



HAL
open science

Communication-Electrochemical Single Nano-Impacts of Electroactive *Shewanella Oneidensis* Bacteria onto Carbon Ultramicroelectrode

Estelle Lebègue, Nazua L. Costa, Ricardo O. Louro, Frédéric Barrière

► **To cite this version:**

Estelle Lebègue, Nazua L. Costa, Ricardo O. Louro, Frédéric Barrière. Communication-Electrochemical Single Nano-Impacts of Electroactive *Shewanella Oneidensis* Bacteria onto Carbon Ultramicroelectrode. *Journal of The Electrochemical Society*, 2020, 167 (10), pp.105501. 10.1149/1945-7111/ab9e39 . hal-02928571

HAL Id: hal-02928571

<https://hal.science/hal-02928571>

Submitted on 6 Apr 2023

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.

Electrochemical Single Nano-Impacts of Electroactive *Shewanella Oneidensis* Bacteria Onto Carbon Ultramicroelectrode

Estelle Lebègue,^{1,z} Nazua L. Costa,² Ricardo O. Louro,² and Frédéric Barrière^{3,z}

¹ Université de Nantes, CNRS, CEISAM UMR 6230, F-44000, Nantes, France

² Universidade NOVA de Lisboa, Instituto de Tecnologia Química e Biológica, António Xavier, 2780-157 Oeiras, Portugal

³ Univ Rennes, CNRS, Institut des Sciences Chimiques de Rennes – UMR 6226, F-35000, Rennes, France

^z estelle.lebegue@univ-nantes.fr and frederic.barriere@univ-rennes1.fr

Supplementary material for this article is available

Abstract

Electrochemical single nano-impacts of electroactive *Shewanella oneidensis* bacteria at a 7 μm diameter carbon fibre ultramicroelectrode in an aqueous potassium phosphate buffer (pH = 7.2) solution containing a redox active probe (potassium ferro- or ferricyanide) is reported. We present chronoamperometric measurements recorded at the ultramicroelectrode polarized at the potential of the steady-state current of the redox probe in solution (oxidation for $\text{K}_4\text{Fe}(\text{CN})_6$ or reduction for $\text{K}_3\text{Fe}(\text{CN})_6$) in the presence of bacteria. The shape of current transients associated to single bacteria nano-impacts is compared and discussed as a function of the redox probe in solution and of the ultramicroelectrode applied potential.

The rapid and selective detection of bacteria at the level of a single cell is a fundamental scientific challenge to progress toward more efficient biosensors and diagnostics.¹⁻⁵ To this end, fast and easy handling systems for detection of bacteria at the single cell level (the highest possible sensitivity) have become reachable with the promising electrochemistry of single nano-impacts.^{3,4,6-11} Electrochemical detection of single bacteria with the nano-impacts method has only been reported in few studies,^{3,4,12-14} four of which involving detection of living label-free bacteria.^{4,12-14} These four reports especially focus on *Escherichia coli* as a model bacterium and have not been extended to electroactive bacteria that perform electrode biocatalytic processes in microbial fuel cells and in other microbial bioelectrochemical systems.^{15,16}

Studies on extracellular electron transfer at the microorganism/electrode interface mainly involve two Gram-negative mesophilic bacteria able to electrically connect their respiratory metabolism to extracellular solid electron acceptors: *Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens*.¹⁵⁻¹⁸ It is well known that *Shewanella oneidensis* biofilms attached to graphitic carbon electrodes transfer electrons at a formal potential of about -0.2 V vs. SHE.¹⁹⁻²¹ The electrochemical nano-impacts method is fully relevant for evaluating the electrochemical activity of a single bacteria cell at an ultramicroelectrode²² under controlled conditions such as in the absence of a biofilm in order to minimize the effect of secreted materials.²³

For single bacteria nano-impacts electrochemical detection, the strategy is to record a chronoamperometric measurement in an aqueous solution containing a redox probe at an ultramicroelectrode polarized at the oxidation or reduction potential of the electrochemically active species and to observe a “current step” when one bacterium impacts the ultramicroelectrode, corresponding to a “blocking effect” (Fig. 1A).^{4,12,13} Recent studies have shown that in these conditions (presence of an aqueous redox probe and bacteria), the response signal expected from single bacterium collision may also be a “current spike” corresponding to either the own electrochemical activity of the bacterium toward the redox probe at the ultramicroelectrode applied potential^{13,14} or a “bouncing effect” of the bacterium that does not stick to the ultramicroelectrode surface (Fig. 1B).¹² The fundamental mechanism that occurs during the single bacterium nano-impact event is not fully understood

and requires more in-depth studies with different bacterial strains.

