# Revealing the effects of three different antimicrobial agents on *E. coli* biofilms by using Soft-Probe Scanning Electrochemical Microscopy

## Supplementary Material

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## SI-1. Characterization of biofilms using state-of-the-art techniques

Laser scanning micrographs (LSMs) in **Fig.S1a,b** show the 3D structure of an *E. coli* biofilm attached to a glass slide in two magnifications. Low magnifications (**Fig.S1a**) show the compact and homogeneous biofilm grown on the glass slide. The higher magnification (**Fig.S1b**) shows the bacteria embedded in the EPS. Scanning electron microscopy (SEM) also shows biofilm morphology in **Fig.S1c,d** and supports the results from LSMs. The fluorescence image of crystal violet, which stained both bacteria and the EPS of the biofilm's formation and biomass, is used to visualize the biofilm's formation and biomass (**Fig.S1d**). The live/dead co-staining of *E. coli* biofilm was investigated by staining with SYTO 9 and propidium iodide (PI). The SYTO 9 stain labels all bacteria green, whereas propidium iodide labels (red color) nonviable bacteria with compromised membranes.

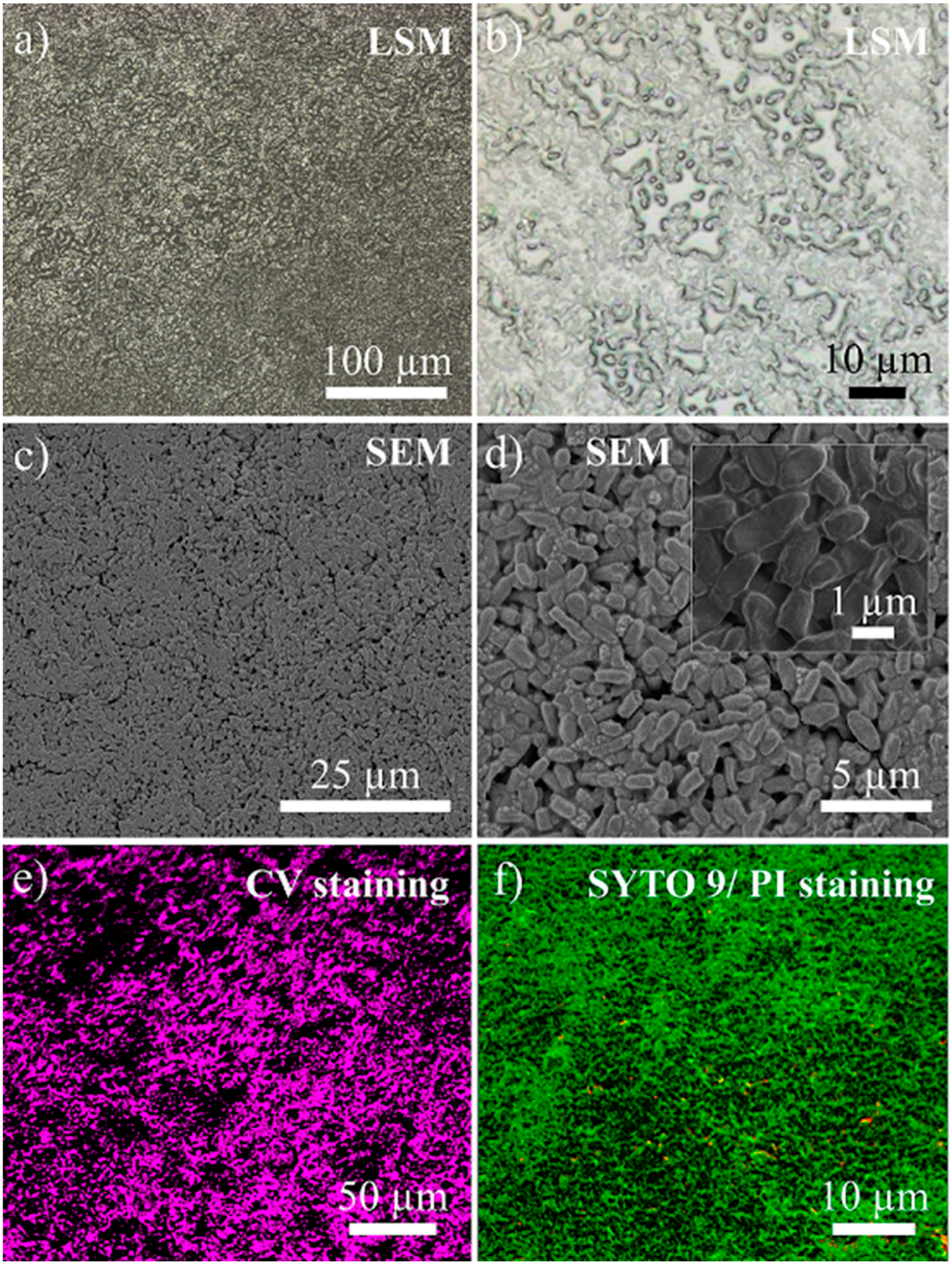


**Fig.S1** Investigate the presence of one-day-old *E. coli* biofilm grown on a coverslip using microscopy: (a-b) laser scanning micrograph in two magnifications. (c-d) SEM images at three different magnifications. e) Fluorescence crystal violet biomass staining. (f) Fluorescence images of SYTO 9/ PI co-staining. SYTO 9 (green color) stained all bacteria, and PI (red color) stained dead bacteria.

**Fig.S2** shows the 3D z-stack fluorescence image of the biofilm, which indicates the 3D structure of the *E. coli* biofilm on the glass slide. The fluorescence image (**Fig.S2a**) shows

mostly the green fluorescence emitted by SYTO 9, indicating that most of the bacteria in the EPS were alive.

