10^4 CFU mL⁻¹.

A conspicuous observation is that the spectra recorded at the lowest concentration (10 CFU mL⁻¹) exhibit features somewhat different from those recorded at higher concentration. In correlation with the microscopy images (**Figure SI8B**), it can be inferred that these differences likely arise from the effect of bacteria aggregation, as already observed by other groups. [58] The spectra obtained after incubation with 10 CFU mL⁻¹ solutions appear less reproducible and more dependent on the bacterial environment, as compared to SERS spectra recorded after incubation with high-concentration solution. However, these variations are less significant than those recorded among various *E. coli* strains, as shown by the PCA displayed in **Figure 6C** where a clear clustering appears, separating the signatures of *E. coli* AAEC185(pUT2002) from those of *E. coli* K12 MG1655. Furthermore, PCA suggests that the bacteria deposited from high-concentration solutions have an environment which might change from isolated bacteria to aggregated clusters. This can be evidenced from the results in **Figure 6C**, where the signatures recorded in these conditions spread between the cluster obtained from experiments using low bacteria concentrations, and the cluster corresponding to high bacteria concentrations mixed with AuNRs and dried on a glass substrate. However, as shown in **Figure SI9**, this view is probably oversimplified, since PCA performed from experiments made using solutions *E. coli* K12 MG1655 in different media and at various concentrations does not reveal any obvious clustering, leading to the conclusion that no statistical difference can be brought forward when analyzing *E. coli* K12 MG1655 deposited on the biochip from solutions at 10^8 and 10 CFU mL⁻¹ either in PBS1X or urine.

4. Conclusion

The construction of an antifouling biochip for the specific capture of uropathogenic *E. coli* strains has been presented here. Further interaction with positively charged Au NRs allowed the detection of the chemical signature of these *E. coli* strains using SERS with high sensitivity. SERS signals of these bacteria could be determined even at 10 CFU mL⁻¹ in urine samples. Thanks to the sensitivity of the SERS response, pre-enrichment steps can be avoided, allowing a diagnosis suited to real world settings to be performed. Indeed, with a total assay time lower than 3 h, from *E. coli* strains incubation on the biochip to the SERS analysis, the approach is suited to sample screening of patients with symptoms of urinal infection. The robustness of the interface appears remarkable, since it could potentially be reused without loss of sensitivity and specificity. In addition, the versatile chemistry developed in this study is suitable for the immobilization of various types of probes by simple amidation offers a rapid and powerful diagnostic tool for the discrimination and multiplexing identification of pathogens.

CRedit author statement

Cristina-Cassiana Andrei: methodology, validation, investigation, writing–original draft; Anne Moraillon: methodology (spotting experiment); Stephanie Lau and Nordin Felidj: resources (choice and synthesis of CTAB-AuNRs); Nao Yamakawa and Julie Bouckaert: methodology and resources (generation and purification of antiFimA proteins; bacteria growth process); Eric Larquet: resources (TEM images); Sabine Szunerits and Anne Chantal Gouget-Laemmel: conceptualization, supervision, project administration and funding acquisition; Rabah Boukherroub: funding acquisition, writing and review & editing; François Ozanam: conceptualization, writing and review & editing.

Declaration of interest statement

There are no conflicts to declare.

ACKNOWLEDGMENTS

C.C Andrei thanks the DGA and Ecole Polytechnique for financial support. We also thank the BIOCI laboratory at Ecole Polytechnique for the laboratory access and facilities for the growth of bacteria and for fruitful discussions. This project has received funding from the Centre National de la Recherche Scientifique (CNRS), the University of Lille and the Europe's Horizon 2020 research and innovation staff exchange (RISE) program under grant agreement No. 690836.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi>.