Based on our expertise and knowledge in both the electrochemical nano-impacts technique²⁴⁻²⁶ and electroactive bacteria studied in microbial fuel cells,^{18,27,28} we present herein first results on electrochemical single collisions at a carbon fibre ultramicroelectrode of *Shewanella oneidensis* as a model electroactive bacteria (Fig. 1). Chronoamperometric measurements were performed in potassium phosphate buffer aqueous solution containing a redox probe (20 mM potassium ferro- or ferricyanide) in the presence of electroactive bacteria at a polarized 7 μm diameter carbon ultramicroelectrode (Fig. 1).

Experimental

Reagents. All chemicals were used as purchased without further purification. Milli-Q water (18.2 M Ω cm) was used to prepare all solutions. Potassium ferricyanide (99.0% min) and potassium ferrocyanide trihydrate (99.0% min) were obtained from Sigma Aldrich. Potassium phosphate monobasic (99.0% min) and potassium phosphate dibasic (99.0% min) were purchased from VWR (USA).

Bacterial strain and growth conditions. Pure culture of *Shewanella oneidensis* MR-1 was grown aerobically in Luria Bertani (LB) medium (Sigma Aldrich). Briefly, 1 vial of culture stock was thawed and 1% of livestock cells was used to inoculate 20 mL culture medium. The cells were incubated overnight at 30 °C with shaking at 150 rpm. After 16 hours growth, the undiluted cells optical density at 600 nm (OD₆₀₀) was 2.0 consistent with the exponential growth phase.²⁹ Live culture of *S. oneidensis* MR-1 grown in LB medium and culture stocks were stored at -80 °C with 50% glycerol.

Electrochemical measurements. Electrochemical experiments were carried out at room temperature (21 \pm 3 °C) with a three-electrode cell placed in a Faraday cage and an Autolab PGSTAT302N potentiostat/galvanostat (Eco Chemie B.V., Netherlands) using Nova 2.1 as electrochemical software (Metrohm). All solutions were deaerated by bubbling argon for 5 minutes before each measurement. For all recorded chronamperometric curves, the sample interval (in sampling time) was 0.1 s. A carbon fibre ultramicroelectrode (7 μm diameter) obtained from IJ Cambria Scientific Ltd. as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl, KCl 3 M reference electrode were used to perform all

electrochemical measurements. Before each experiment, carbon ultramicroelectrodes were gently polished using a wetted fine grid silicon carbide paper from Struers (4000-grid SiC) and washed several times with ultrapure water. The *S. oneidensis* bacteria solution injected in the electrochemical cell before chronoamperometric measurements was prepared by 15 min centrifugation at 3000 rpm of 1 mL culture stock solution to remove the LB growth medium and resuspension during 15 min in an aqueous solution of 20 mM potassium phosphate buffer (pH 7.2). The bacteria solution in potassium phosphate buffer was prepared just before the electrochemical nano-impact experiments and used immediately within 1 hour maximum.