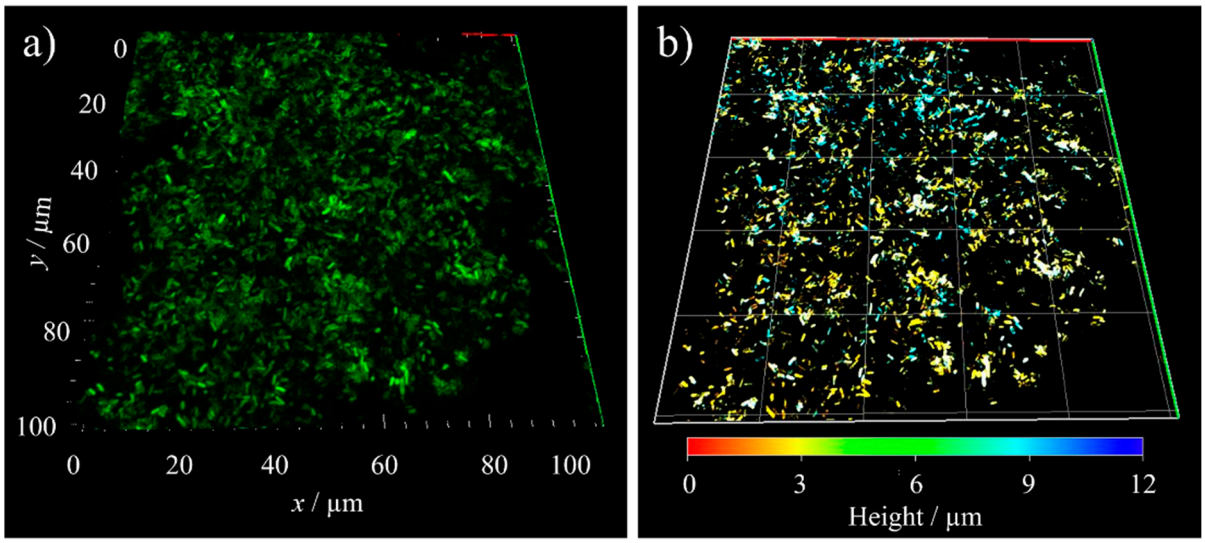


**Fig.S2** (a) 3D structure of one day old *E. coli* biofilm on the coverslip. (b) Height profile of the biofilm. The color bar indicates the height of the biofilm.

**SI-2. Redox potentials of important redox couples in the electron transport chain of *E.coli*.**

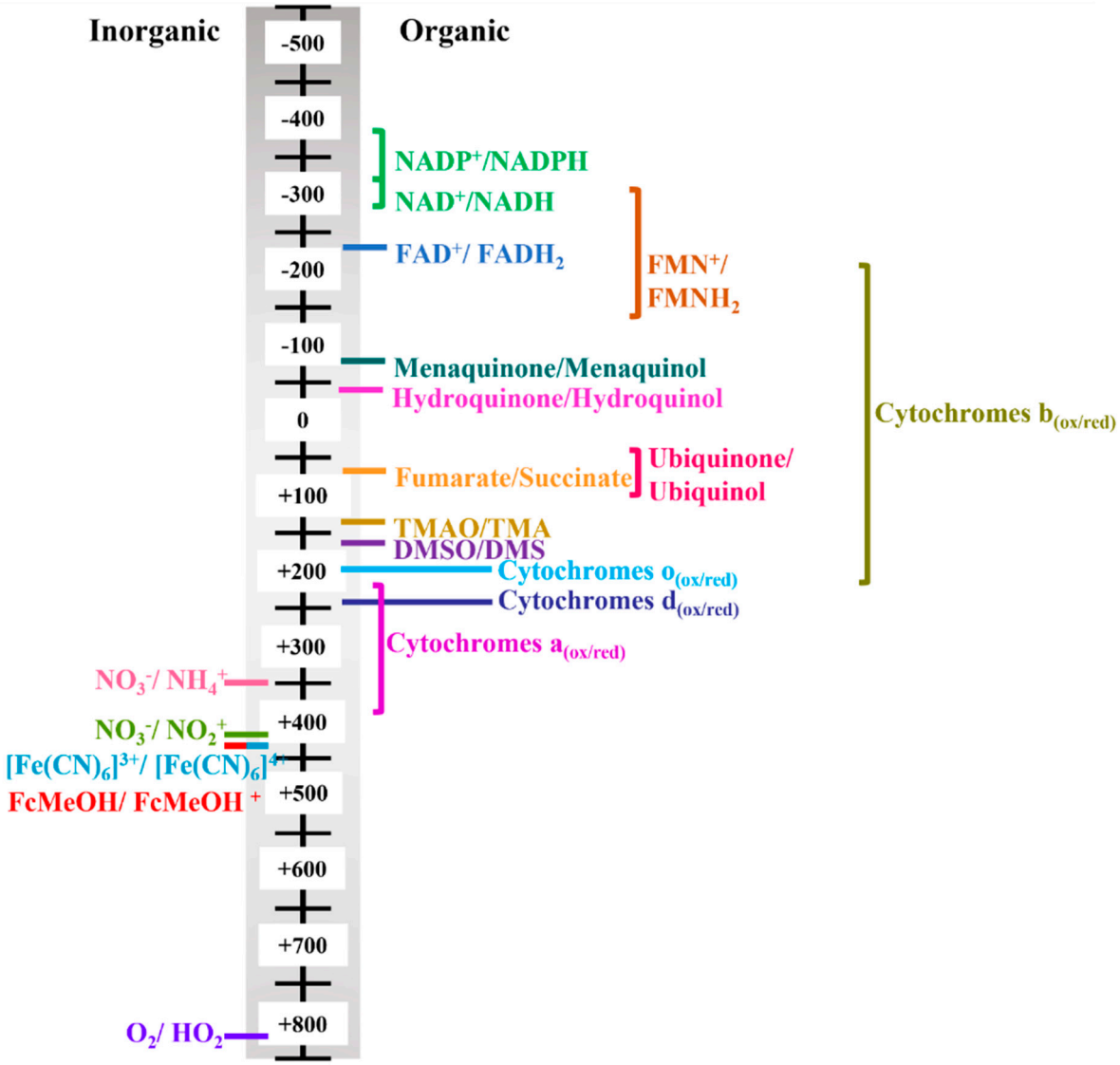


**Fig.S3** Redox potentials of important redox couples in the electron transport chain of *E.coli*. Standard redox potentials ($E^{0'}$ [mV, 25°C, pH 7]) are indicated. Physiological or environmental conditions are known to shift the potential from the $E^{0'}$, redox windows are indicated [1].