References

- [1] V.P. O'Brien, D.A. Dorsey, T.J. Hannan, S.J. Hultgren, Host restriction of *Escherichia coli* recurrent urinary tract infection occurs in a bacterial strain-specific manner, *PLoS Pathog.* 14 (2018) e1007457.
- [2] L.K. McLellan, D. A., D.A. Hunstad, Urinary Tract Infection: Pathogenesis and Outlook, *Trends Mol Med.* 22 (2016) 946–957.
- [3] M. Íñigo, A. Coello, G. Fernández-Rivas, B. Rivaya, J. Hidalgo, M.D. Quesada, e. al, Direct identification of urinary tract pathogens from urine samples, combining urine screening methods and matrix-assisted laser desorption ionization-time of flight mass spectrometry, *J. Clin. Microbiol.* 54 (2016) 988–993.
- [4] M. Amiri, A. Bezaatpour, H. Jafari, R. Boukherroub, S. Szunerits, Electrochemical Methodologies for the Detection of Pathogens, *ACS Sens.* 3 (2018) 1069-1086.
- [5] A.L. Furst, M.B. Francis, Array sensing using optical methods for detection of chemical and biological hazards, *Chem Rev.* 119 (2019) 700-726.
- [6] K.L. Diehl, E.V. Anslyn, Array sensing using optical methods for detection of chemical and biological hazards, *Chem. Soc. Rev.* 42 (2013) 8596-8611.
- [7] T. Mocan, C.T. Matea, T. Pop, O. Mosteanu, A.D. Buzoianu, C. Puia, C. Iancu, L. Mocan, Development of nanoparticle-based optical sensors for pathogenic bacterial detection, *J. Nanobiotechnol.* 15 (2017).
- [8] V. Templier, T. Livache, S. Boisset, M. Maurin, S. Slimani, R. Mathey, Y. Roupioz, Biochips for Direct Detection and Identification of Bacteria in Blood Culture-Like Conditions, *Sci. Rep.* 7 (2017), 9457.
- [9] S. Bouguelia, Y. Roupioz, S. Slimani, L. Mondani, M.G. Casabona, C. Durmort, T. Vernet, R. Calemczuk, T. Livache, On-chip microbial culture for the specific detection of very low levels of bacteria, *Lab. Chip* 13 (2013) 4024-4032.
- [10] E. Primiceri, M.S. Chiriaco, F. de Feo, E. Santovito, V. Fusco, G. Maruccio, A multipurpose biochip for food pathogen detection, *Anal. Meth.* 8 (2016) 3055-3060.
- [11] A. Mader, K. Gruber, R. Castelli, B.A. Hermann, P.H. Seeberger, J.O. Raedler, M. Leisner, Discrimination of *Escherichia coli* Strains using Glycan Cantilever Array Sensors, *Nano Lett.* 12 (2012) 420-423.

