Supplementary Information (SI) for Analytical Methods.
This journal is © The Royal Society of Chemistry 2024

Supplimenary information

Ultrasensitive detection of E. coli using bioinspired based platform

Sawsan Almohammed [a, b], Tristan Nolan [c], Niamh Martin [c], Wim G. Meijer [c], Brian J. Rodriguez * [a, b], and James H. Rice * [a]

[a] School of Physics, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland
[b] Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland
[c] School of Biomolecular and Biomedical Science, University College Dublin, Belfield, Dublin, D04 V1W8, Ireland

Materials and Methods

Preparation of FF-PNT Solution

The nanotubes (FF-PNTs) were formed by dissolving L-diphenylalanine peptide (Bachem) in 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma-Aldrich). The initial concentration of 100 mg/mL was then diluted in deionized water to a final concentration of 2 mg/mL. Fresh stock solutions were prepared for each experiment.

Preparation of FF-PNT/Ag NP Template

FF-PNT/Ag NP templates were prepared using 2 mg/mL of the FF-PNT solution, which was heated at 100 °C for 2 minutes. A silver nanoparticle (Ag NP) solution with a concentration of 0.02 mg/mL in water was then added to the heated FF-PNT solution in a 1:1 ratio and stirred for 3 minutes. Subsequently, 40 µL of the mixed solution was placed on either a coverslip or a gold electrode-patterned Si substrate to create an aligned FF-PNT/Ag NP template on the SiO_2 layer formed by UV/ozone treatment. For the control sample of Ag NPs on a Si substrate, 60 µL of Ag NPs (0.02 mg/mL) was diluted with 60 µL of water, and then 40 µL of the solution was deposited on the Si substrate.

Preparation of Si Substrate with Gold Electrode

Si wafers (SiMat), cut to 2 cm × 1 cm, were cleaned by immersion in acetone for 2 minutes, followed by washing with ethanol and isopropanol (Sigma-Aldrich). The substrates were rinsed with deionized water and blown dry with nitrogen. To fabricate interdigitated gold electrode pairs on the Si substrate, gold was sputtered through a 3D-printed mask with an opening of approximately 0.1 mm. For a patterned region, a mask with a 1 mm opening was positioned over the gold electrode, allowing the nanotubes to align during the self-assembly process.
Scanning Electron Microscopy

Scanning electron microscopy (SEM) images were acquired on a scanning electron microscope (JSM-7600F). Prior to imaging, a thin (approximately 10 nm) layer of gold was sputtered onto the samples (Hummer IV, Anatech USA).

Culture Media and Bacterial Cultivation Conditions

Escherichia coli was cultured in LB media to an OD of 0.6 (~10^8 CFU/mL). Dilutions ranging from 10^8 to 10^1 CFU/mL were prepared by washing with PBS, Ringer saline solution, and NaCl as described in previous studies.[6,27] A human blood sample, donated by a volunteer, was mixed with *E. coli* samples by adding a drop of blood (around 5 µL) and then drop-casting onto the FF-Ag NP template for surface-enhanced Raman spectroscopy (SERS) measurements.

Raman Measurements

SERS measurements were conducted using a customized Raman system consisting of an inverted optical microscope (IX71, Olympus), a green laser with a beam splitter and long pass filter (RazorEdge, Semrock), a spectrograph (SP-2300i, Princeton Instruments), and a CCD camera (IXON, Andor). A 50× objective was used to focus the 532 nm wavelength laser with 5 mW incident power. Raman spectra were collected with an exposure time of 1 s, and the average of five measurements was reported. Toluene was used for calibration of the Raman signal over the spectral window. SERS measurements were carried out in situ under an applied electric field from 0 to 20 V in 5 V increments.

