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Electrochemical Detection of Single E. coli Bacteria Labeled with Silver Nanoparticles

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Abstract: **A proof-of-concept for the electrochemical detection of single *Escherichia coli* bacteria decorated with silver nanoparticles is reported. Impacts of bacteria with an electrode - held at a suitably oxidizing potential - lead to an accompanying burst of current with each collision event. The frequency of impacts scales with the concentration of bacteria and the charge indicates the extent of decoration.**

The development of fast and sensitive methods for bacterial detection is the target of much current nano-research.[1–5] The exquisite inherent sensitivity of amperometry makes this a potentially attractive approach, but hitherto largely unexplored. In this paper we provide proof-of-concept of a novel electrochemical method which allows the detection of single bacteria and the estimation of their concentration. Recently, this method was established for the detection of individual silver NPs (AgNPs) in solution by means of anodic particle coulometry.[6] In these experiments, the diffusional Brownian motion of the NPs in solution randomly brings them in contact with an inert electrode held under potentiostatic control. If the electrode is held at a suitably oxidizing potential, the collision between the NP and the electrode leads to a short burst of current, as a result of AgNP oxidation. In this paper we utilize the well documented phenomenon in which AgNPs adhere to *Escherichia coli (E. coli)*.[7–10] and show that *single E. coli* cells, 'tagged' with AgNPs, can be reliably detected via this method.

First, the attachment of AgNPs to bacteria is demonstrated. Citrate capped AgNPs (diameter of 9.4±3.6 nm) were first characterized by UV-Vis measurements (for SEM images see[11]), with and without *E. coli* ($\sim 2 \times 0.5\ \mu m^2$). As can be seen from **Figure 1**a, the AgNPs (C = 10 nM) in the absence of *E. coli* exhibit an absorption peak at 393 nm in water (black spectra). Next, the AgNPs were measured in 0.1 M KCl to provide a physiological platform together with a supporting electrolyte needed for the latter electrochemical measurements. First, the peak maximum at 393 nm vanishes almost completely when no *E. coli* cells are present in the electrolyte solution (brown bottom spectra). The absorption spectra of the AgNPs in 0.1 M KCl are quenched due to ionic strength induced aggregation/agglomeration.[12] Second, addition of AgNPs to 0.1 M KCl solution *containing* 0.3 pM *E. coli* showed negligible (~15%) loss in the absorption peak accompanied by a small red shift to 399 nm (red spectra). The shift in the absorbance peak maximum is similar to previously reported work [13] and might be ascribed to several processes such as surface plasmon coupling, aggregation between closely spaced NPs or a capping agent replacement.[14] The inset to **Figure 1**a presents a "titration curve" of the absorption peak of 10 nM AgNP in solution containing different *E. coli* concentrations. The UV-Vis absorption data indicates that there is no change in the absorption peak maxima when ≥0.3 pM *E. coli* cells are present in the solution (molarity ratio of ≤30,000:1 of AgNP:*E. coli*). Therefore, below a molarity ratio of 30,000:1 (*AgNP:E. coli*), the cellular environment stabilizes the AgNPs, despite the high ionic strength of the solution. The high total number of AgNP per single *E. coli* is

plausible, taking into account a single cell volume of ~ 1μm^3 [15], which is larger than the total volume of 30,000 AgNPs (~ 0.01 μm^3). The absorption data shows that above a 30,000:1 ratio of AgNP:*E. coli*, the *E. coli* cells no longer stabilize the AgNP fully (i.e., *E. coli* cells were "saturated" with AgNPs). This saturation is expressed by an attenuation of the absorption peak and larger deviations in the measured absorption, which occurs due to AgNP agglomeration/aggregation and the higher sensitivity to *E. coli* concentration, respectively (pink spectra, see **Figure S**1 for absorption spectra at concentrations of 0.075-3 pM *E. Coli*).