- [12] X.H. Xie, H.B. Pu, D.W. Sun, Recent advances in nanofabrication techniques for SERS substrates and their applications in food safety analysis, *Crit. Rev. Food Sci. Nutr.* 58 (2018) 2800-2813.
- [13] R.M. Jarvis, R. Goodacre, Discrimination of bacteria using surface-enhanced Raman spectroscopy, *Anal. Chemistry* 76 (2004) 40-47.
- [14] R.M. Jarvis, R. Goodacre, Characterisation and identification of bacteria using SERS, *Chem. Soc. Rev.* 37 (2008) 931-936.
- [15] X. Zhao, M. Li, Z. Xu, Detection of Foodborne Pathogens by Surface Enhanced Raman Spectroscopy, *Front. Microbiol.* 9 (2018), 1236.
- [16] Y. Wang, B. Yan, L. Chen, Novel Optical Nanoprobes for Bioanalysis, *Chem. Rev.* 113 (2013) 1391-1428.
- [17] B. Guven, N. Basaran-Akgul, E. Temur, U. Tamer, I.H. Boyaci, SERS-based sandwich immunoassay using antibody coated magnetic nanoparticles for *Escherichia coli* enumeration, *Analyst* 136 (2011) 740-748.
- [18] W. Gao, B. Li, R. Yao, Z. Li, X. Wang, X. Dong, H. Qu, Q. Li, N. Li, H. Chi, B. Zhou, Z. Xia, Intuitive Label-Free SERS Detection of Bacteria Using Aptamer-Based in Situ Silver Nanoparticles Synthesis, *Anal. Chem.* 89 (2017) 9836-9842.
- [19] T. Szymborski, E. Witkowska, W. Adamkiewicz, J. Waluk, A. Kaminska, Electrospun polymer mat as a SERS platform for the immobilization and detection of bacteria from fluids *Analyst* 139 (2014) 5061-5064.
- [20] N.A. Mungroo, G. Oliveira, S. Neethirajan, SERS based point-of-care detection of food-borne pathogens, *Microchim. Acta* 183 (2016) 697-707.
- [21] I.F. Cheng, T.-Y. Chen, R.-J. Lu, H.-W. Wu, Rapid identification of bacteria utilizing amplified dielectrophoretic force-assisted nanoparticle-induced surface-enhanced Raman spectroscopy, *Nanoscale Res. Lett.* 9 (2014) 1-8.
- [22] M. Knauer, N.P. Ivleva, X.J. Liu, R. Niessner, C. Haisch, Surface-Enhanced Raman Scattering-Based Label-Free Microarray Readout for the Detection of Microorganisms, *Anal. Chem.* 82 (2010) 2766-2772.
- [23] M. Knauer, N.P. Ivleva, R. Niessner, C. Haisch, A flow-through microarray cell for the online SERS detection of antibody-captured *E. coli* bacteria, *Anal. Bioanal. Chem.* 402 (2012) 2663-2667.
- [24] H. Zhou, D. Yang, N.E. Mircescu, N.P. Ivleva, K. Schwarzmeier, A. Wieser, S. Schubert, R. Niessner, C. Haisch, Surface-enhanced Raman scattering detection of bacteria on microarrays at single cell levels using silver nanoparticles, *Microchim. Acta* 182 (2015) 2259-2266.
- [25] A.E. Grow, L.L. Wood, J.L. Claycomb, P.A. Thompson, New biochip technology for label-free detection of pathogens and their toxins, *J. Microbiol. Meth.* 53 (2003) 221-233.
- [26] P.A. Mosier-Boss, Review on SERS of Bacteria, *Biosensors-Basel* 7 (2017), 51-77.
- [27] W.E. Huang, R.I. Griffiths, I.P. Thompson, M.J. Bailey, A.S. Whiteley, Raman microscopic analysis of single microbial cells, *Anal. Chem.* (2004) 4452-4458.
- [28] S. Stockel, J. Kirchhoff, U. Neugebauer, P. Rosch, J. Popp, The application of Raman spectroscopy for the detection and identification of microorganisms, *J. Raman Spectrosc.* 47 (2016) 89-109.
- [29] S.D. Knight, J. Bouckaert, Structure, Function, and Assembly of Type 1 Fimbriae, *Top Curr Chem.* 288 (2009) 67-107.
- [30] R. Jijie, K. Kahlouche, A. Barras, N. Yamakawa, J. Bouckaert, T. Gharbi, S. Szunerits, R. Boukherroub, Reduced graphene oxide/polyethylenimine based immunosensor for the selective and sensitive electrochemical detection of uropathogenic *Escherichia coli*, *Sensors Actuat. B Chem.* 260 (2018) 255-263.