Substrate formation

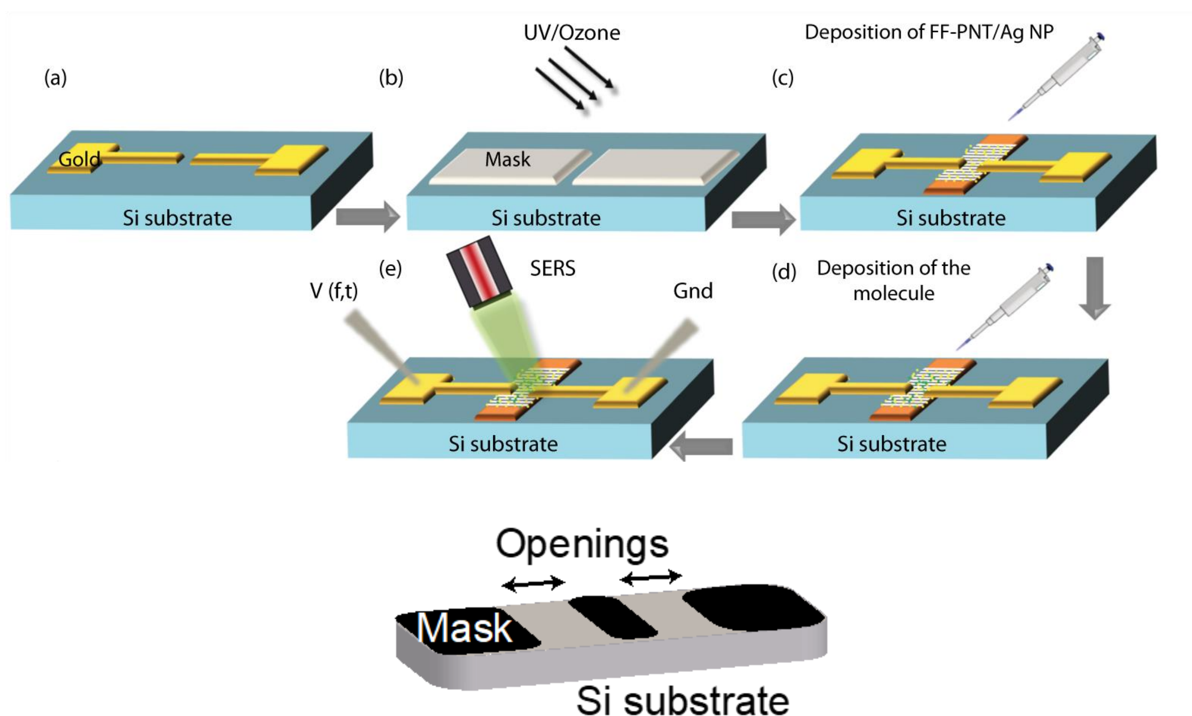

Figure S1. (Top) Process to align the FF-PNT/Ag NP template on the microfabricated gold electrode device. Steps include: (a) gold coating through a 3D-printed mask with an opening size of approximately 0.1 mm, (b) exposure to UV/ozone through a physical mask, (c) deposition of the FF-PNT/Ag NP solution, (d) deposition of the probe molecule on the aligned, dried template, and (e) performing SERS and EL-SERS measurements. **(Bottom)** Schematic of the mask, with the white region indicating the opening where metal is vapor-deposited to form the two parallel electrodes. A comprehensive study on the effects of gap distance and FF-PNT formation on the substrate is presented in Almohammed, S., Oladapo, S.O., Ryan, K., Kholkin, A.L., Rice, J.H., and Rodriguez, B.J. (2016). "Wettability Gradient-Induced Alignment of Peptide Nanotubes as Templates for Biosensing Applications," *RSC Advances*, 6(48), pp. 41809-41815.

Optical imaging of the substrate

To investigate the arrangement of peptide nanotubes on the substrate surface, optical contrast microscopy imaging was performed (Fig. S1, below). The alignment of the peptide nanotubes on the substrate surface was assessed using Fast Fourier Transform (FFT) analysis, as outlined in *RSC Advances*, 6(48), pp. 41809-41815. The degree of alignment was quantified by calculating the average full-width at half-maximum (FWHM) of the radial summation of the FFT of the optical micrographs, determined through a Gaussian fit (see Fig. S2 below). Upon alignment of the peptide nanotubes (PNTs), the FWHM decreased from 68° to 17°, indicating a higher degree of alignment.