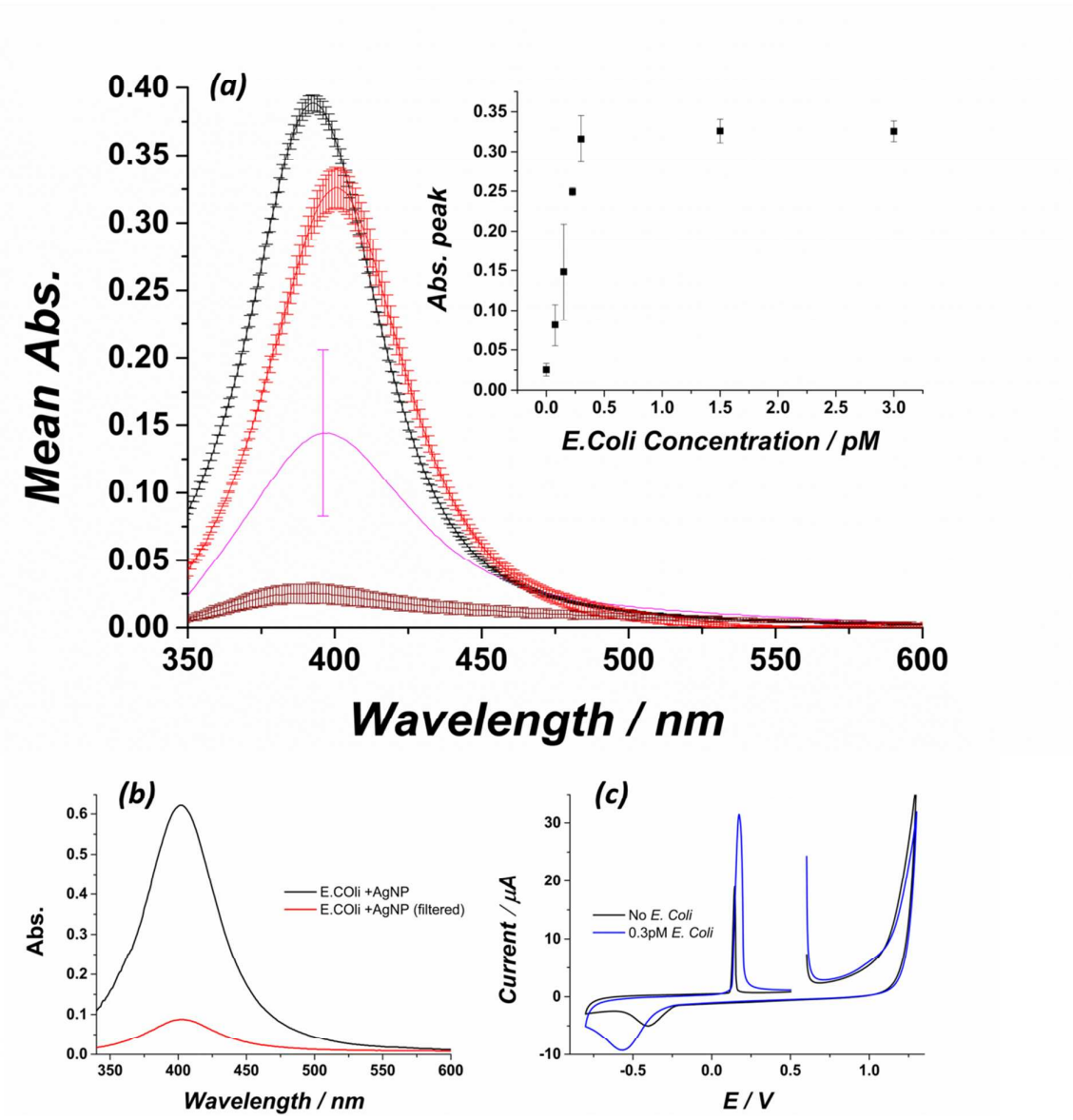

Figure 1. **(a) Absorption spectra of 10 nM AgNPs in water (black), in 0.1 M KCl solution (brown) and in 0.1 M KCl solution containing 0.3 pM (red) or 0.15 pM (pink) *E. coli* cells. (Inset) "Titration curve" of the absorption peak (at 399 nm) of 10 nM AgNPs in 0.1M KCl solution containing different concentrations of *E. coli*. (b) Absorption spectra of 2 nM AgNPs in 0.1 M KCl solution containing 0.3 pM *E. coli*, before and after filtration with 0.2 μm filter (measurement was done in a cuvette with 1cm optical length). (c) Cyclic voltammetry of bare and *E. coli* modified glassy carbon electrode (10 μL of 0.3 pM *E. coli* drop casted).**

The electrode was exposed for 10 minutes to a solution containing 10 nM AgNPs (in 20 mM KCl) and scanned from 0.6 V to 1.3 V to -0.8 V to 0.5 V vs. SCE.