- [31] F. Halouane, R. Jijie, D. Meziane, C. Li, S. Singh, J. Bouckaert, J. Juraszek, S. Kurungot, A. Barras, M. Li, R. Boukherroub, S. Szunerits, Selective isolation and eradication of *E. coli* associated with urinary tract infections using anti-fimbrial modified magnetic reduced graphene oxide nanoheaters, *J. Mater. Chem. B* 5 (2017) 8133-8142.
- [32] B. Nikoobakht, M.A. El-Sayed, Preparation and growth mechanism of gold nanorods (NRs) using seed-mediated growth method, *Chem. Mater.* 15 (2003) 1957-1962.
- [33] I. Solomon, M.P. Schmidt, H. Tranquoc, selective low-power plasma decomposition of silane-methane mixtures for the preparation of methylated amorphous-silicon, *Phys. Rev. B* 38 (1988) 9895-9901.
- [34] S. Sam, L. Touahir, J.S. Andresa, P. Allongue, J.N. Chazalviel, A.C. Gouget-Laemmel, C. Henry de Villeneuve, A. Moraillon, F. Ozanam, N. Gabouze, S. Djebbar, Semiquantitative Study of the EDC/NHS Activation of Acid Terminal Groups at Modified Porous Silicon Surfaces, *Langmuir* 26 (2010) 809-814.
- [35] P.H. Hirel, F. Leveque, P. Mellot, F. Dardel, M. Panvert, Y. Mechulam, G. Fayat, Genetic engineering of methionyl-tRNA synthetase: *in vitro* regeneration of an active synthetase by proteolytic cleavage of a methionyl-tRNA synthetase- β -galactosidase chimeric protein, *Biochimie* 70 (1988) 773-782.
- [36] Y. Mechulam, S. Blanquet, G. Fayat, Dual Level Control of the Escherichia coli pheST-himA Operon Expression tRNAPhe-dependent Attenuation and Transcriptional Operator-repressor Control by himA and the SOS Network, *J. Mol. Biol.* 197 (1987) 453-470.
- [37] H.B. Yan, R.S. Yalagala, F.Y. Yan, Fluorescently labelled glycans and their applications, *Glycoconj. J.* 32 (2015) 559-574.
- [38] F.C. Minion, S.N. Abraham, E.H. Beachey, J.D. Goguen, The genetic determinant of adhesive function in type 1 fimbriae of Escherichia coli is distinct from the gene encoding the fimbrial subunit, *J. Bacteriol.* 165 (1989) 1033-1036.
- [39] J.P. Furste, W. Pansegrau, R. Frank, H. Blocker, P. Scholz, M. Bagdasarian, E. Lanka, Molecular-cloning of the plasmid RP4 primase region in a multi-host-range TACP expression vector, *Gene* 48 (1986) 119-131.
- [40] I.C. Blomfield, M.S. McClain, B.I. Eisenstein, Type 1 fimbriae mutants of Escherichia coli k12: Characterization of recognized afimbriate strains and construction of new fim deletion mutants, *Mo. Microbiol.* 5 (1991) 1439-1445.
- [41] Y. Eshdat, F.J. Silverblatt, N. Sharon, Dissociation and reassembly of escherichia coli type 1 pili, *J. Bacteriol.* 148 (1981) 308-314.
- [42] M.S. Hanson, C.C. Brinton Jr, Identification and characterization of *E. coli* type-1 pilus tip adhesion protein, *Nature* 332 (1988) 265-268.
- [43] S. Miquel, E. Peyretailade, L. Claret, A. de Vallée, C. Dossat, B. Vacherie, e. H. Zineb, B. Segurens, V. Barbe, P. Sauvanet, C. Neut, J.F. Colombel, C. Medigue, F.J. Mojica, P. Peyret, R. Bonnet and A. Darfeuille-Michaud, Complete genome sequence of Crohn's disease-associated adherent-invasive *E. coli* strain LF82, *PLoS One* 17 (2010) e12714.
- [44] P. Allongue, D. Aureau, R. Boukherroub, J.N. Chazalviel, E. Galopin, A.C. Gouget-Laemmel, C. Henry de Villeneuve, A. Moraillon, J. Niedziółka-Jönsson, F. Ozanam, J. Salvador Andresa, S. Sam, I. Solomon, S. Szunerits, Molecular monolayers on silicon as substrates for biosensors, *Bioelectrochem.* (2010) 17-25.
- [45] L. Touahir, A. Moraillon, P. Allongue, J.N. Chazalviel, C.H. de Villeneuve, F. Ozanam, I. Solomon, A.C. Gouget-Laemmel, Highly sensitive and reusable fluorescence microarrays based on hydrogenated amorphous silicon-carbon alloys, *Biosens. Bioelectron.* 25 (2009) 952-955.