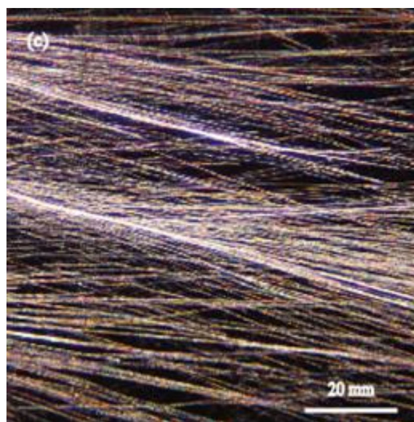

Figure S2. (a) SEM image of an aligned FF-PNT/Ag NP substrate.

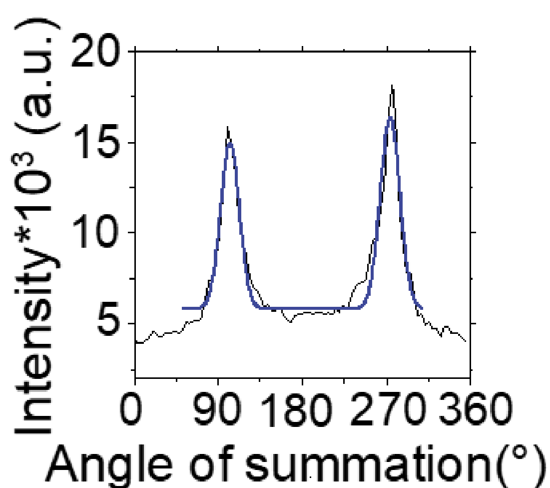

Figure S3. Radial summation of the FFT intensity versus angle showing Gaussian fits (blue line) used to determine the FWHM, i.e., the degree of alignment.

Operation of applying an electric field for SERS enhancement

Previous studies, such as *Nature Communications*, 10(1), p. 2496 (2019), have outlined the methodology we are using to enhance the SERS signal for various probe molecules. To further validate this approach, we conducted studies using glucose, as described in the main paper. As shown in Fig. S3 below, applying a DC electric field significantly increases the SERS signal. An applied voltage in the range of 10 to 20 V enhances the overall SERS signal by approximately fivefold (Fig. S3), with a peak-to-peak ratio that is enhanced by nearly tenfold (see Fig. 1(c) in the main paper).

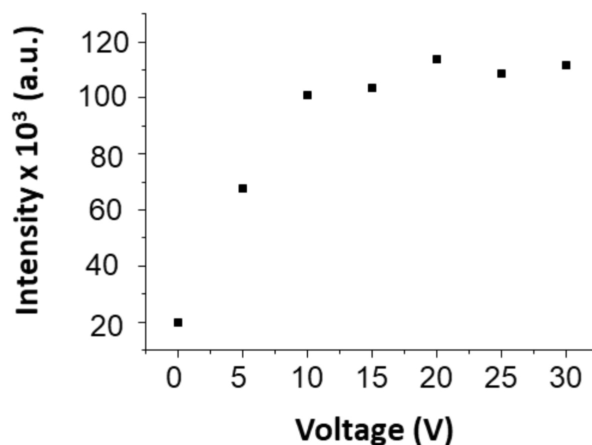

Figure S4. Applying an electric field using an FF-PNT/Ag NP template, with glucose as the probe molecule. Plot showing SERs signal intensity Vs applied voltage.