## SI-3. Sodium azide treatment of *E.coli* biofilm

**Table S1** details SECM currents at the contact point of the soft probe with the biofilm surface.

| Sample | $I_{Norm} = I/I_{Bulk}$ |
|---|---|
| **Non treated, Sample 1** | 0.62 |
| **Non treated, Sample 2** | 0.60 |
| **Non treated, Sample 3** | 0.64 |
| **5 min, Sample 1** | 0.43 |
| **5 min, Sample 2** | 0.46 |
| **5 min, Sample 3** | 0.45 |
| **15 min, Sample 1** | 0.38 |
| **15 min, Sample 2** | 0.30 |
| **15 min, Sample 3** | 0.36 |

**Table S2** details the SECM x-line scan data of **Fig.S4**.

| Position | *Time* | $I_{bulk}$ / nA | $I_{Plastic}$ / nA | $I_{bulk}^{*}$ / nA | $I_{mean,FB\text{-}image}$ / nA | $I^{*}_{mean}$ |
|---|---|---|---|---|---|---|
| **Position 1** | **non** | 3.74 | 0.10 | 3.64 | (2.08 ± 0.005) ($N$ = 81) | 0.57 ($N$ = 81) |
| | **5** | 3.69 | 0.09 | 3.60 | (1.47 ± 0.006) ($N$ = 81) | 0.41 ($N$ = 81) |
| | **15** | 3.66 | 0.11 | 3.55 | (1.06 ± 0.01) ($N$ = 81) | 0.26 ($N$ = 81) |
| **Position 2** | **non** | 3.74 | 0.10 | 3.64 | (2.09 ± 0.009) ($N$ = 81) | 0.58 ($N$ = 81) |
| | **5** | 3.68 | 0.09 | 3.59 | (1.44 ± 0.02) ($N$ = 81) | 0.40 ($N$ = 81) |
| | **15** | 3.66 | 0.11 | 3.55 | (1.24 ± 0.19) ($N$ = 81) | 0.35 ($N$ = 81) |
| **Position 3** | **non** | 3.74 | 0.10 | 3.64 | (2.11 ± 0.007) ($N$ = 81) | 0.58 ($N$ = 81) |
| | **5** | 3.69 | 0.09 | 3.59 | (1.47 ± 0.03) ($N$ = 81) | 0.41 ($N$ = 81) |
| | **15** | 3.66 | 0.11 | 3.55 | (1.22 ± 0.20) ($N$ = 81) | 0.35 ($N$ = 81) |
| **Position 4** | **non** | 3.74 | 0.10 | 3.64 | (2.08 ± 0.02) ($N$ = 81) | 0.57 ($N$ = 81) |
| | **5** | 3.69 | 0.09 | 3.59 | (1.47 ± 0.03) ($N$ = 81) | 0.41 ($N$ = 81) |
| | **15** | 3.66 | 0.11 | 3.55 | (1.21 ± 0.20) ($N$ = 81) | 0.34 ($N$ = 81) |

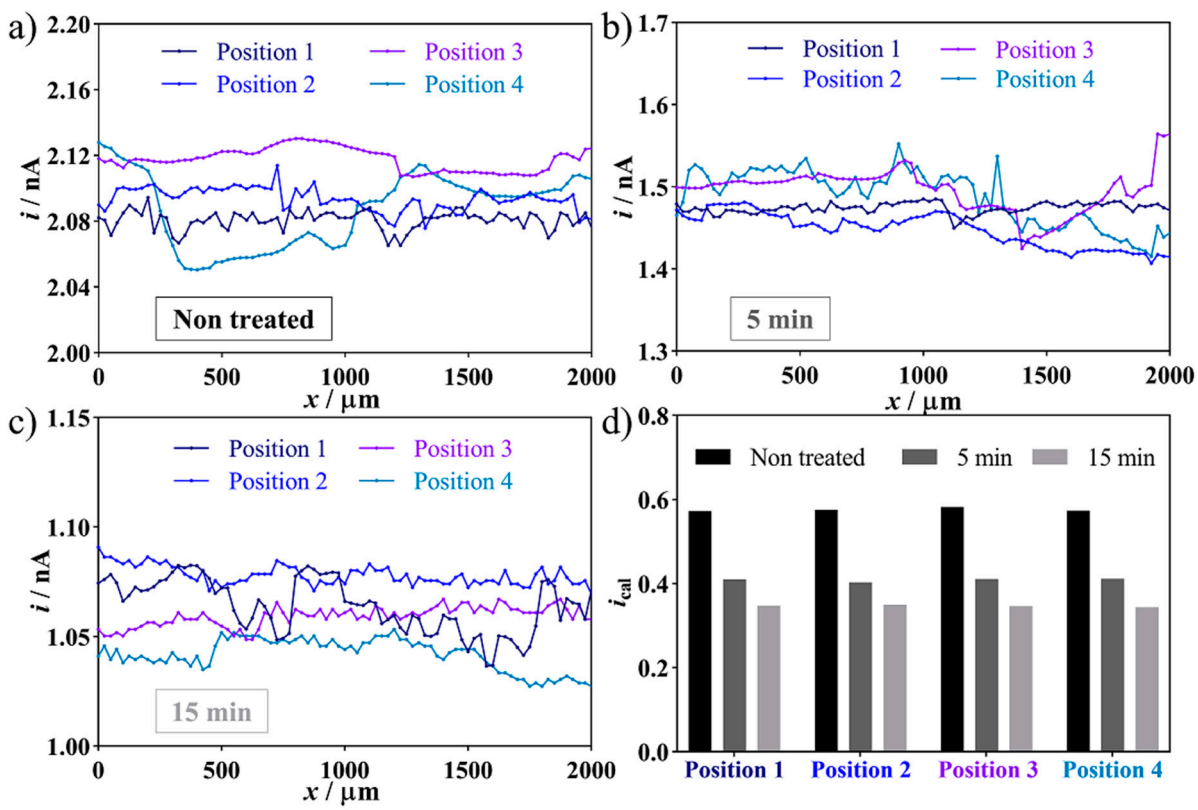


**Fig.S4** *X*-line scans SECM in four separated positions with a lateral distance of 250 µm before sodium azide treatment (a), after 5 min (b), and after 15 min of sodium azide treatment. (d) Calibrated mean currents ± standard deviation of three SECM feedback line scan over one *E. coli* biofilm before and after 5 min and 15 min incubation of the biofilm in sodium azide containing solution, grouped by (b) line scan position and (c) grouped by treatment time. Experimental details for *x-line* SECM scans: working potential $E_T$ = 0.5 V, probe translation speed = 25 µm $s^{-1}$, step size = 10 µm, 2.5 mM FcMeOH in 100 mM PBS (pH 7.4).