The UV-Vis absorption measurements support the decoration of *E. coli* with AgNPs. Upon filtration of *E. coli* from solution containing the cells and AgNPs, the absorption peak was substantially attenuated (**Figure 1**b). The strong decrease in the absorption peak (~90% loss) confirms that an intimate contact between AgNPs and E. coli is established.

Further and direct evidence for the high affinity of AgNPs to *E. coli* was obtained by voltammetry.[16] *E. coli* films were deposited on a glassy carbon electrode (diameter 3 mm) by drop casting 10 μL of various *E. coli* concentrations and drying the drop under continues flow of N_2. The modified electrode was introduced to a 20 mM KCl solution containing 10 nM AgNPs for ten minutes, followed by a voltammetric scan. The working electrode was first swept to high anodic potential (from 0.6 V up to 1.3 V vs. SCE) to allow oxidation of the AgNP connected to the cell. The results shown in **Figure 1**c indicate that the unmodified (bare) electrode had accumulated NPs during the ten minutes of exposure to the solution, reflected by a small reductive wave (black curve). The existence of small percentage of free silver ions in the solution, might contribute to the signal observed. However, after drop casting and drying 0.3 pM *E. coli* on the electrode, significantly larger stripping charges are seen (blue curve), again *revealing in-situ the "sticking" properties of E. coli towards AgNPs.* The latter result indicates that AgNP coated bacteria provide an electrochemically active platform. Interaction with an electrode may initiate the following reaction:

$$Ag_{N(NP)} - Ne^- \rightarrow NA_g{}^+(aq)$$

where the overall number of atoms 'N' oxidized, reflects the number of nanoparticles Ag_N attached to individual bacteria with the electrode.

After establishing *E. coli* decoration with AgNPs in solution, single *E. coli* detection by means of anodic particle coulometry was investigated. A cylindrical carbon fiber served as the working electrode, which provided a large electrochemically accessible area of ~$2.2 \times 10^4\ \mu m^2$. A saturated calomel electrode and a graphite rod served as the reference and counter electrodes, respectively. The current-time plot of the AgNPs in 0.1 M KCl solution in the absence of *E. coli* (E=1.3 V vs. SCE), is shown in **Figure 2**a (black). As can be seen, a spikeless chronoamperometric signal is obtained upon recording of solution containing AgNP solely (all measurements were preformed ten minutes after AgNPs injection[a]). Note that no oxidative spikes arise from possible solvent breakdown at this applied potential. We chose to coat the *E. coli* with *intentionally* small AgNPs, since the total current arising from an individual AgNP (~$4 \times 10^{-15}\ C$)[b] could not be detected (within the noise level of the system). Only current signals arising from bacteria multiply coated with AgNPs would be seen. After injection of AgNPs to the solution containing *E. coli* and allowing them to interact for ten minutes,[17] electrochemical signals appear (red).

Typical spikes are shown in **Figure 2**b. Each of the current spikes recorded reflects Faradaic charge transfer from a single bacterium multiply coated with AgNP. The duration of the spikes is limited by the pre-amplifier response (See **Figure S**3 for further details). The calculated total charge transferred per impact was $1.2 \pm 0.5 \times 10^{-12}\ \boldsymbol{C}$. Taking into account a calculated charge of $4 \times 10^{-15}\ C$ per individual 9.6 nm AgNP, this corresponds to about $295 \pm 125\ AgNP$s per single *E. coli*. The frequency of the spikes can be approximated theoretically (for a full explanation see [18]), since the process is governed by the Brownian motion of the cells, diffusing freely in the solution. Theoretical estimation of the impact frequency using a reported diffusion coefficient of $2 \times 10^{-13}\ m^2/s$[19] for nonmotile *E. coli* and a cell molarity of ~0.3 pM predicts ~ 0.4 ± 0.2 impacts/s. Our experimental results produced 0.25 ± 0.11 impacts/s, in reasonable agreement with the theoretical estimate. Increasing the molarity of *E. coli* and AgNP (while keeping the molarity ratio of bacteria and AgNP constant) increased the average impact frequency (**Figure 2**c and **Figure S**2). This correlation between frequency and *E. coli* concentration, supports the conclusion that the observed spikes are indeed due to AgNPs decorated bacteria impacting the electrode.