SERs studies of E. Coli

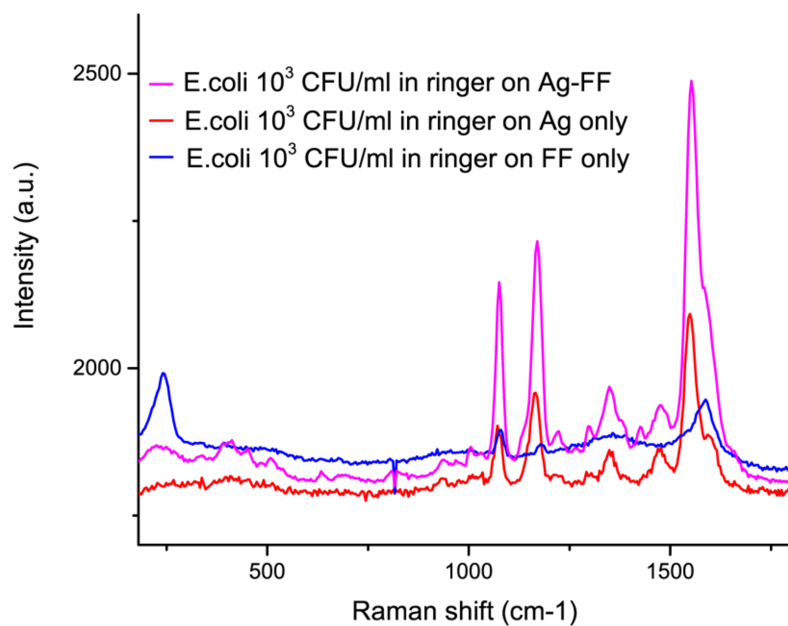

Figure S5. SERS and Raman spectra form E. Coli on three different substrates. The FF-PNT/Ag NP (Ag-FF) is the most intense regarding SERS signal strength. No discernable Raman bands for the bacteria can be detected using the peptide as the substrate.

Demonstration of the wider applicability of the method

We applied the methodology to detect biologically relevant molecules to further demonstrate the wider applicability of the approach.