**Table S3** Details about quantification intensity data of **Fig.2.**

| Time of incubation | Stain | Mean | Standard deviation | Area | Mean |
|---|---|---|---|---|---|
| **Non treated** | **SYTO 9** | 47.92 | 20.40 | 213904 | 43.37 |
| | **PI** | 3.51 | 8.83 | 217622 | 6.15 |
| | **CV** | 98.31 | 29.87 | 104949 | 96.01 |
| **5 min** | **SYTO 9** | 43.37 | 19.09 | 218556 | -8.59 |
| | **PI** | 6.15 | 7.79 | 218089 | 13.03 |
| | **CV** | 96.01 | 24.36 | 104619 | -6.02 |
| **15 min** | **SYTO 9** | 41.66 | 12.11 | 221370 | -21.20 |
| | **PI** | 8.28 | 14.92 | 222312 | 66.67 |
| | **CV** | 60.48 | 41.19 | 104949 | -21.03 |

## SI-4. Silver nanoparticles treatment of *E.coli* biofilm

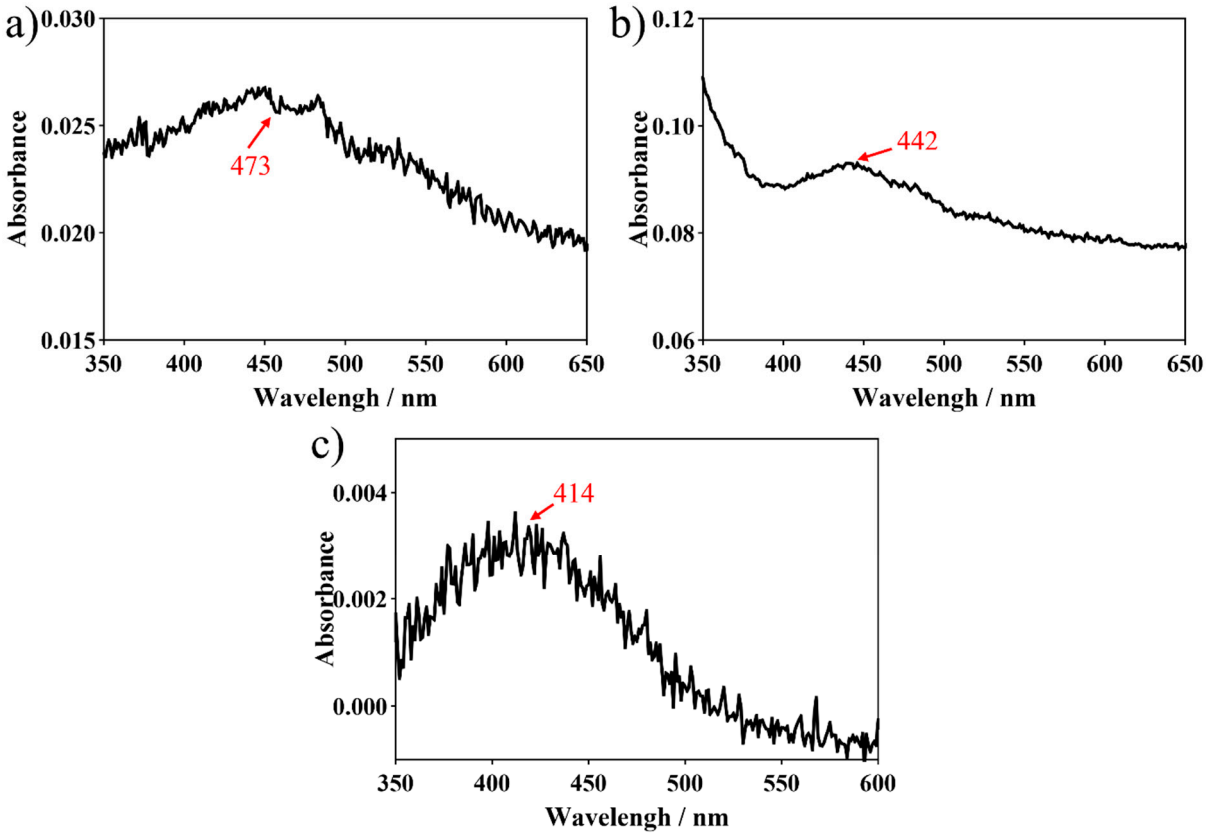


**Fig.S5** UV-Vis spectra of (a) AgNPs capped with citrate, PVP capped AgNPs in (b) water, and (c) EG just aftersynthesis