- [46] A. Faucheux, A.C. Gouget-Laemmel, C. Henry de Villeneuve, R. Boukherroub, F. Ozanam, P. Allongue, J.-N. Chazalviel, Well-Defined Carboxyl-Terminated Alkyl Monolayers Grafted onto H-Si(111): Packing Density from a Combined AFM and Quantitative IR Study, *Langmuir* 22 (2006) 153-162.
- [47] J. Yang, A. Moraillon, A. Siriwardena, R. Boukherroub, F. Ozanam, A.C. Gouget-Laemmel, S. Szunerits, Carbohydrate Microarray for the Detection of Glycan-Protein Interactions Using Metal-Enhanced Fluorescence, *Anal. Chem.* 87 (2015) 3721.
- [48] J. Yang, J.-N. Chazalviel, A. Siriwardena, R. Boukherroub, F. Ozanam, S. Szunerits, A.C. Gouget-Laemmel, Quantitative Assessment of the Multivalent Protein-Carbohydrate Interactions on Silicon, *Anal. Chem.* 86 (2014) 10340.
- [49] J.B. Delehanty, F.S. Ligler, A microarray immunoassay for simultaneous detection of proteins and bacteria, *Anal. Chem.* 74 (2002) 5681-5687.
- [50] Y.M. Ou, X.H. Wang, K.Q. Lai, Y.Q. Huang, B.A. Rasco, Y.X. Fan, Gold Nanorods as Surface-Enhanced Raman Spectroscopy Substrates for Rapid and Sensitive Analysis of Allura Red and Sunset Yellow in Beverages, *J. Agri. Food Chem.* 66 (2018) 2954-2961.
- [51] I. Haidar, G. Levi, L. Mouton, J. Aubard, J. Grand, S. Lau-Truong, D.R. Neuville, N. Felidj, L. Boubekeur-Lecaque, Highly stable silica-coated gold nanorods dimers for solution-based SERS, *Phys. Chem. Chem. Phys.* 18 (2016) 32272-32280.
- [52] X.P. Chen, M.Q. Tang, Y. Liu, J.Q. Huang, Z.Y. Liu, H.Y. Tian, Y.T. Zheng, M.L. de la Chapelle, Y. Zhang, W.L. Fu, Surface-enhanced Raman scattering method for the identification of methicillin-resistant *Staphylococcus aureus* using positively charged silver nanoparticles, *Microchim. Acta* 186 (2019), 102.
- [53] K. Nakata, T. Tsuchido, Y. Matsumura, J., Antimicrobial cationic surfactant, cetyltrimethylammonium bromide, induces superoxide stress in *Escherichia coli* cells, *Appl. Microbiol.* 110 (2011) 568-579.
- [54] S.L. Chen, C.S. Hung, J.S. Pinkner, J.N. Walker, C.K. Cusumano, Z. Li, J. Bouckaert, J.I. Gordon, S.J. Hultgren, Positive selection identifies an in vivo role for fimh during urinary tract infection in addition to mannose binding, *Proc. Natl. Acad. Sci. U.S.A* 106 (2009) 22439-22444.
- [55] E. Witkowska, D. Korsak, A. Kowalska, A. Janeczek, A. Kaminska, Strain-level typing and identification of bacteria - a novel approach for SERS active plasmonic nanostructures, *Anal. Bioanal. Chem.* 410 (2018) 5019-5031.
- [56] B. Guven, M. Eryilmaz, A. Uzer, I.H. Boyaci, U. Tamer, R. Apak, Surface-enhanced Raman spectroscopy combined with gold nanorods for the simultaneous quantification of nitramine energetic materials, *RSC Advances* 7 (2017) 37039-37047.
- [57] Y. Tian, P. Wu, Q. Liu, X. Wu, X. Hou, Mapping for total surface-enhanced Raman scattering to improve its quantification analysis, *Talanta* 161 (2016) 151-156.
- [58] K.C. Schuster, E. Urlaub, J.R. Gapes, Single-cell analysis of bacteria by Raman microscopy: spectral information on the chemical composition of cells and on the heterogeneity in a culture, *J. Microbiol. Methods* 42 (2000) 29-38.