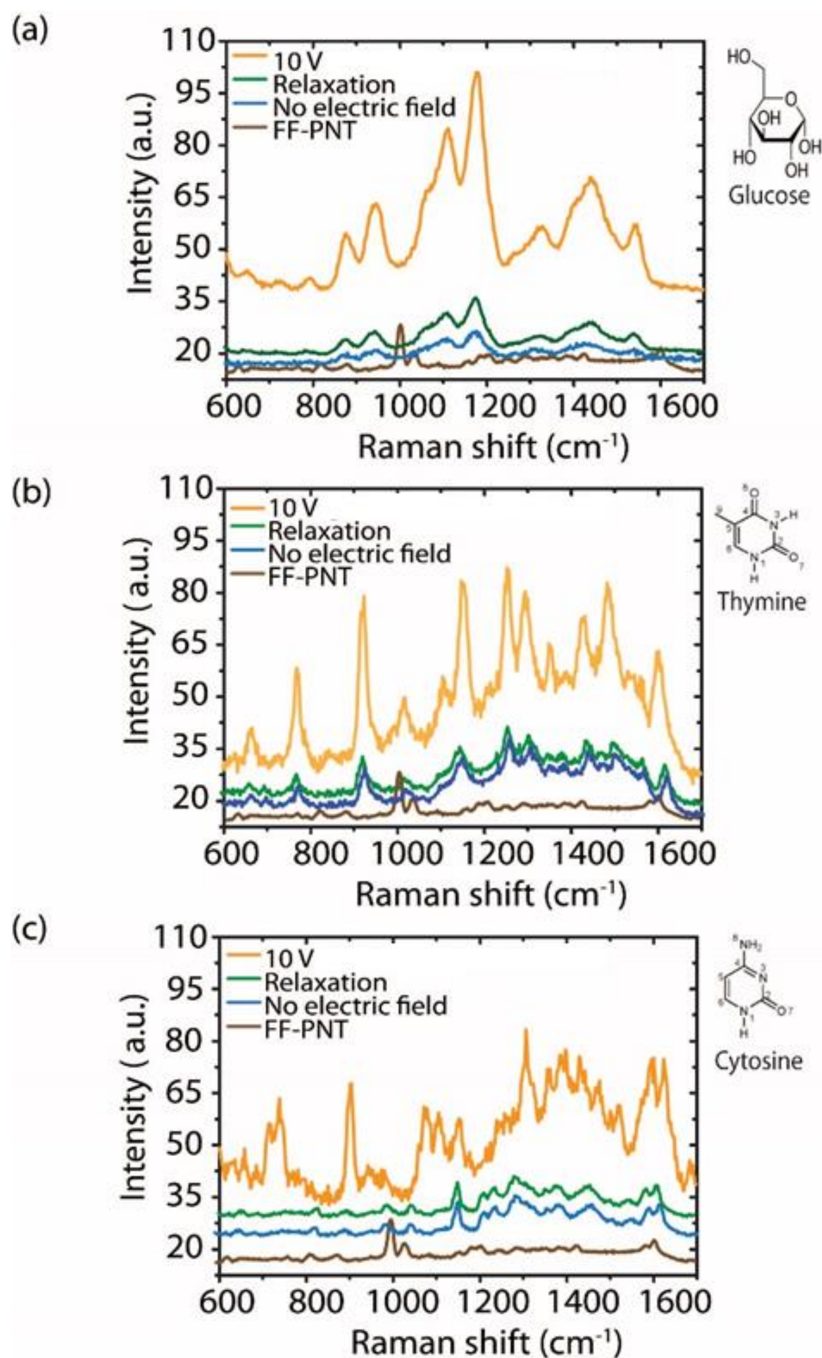

Figure S6. EL-SERS measurements of (a) glucose and DNA based molecules (b) thymine, (c) cytosine, demonstrating field-enhanced sensing using the FF-PNT/Ag NP template.

To further demonstrate the applicability of this methodology, we applied our template to detect bacteria (*E. coli*) in blood as a proof-of-principle study (Fig. S7). *E. coli* was spiked into blood at varying concentrations, from 10^6 to 10^2 CFU/mL, and deposited onto the substrate (Fig. S7(a)). The bacteria sample was drop-cast onto the template and allowed to dry at room temperature before performing SERS measurements. SERS spectra of *E. coli* alone, *E. coli* spiked into blood (at a concentration of 10^3 CFU/mL), and blood alone were recorded (Fig. S7(b)). Raman band intensities from the SERS spectra of blood and blood with *E. coli* were plotted (Fig. S7(c)). The plot shows that the Raman band at 1090 cm^{-1} (C–C skeletal and C–O–C stretching from glycosidic linkage) and the band at 2997 cm^{-1} (Amide B) serve as markers for the presence of bacteria. As the concentration of *E. coli* decreased, associated bacterial bands began to diminish, with detectable bacterial presence at concentrations as low as 10^2 CFU/mL in blood (Fig. S7(d)).