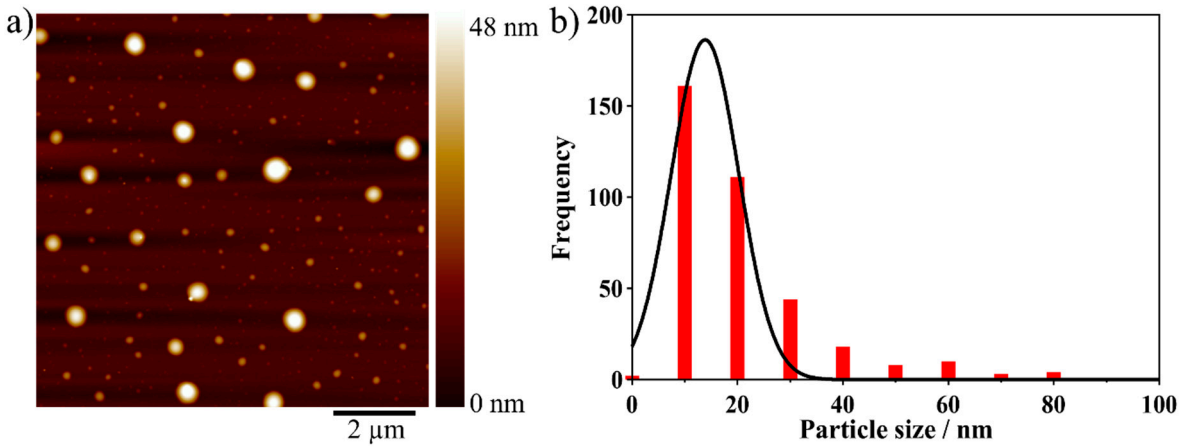


**Fig.S6** (a) AFM map of citrate capped AgNP and (b) plot of particle size distribution of citrate capped AgNP.

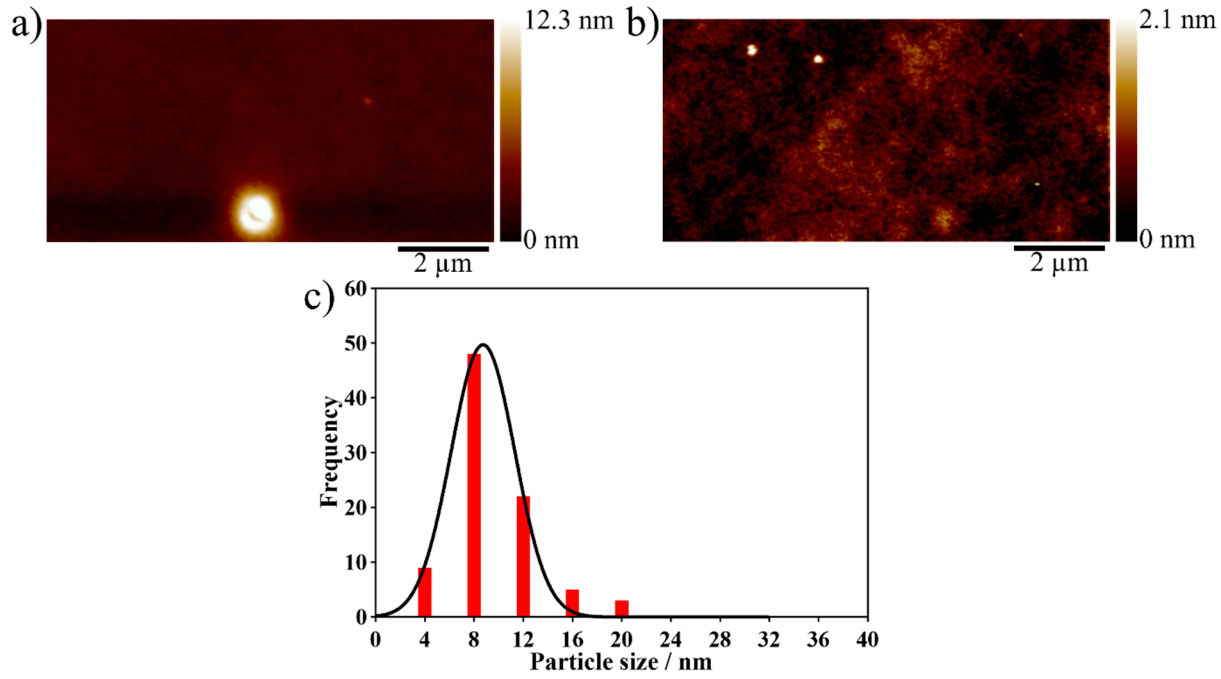


**Fig.S7** (a) and (b)AFM map of PVP capped AgNP in water in two areas of sample and (c) plot of particle size distribution of citrate capped AgNP.

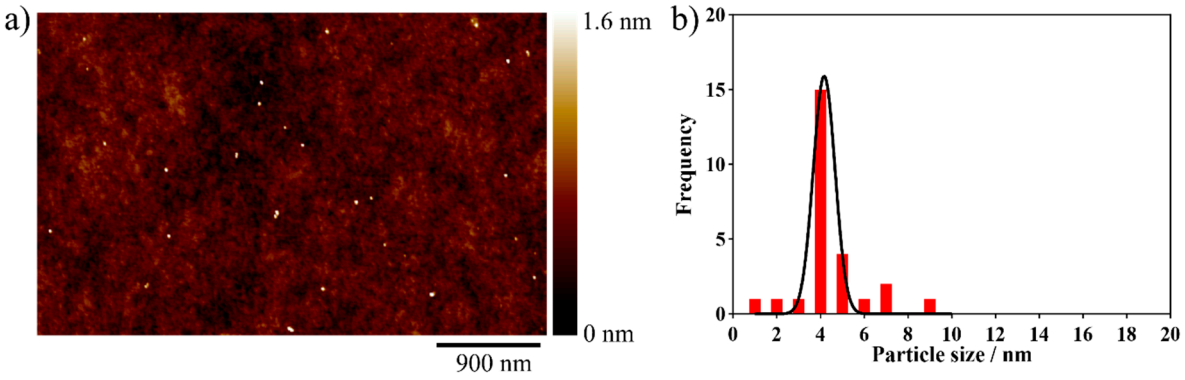


**Fig.S8** (a) AFM map of PVP capped AgNP in EG and (b) plot of particle size distribution of citrate capped AgNP.

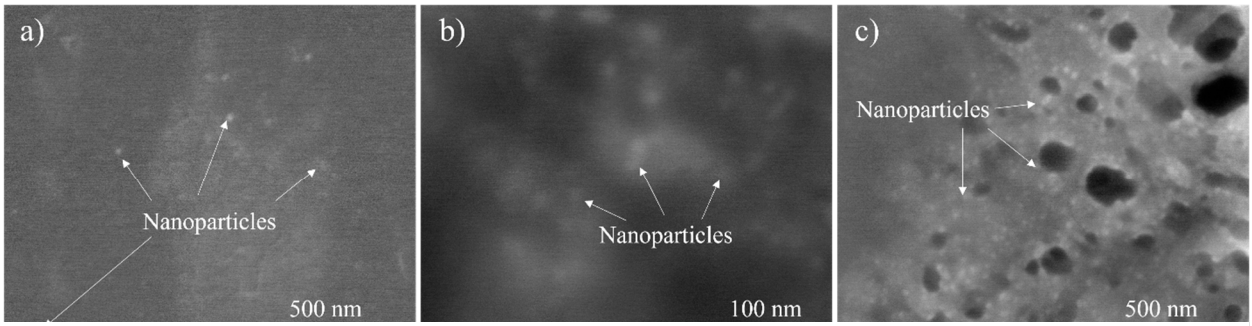


**Fig.S9** SEM images of (a) AgNPs capped with citrate, PVP capped AgNPs in (b) water, and (c) EG.

For the further analysis of silver nanoparticles the Energy-dispersive X-ray spectroscopy (EDX) elemental mapping measurement was done on the diluted sample. For sample preparation the solution were spin coated on 50 nm $SiO_2$ in 6000 rpm for 3 minutes.