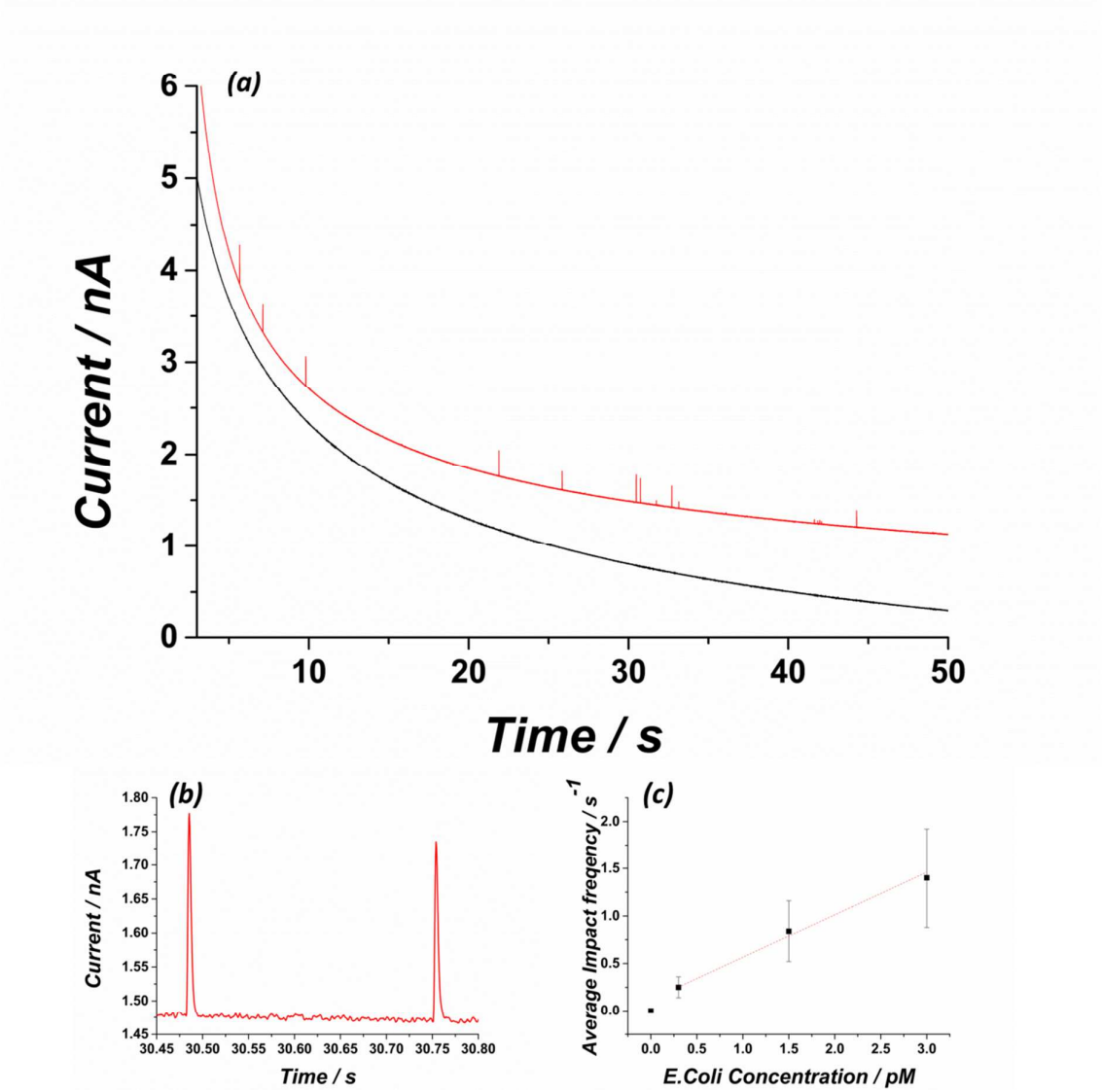

***Figure 2*. Representative chronoamperometric responses for a carbon fiber electrode potentiostated at +1.3 V vs. SCE in 0.1 M KCl and AgNPs. (a) Reference ('blank') chronoamperometric measurement of 1 nM AgNPs without *E. coli* cells (black curve) and with approximately 0.3 pM *E. coli* (red curve). The 'blank' plot was offset in y-axis, for clarity. (b) Current spikes seen with expanded time scale. (c) Average spike frequency (number of spikes / s) as a function of cell concentration with a constant AgNP:*E. Coli* ratio of 3000:1.**