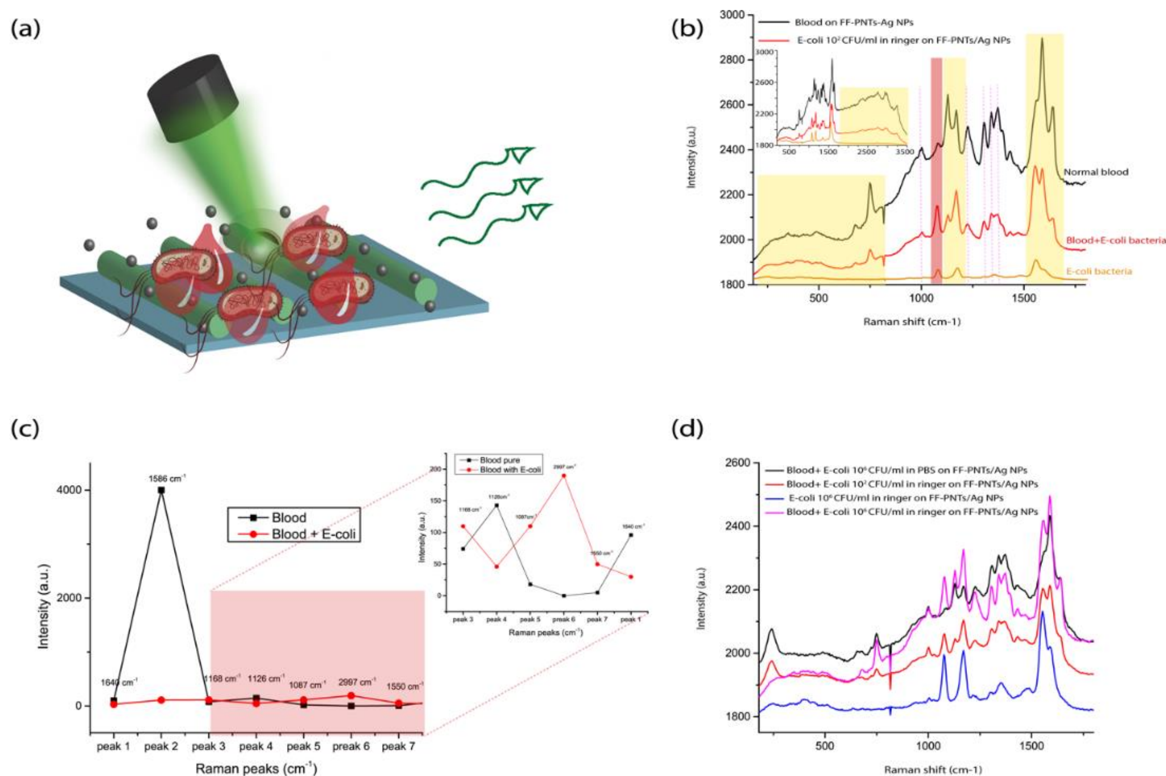

Figure. S7. (a) Schematic drawing of the peptide nanotubes-metal nanoparticles sensor platform for detection of bacteria (*E. coli*) in a blood sample. (b) SERS spectra of *E. coli* on the peptide-silver nanoparticle substrate, where the bacteria was prepared in water and in blood. Shown also is a SERS spectrum of blood only. (c) Plot of Raman band intensities for blood vs blood with *E. coli* spiked at a concentration of 10^3 CFU/ml. (d) SERS spectra of *E. coli* spoked at different concentrations into a blood sample. Insert shows an extended wavelength range for the same SERS spectra, extending the wavelength range to c.a. 3500 cm^{-1}.

SERS band assignments for *E coli*

Wavenumber (cm^{-1})	Assignment	Significance
730	Glycosidic ring vibrations or adenine	Presence of nucleic acids or carbohydrates
1001	Phenylalanine	Used as an internal standard; indicative of protein content
1035	C=C stretching	Reflects the structural integrity of proteins within the cell
1150	C-C deformation	Associated with lipid membranes; indicates membrane integrity and composition
1330	CH2/CH3 wagging modes	Purine bases of nucleic acids
1360	CH2 bending (lipids)	Indicates membrane integrity and composition
1462	CH2/CH3 wagging modes	Deformation of proteins and lipids
1655	Amide I band (C=O stretching)	Indicates protein secondary structure; important for understanding protein folding and function
2850-2950	CH stretching modes of lipids	Reflects lipid composition and membrane dynamics, crucial for assessing cell viability and metabolic state
2930	CH2 stretching	Associated with fatty acid chains in membranes

Table S1. SERS band assignments, taken from Zhou, H.; Yang, D.; Ivleva, N. P.; Mircescu, N. E.; Niessner, R.; Haisch, C. SERS Detection of Bacteria in Water by in Situ Coating with Ag Nanoparticles. Anal. Chem. 2014, 86 (3), 1525–1533. https://doi.org/10.1021/ac402935p.
Zhao, Y., Zhang, Z., Ning, Y., Miao, P., Li, Z. and Wang, H. Simultaneous quantitative analysis of Escherichia coli, Staphylococcus aureus and Salmonella typhimurium using surface-enhanced Raman spectroscopy coupled with partial least squares regression and artificial neural networks. Spectrochimica Acta Part A. 2023, 122510 (293).