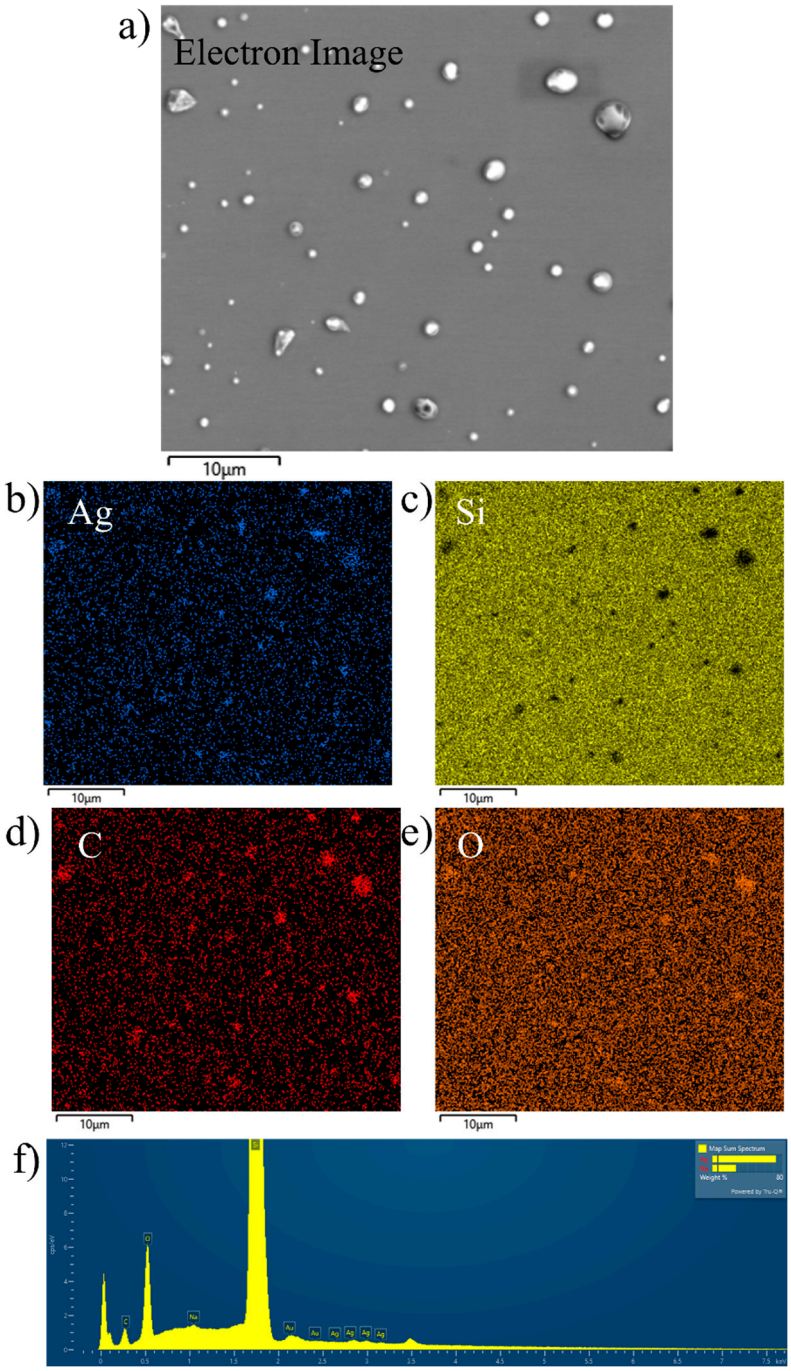


**Fig.S10** EDX elemental mapping of AgNPs capped with citrate: (a) SEM image. Elemental mapping of (b) Ag, (c) Si, (d) C, and (e) O. (f) EDX analysis.