A full analysis of the average total charge per spike transferred as a function of applied potential is shown in **Figure** 3 (for chronoamperometric responses under various applied potentials and after filtering the *E. coli,* see **Figure S**4 and experimental). A total charge of 1.2 ± 0.5 pC was observed at a threshold voltage above ca. 1 V. The data in **Figure 3** resembles a voltammogram as measured in conventional molecular voltammetry with a half wave potential at ca. 1.1 V vs. SCE. Such a value is considerably positive of that previously reported,[20] for "clean" AgNPs (uncoupled to bacteria). This is consistent with the voltammetry seen in Figure 1c and possibly reflects a kinetic barrier due to incorporation of AgNPs into the cell wall of the *E. coli* and consequence AgNP stabilization and inhibition of electron transfer. This is further supported by the small increasing charge seen at applied potentials between 0.4 - 1 V vs. SCE, which may reflect exposed AgNPs that are able to be oxidized at lower anodic potentials.

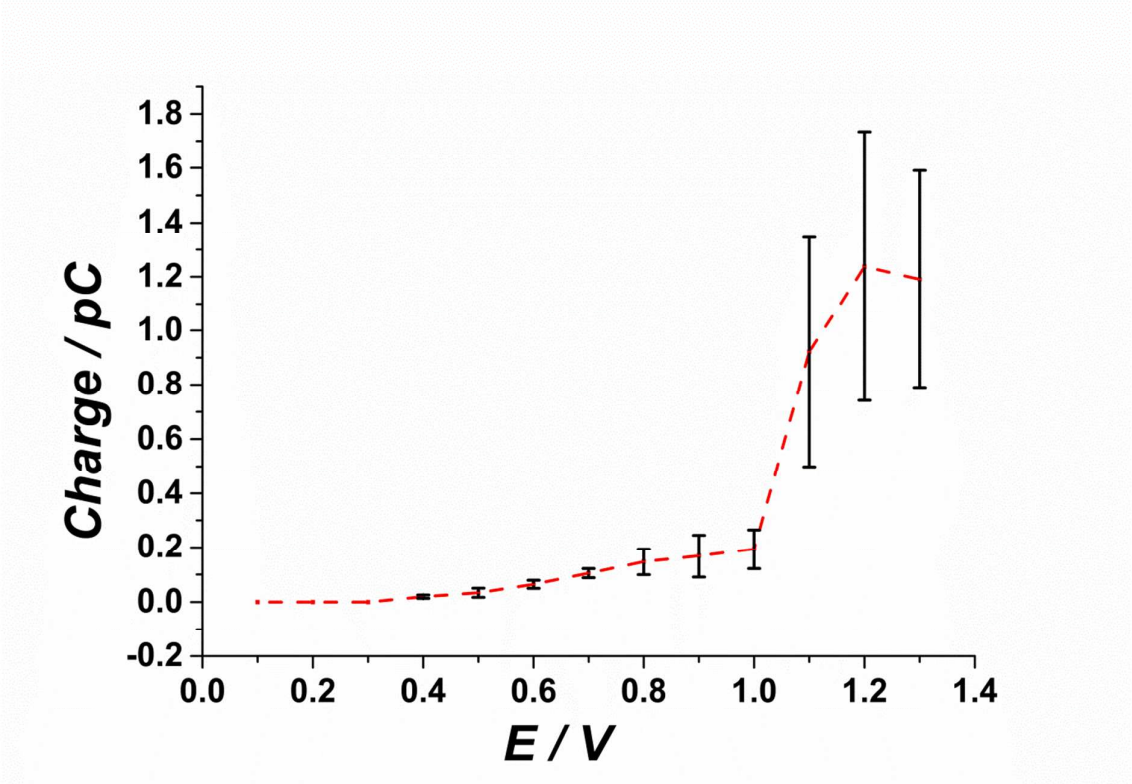

Figure 3. **Measured average total charge transferred (dashed line) as a function of the applied electrode potential, where the depicted error bars represent the uncertainty of the mean.**

We further investigated the response of 10 nM AgNPS in *0.3 pM E. coli* solution (Molarity ratio of 30000:1). The electrochemical results shown in **Figure S**5 indicate that the average total charge increased from 1 pC to ~ 9 pC, as anticipated from a larger AgNPs coverage of the bacteria (for further details see ESI). Hence, the average charge detected at each bacteria/electrode impact reflects the extent of *E. coli* decoration with AgNP.