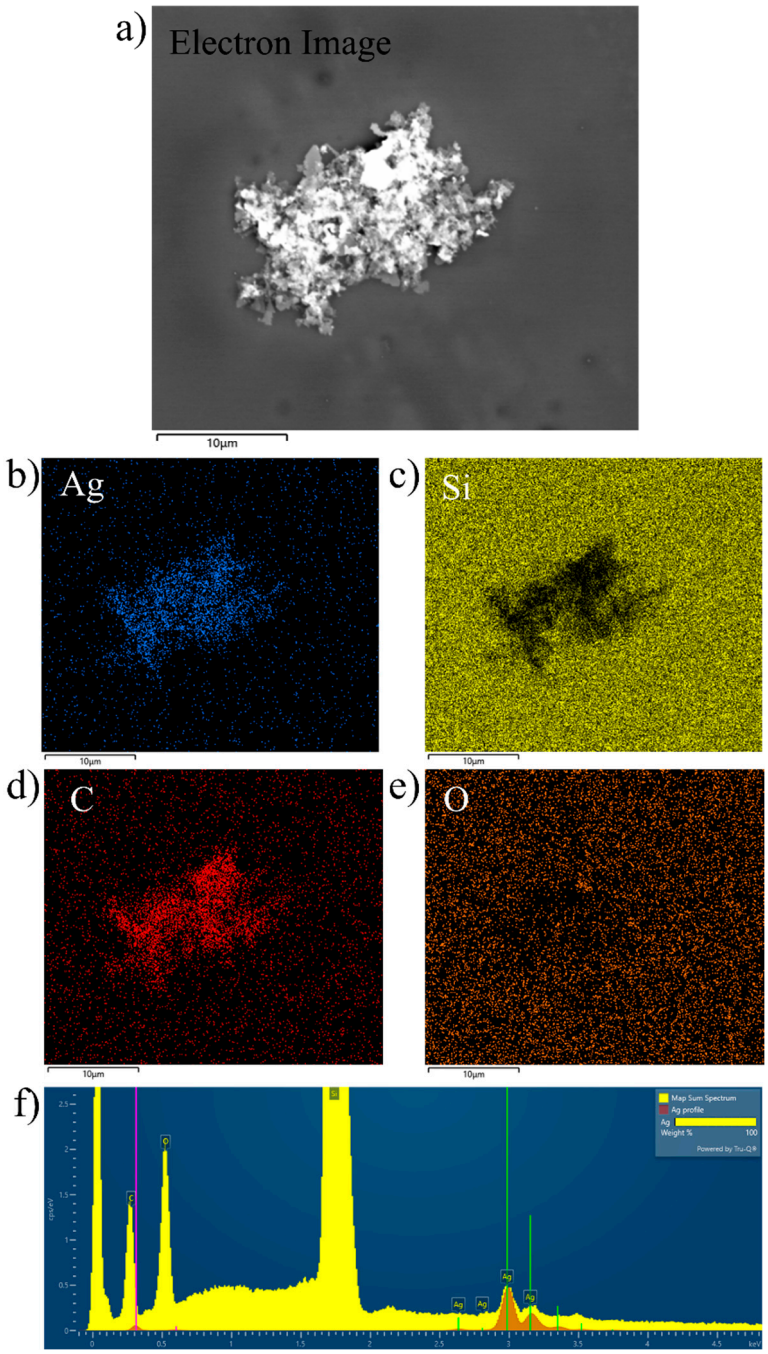


**Fig.S11** EDX elemental mapping of PVP capped AgNPs inwater: (a) SEM image. Elemental mapping of (b) Ag, (c) Si, (d) C, and (e) O. (f) EDX analysis.

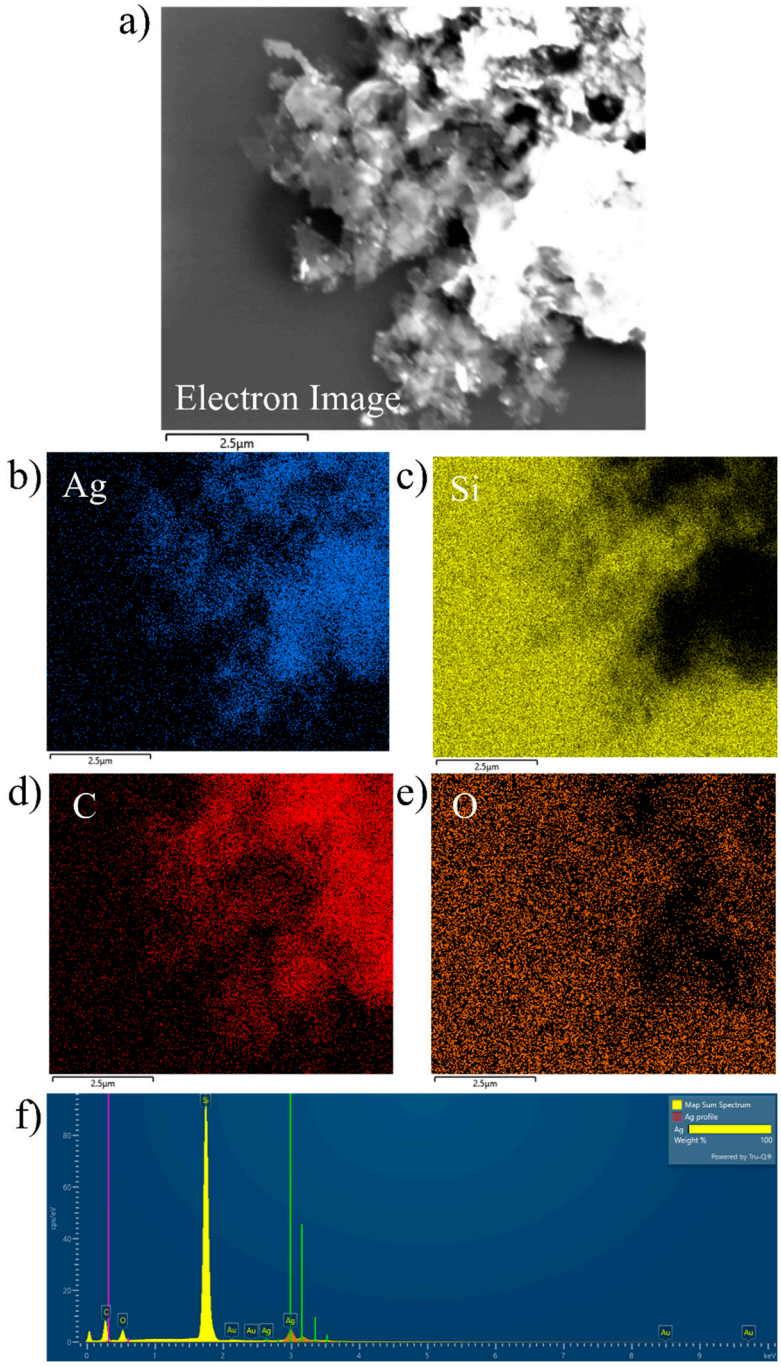


**Fig.S12** EDX elemental mapping of PVP capped AgNPs in EG: (a) SEM image. Elemental mapping of (b) Ag, (c) Si, (d) C, and (e) O. (f) EDX analysis.

**Table S4** Details of SECM *z*-line scan data at the approached point of **Fig.4**.

| Concentration of $AgNO_3$ / µg/mL | Treatment | AgNPs | Repetition | $I_{Norm} = I/I_{Bulk}$ |
|---|---|---|---|---|
| 0.1 | Non treated | AgNPs/Citrate | 1 | 0.59 |
| | | | 2 | 0.64 |
| | | | 3 | 0.64 |
| | | AgNPs/PVP/Water | 1 | 0.55 |
| | | | 2 | 0.54 |
| | | | 3 | 0.59 |
| | | AgNPs/PVP/EG | 1 | 0.57 |
| | | | 2 | 0.60 |
| | | | 3 | 0.58 |
| | Treated | AgNPs/Citrate | 1 | 0.48 |
| | | | 2 | 0.48 |
| | | | 3 | 0.46 |
| | | AgNPs/PVP/Water | 1 | 0.36 |
| | | | 2 | 0.37 |
| | | | 3 | 0.39 |
| | | AgNPs/PVP/EG | 1 | 0.16 |
| | | | 2 | 0.15 |
| | | | 3 | 0.19 |
| 1 | Non treated | AgNPs/Citrate | 1 | 0.65 |
| | | | 2 | 0.54 |
| | | | 3 | 0.55 |
| | | AgNPs/PVP/Water | 1 | 0.56 |
| | | | 2 | 0.63 |
| | | | 3 | 0.59 |
| | | AgNPs/PVP/EG | 1 | 0.56 |
| | | | 2 | 0.52 |
| | | | 3 | 0.60 |
| | Treated | AgNPs/Citrate | 1 | 0.28 |
| | | | 2 | 0.22 |
| | | | 3 | 0.26 |
| | | AgNPs/PVP/Water | 1 | 0.24 |
| | | | 2 | 0.16 |
| | | | 3 | 0.18 |
| | | AgNPs/PVP/EG | 1 | 0.05 |
| | | | 2 | 0.05 |
| | | | 3 | 0.06 |

**Table S5** Details of the average of SECM *z*-line scan data at the approached point of **Table S4**. *N* = sample number.

| Concentration of $AgNO_3$ / µg/mL | Treatment | AgNPs | Average of $I_{Norm} = I/I_{Bulk}$ |
|---|---|---|---|
| 0.1 | Non treated | AgNPs/Citrate | (0.62 ± 0.02) (*N* = 3) |
| | | AgNPs/PVP in water | (0.56 ± 0.02) (*N* = 3) |
| | | AgNPs/PVP in EG | (0.58 ± 0.01) (*N* = 3) |
| | Treated | AgNPs/Citrate | (0.47 ± 0.01) (*N* = 3) |
| | | AgNPs/PVP in water | (0.37 ± 0.01) (*N* = 3) |
| | | AgNPs/PVP in EG | (0.16 ± 0.01) (*N* = 3) |
| 1 | Non treated | AgNPs/Citrate | (0.58 ± 0.05) (*N* = 3) |
| | | AgNPs/PVP in water | (0.60 ± 0.02) (*N* = 3) |
| | | AgNPs/PVP in EG | (0.56 ± 0.03) (*N* = 3) |
| | Treated | AgNPs/Citrate | (0.25 ± 0.03) (*N* = 3) |
| | | AgNPs/PVP in water | (0.19 ± 0.04) (*N* = 3) |
| | | AgNPs/PVP in EG | (0.06 ± 0.005) (*N* = 3) |

**Table S6** Details about quantification intensity data of **Fig.5**.

| Stain | Non treated | | | AgNPs | | | |
|---|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Area | Mean | Standard deviation | Area | Changes / % |
| SYTO 9 | 47.69 | 19.81 | 212517 | 39.10 | 17.45 | 218085 | -15 |
| PI | 2.908 | 1.87 | 212517 | 24.70 | 24.36 | 218085 | 90 |
| CV | 89.65 | 15.60 | 213443 | 18.51 | 26.22 | 213443 | -57 |

## SI-5. Flash light treatment of *E.coli* biofilm

**Table S7** Details of SECM *z*-line scan data at the approached point of **Fig.6**.

| Treatment | Flashlight | Repetition | $I_{Norm} = I/I_{Bulk}$ |
|---|---|---|---|
| **550 V, 1 shot** | **Non treated** | 1 | 0.85 |
| | | 2 | 0.86 |
| | | 3 | 0.85 |
| | **Treated** | 1 | 0.67 |
| | | 2 | 0.68 |
| | | 3 | 0.68 |
| **550 V, 3 shots** | **Non treated** | 1 | 0.86 |
| | | 2 | 0.87 |
| | | 3 | 0.86 |
| | **Treated** | 1 | 0.20 |
| | | 2 | 0.18 |
| | | 3 | 0.21 |
| **550 V, 5 shots** | **Non treated** | 1 | 0.84 |
| | | 2 | 0.85 |
| | | 3 | 0.86 |
| | **Treated** | 1 | 0.09 |
| | | 2 | 0.08 |
| | | 3 | 0.12 |

**Table S8** Details of the average of SECM *z*-line scan data at the approached point of **Table S7**.

| Treatment | Sample | $I_{Norm} = I/I_{Bulk}$ |
|---|---|---|
| **550 V, 1 shot** | **Non treated** | 0.85 |
| | **Treated** | 0.68 |
| **550 V, 3 shots** | **Non treated** | 0.86 |
| | **Treated** | 0.19 |
| **550 V, 5 shots** | **Non treated** | 0.85 |
| | **Treated** | 0.10 |

**Table S9** Details about quantification intensity data of **Fig.7**.

| Stain | SYTO 9 | | | | PI | | | |
|---|---|---|---|---|---|---|---|---|
| | **Mean** | **Standard deviation** | **Area** | **Changes / %** | **Mean** | **Standard deviation** | **Area** | **Changes / %** |
| **Non treated** | 46.52 | 31.50 | 429016 | - | 6.67 | 11.90 | 429016 | - |
| **1 shot** | 35.56 | 17.66 | 429016 | -32 | 27.58 | 11.80 | 429016 | +21 |
| **3 shots** | 21.52 | 19.88 | 429016 | -47 | 11.90 | 28.26 | 429016 | +63 |
| **5 shots** | 15.83 | 23.28 | 429016 | -50 | 18.83 | 25.78 | 429016 | +79 |

## References

[1] F. Kracke, I. Vassilev, J.O. Krömer, Microbial electron transport and energy conservation–the foundation for optimizing bioelectrochemical systems, Frontiers in microbiology, 6(2015) 575. https://doi.org/10.3389/fmicb.2015.00575.