Conclusions

The anodic particle coulometry technique has the inherent ability to detect single particles, such as bacteria, at low concentrations. Here, using this technique, a proof of concept for fast detection of single bacteria is demonstrated, with *E. coli* cells served as an example due to their know ability to append AgNPs. In less than 1 min scan, single bacteria from solution containing 0.3 pM cells are seen every 4 s (on average). Moreover, the frequency of impacting bacteria holds information on the concentration of bacteria present in an examined solution. The detected charge of 1.2 pC arising from single bacteria is about two orders of magnitude higher than the noise level. This is based on the large accessible surface area of the bacteria, the high affinity of AgNP towards it together with the AgNP electrochemical activity.

Experimental Section

AgNPs of 9.4 ± 3.6 nm were synthesized according to the literature [11,21] and as described in the ESI. Sodium nitrate (>99.5%, $NaNO_3$) was supplied by Fisons Scientific Equipment, Loughborough, UK. Trisodium citrate (>99%, $Na_3C_6H_5O_7$) was ordered from BDH Laboratory Supplies, Poole, UK. Silver nitrate (>99%, $AgNO_3$) and pottesium chloride were obtained from Sigma-Aldrich, Dorset, UK. Sodium borohydride (99%, $NaBH_4$) was supplied by Fisher Scientific, Loughborough, UK. All solutions were made with ultrapure water from Millipore with resistivity not less than 18.2 M cm at 298K. *E. coli* strains (BL21, courtesy of

Prof. C.J Schofield, Oxford) were grown from a starter culture stored at -80 C, using a previously reported protocol.[22] Next, colonies were recovered and grown overnight in a 50 mL 2YT growth media. After cell recovery and growth, 1 mL were inserted into a fresh 2YT media and incubated for approximately 3 hours. The optical density (O.D) at 600 nm was measured during growth until an $O.D_{600}$ of ~1 was achieved. This corresponds to about $0.5 - 1 \times 10^9$ cells /mL (not to be confused with number of colony forming units).[23] Next, a chosen volume (1-10 mL) of the culture was centrifuge at 4000 rpm for 20 min at 4^0C. The cells were washed twice with 0.1 M KCl and suspended gently with 0.1 M KCl (the electrolyte used for the electrochemical experiments).

The characterization of the silver nanoparticle suspension with and without *E. coli* cells was performed by UV–Vis spectroscopy (U-2001, Hitachi, Mannheim, Germany) as reported earlier.[11] For all measurements a quartz cell with a 1mm optical path was used, unless specified otherwise. A blank was used with the same solution conditions in the absence of AgNPs. Filtering of the *E. oli* from the solution containing *E. coli* and AgNP was done using a 0.2μm filter (PVDF filter, Whatman).

For the electrochemical measurements, a $7\,\mu m$ diameter carbon fiber (Goodfellow Cambridge Ltd.), with approximately 1 mm length was used as a working electrode, as reported earlier.[18] All solutions (0.1 M KCl) were made by using ultrapure water of resistivity 18.2 MΩ.cm (Millipore). Experiments were conducted at 293±2 K within a Faraday cage by using a saturated calomel reference electrode (SCE) and a graphite rod counter electrode. For

cyclic voltammetry measurements, an Autolab PGSTAT 12 was used together with a macro glassy carbon electrode (diameter = 3 mm). For the chronoamperometric measurements data was recorded with a three-electrode EPC10 head-stage and Patchmaster software (HEKA Elektronik Dr Schulze GmbH, Lambrecht, Germany). Signals were filtered with the built-in 30 kHz (Bessel) and 0.1-10 kHz filters of the EPC10 at recording time. Impact spikes were analysed using Origin v.8.1 program (www.OriginLab.com) and SignalCounter software developed by Dario Omanovic (Centre for Marine and Environmental Research, Ruder Boskovic Institute, Croatia).

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Keywords: single bacterium detection · nano-electrochemistry · UV-Vis · nano-impact method · silver nanoparticles.

[a] For the first few minutes adventitious spikes do appear, likely caused due to NP agglomeration, see Figure S2).

[b] The charge from individual AgNP was calculated based on estimation that each NP contains ~25,500 silver atoms, taking into account 9.4 nm AgNP spherical NP with atom density (fcc) of 10.5 g/cm^3.

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