Results and Discussion

The applied potentials for the chronoamperometric measurements presented in Fig. 2 is +0.6 V vs. Ag/AgCl for $K_4Fe(CN)_6$ and -0.3 V vs. Ag/AgCl for $K_3Fe(CN)_6$ and correspond to the steady-state current of the aqueous redox probes at a CF UME as determined by cyclic voltammetry (Fig. S1). In the absence of bacteria in solution, no current step or spike is observed over a 200 s polarization time at either +0.6 V or -0.3 V (Fig. 2 Top, blue and orange curves respectively). After addition of *S. oneidensis* bacteria in solution containing $K_4Fe(CN)_6$ as redox probe (Fig. 2 Bottom, blue curve), the stair step current response is detected and assigned to single bacteria nano-impacts onto the CF UME surface, which locally block the flux of $Fe(CN)_6^{4-}$ to the electrode surface. The magnitude of the limiting faradic current decrease (a few percent) by the stair step (or blip, *vide infra*) is consistent with the relative size of *Shewanella* cells (commonly 1 to 2 μm^2) to that of the electrode (ca. 38 μm^2). Here, a current decrease in the shape of stair steps is observed and indicates that most of the bacteria that impact stick and remain on the CF UME surface after collision (except for occasional current spikes or bouncing events of single cells sometimes observed at the beginning of the chronoamperogram, e.g. at ca. 19 and 52 s on Fig. 2 Bottom, blue curve). Note that the lower initial current recorded in the chronoamperometric curve (12.75 nA) compared with that observed in the absence of bacteria (22.30 nA) is assigned to a slight dilution of ferrocyanide after the addition of bacteria and to the variability in the surface state and geometry of the CF UME upon mechanical polishing between each measurement. Control experiments performed at different potential values showed that the stair step current

response related to discrete bacteria nano-impacts in $\text{K}_4\text{Fe}(\text{CN})_6$ aqueous redox probe solution is detected for any applied potential corresponding to the steady-state current of $\text{Fe}(\text{CN})_6^{4-}$ oxidation ($E_{\text{app.}} \geq +0.3 \text{ V vs. Ag/AgCl}$) at a CF UME (Fig. S2, blue curves). These observations with *S. oneidensis* in $\text{K}_4\text{Fe}(\text{CN})_6$ are in agreement with previous works reporting blocking electrochemical nano-impacts of single *Escherichia coli* bacteria in potassium ferrocyanide aqueous solutions.^{4,12,13} The concentration of *S. oneidensis* bacteria in the electrochemical cell is estimated at ca. $1.5 \times 10^8 \text{ CFU mL}^{-1}$ (250 fM) after dilution of the stock solution with $\text{OD600} = 2.0$.³⁰ The experimental frequency f_{exp} of single current step in the chronoamperometric curve is estimated at $0.07 \pm 0.02 \text{ Hz}$ based on three different electrochemical measurements performed in the same experimental conditions over 200 s (Fig. 2 Bottom, blue curve). This frequency value corresponds to a bacteria concentration C_{bac} of about 16.6 pM according to Equation 1 below (in conditions where the mass transport is controlled by diffusion).^{9,14}

$$C_{\text{bac}} = f_{\text{exp}}/4Dr_e$$

[1]

In Equation 1, D is the diffusion coefficient of bacteria ($5 \times 10^{-9} \text{ cm}^2 \text{ s}^{-1}$)³¹ and r_e is the ultramicroelectrode radius ($r_e = 3.5 \text{ }\mu\text{m}$). The bacteria concentration C_{bac} estimated from the experimental frequency f_{exp} of current steps (Fig. 2 Bottom, blue curve) and Equation 1 is two orders of magnitude higher than the concentration determined from OD600. First, this discrepancy may be rationalized by the fact that collision experiments do not discriminate between living and dead bacteria.^{4,12,13} Therefore, it is plausible that collisions of dead cells (in addition to living ones) contribute to the higher than expected experimental collision frequency. Furthermore, it has been reported that positively charged surfaces are more favourable for the formation of catalytic biofilms of microbial fuel cell electrodes because of the electrostatic interaction with the negatively charged surface of most electroactive bacteria.³²⁻³⁴ In addition to the electrostatic attraction between the negatively charged surface of *S. oneidensis* and the positively charged CF UME surface (at $E_{\text{app.}} \geq +0.3 \text{ V vs. Ag/AgCl}$) the higher frequency of current steps from single cell nano-impacts onto CF UME may also be supported by the following biological effect: *S. oneidensis* MR-1 is known to sense and respond to electron acceptor gradients in its environment.³⁵ Indeed, an increase in *S.*

oneidensis motility (cell swimming speed) has been reported when cells are exposed to high enough applied potentials at the working electrode of a micro-electrochemical cell (electrokinesis).³⁶ *S. oneidensis* is a very versatile microorganism that shows growth with a polar flagellum at a broad range of temperatures and pH values.³⁷ With respect to motility, the electrokinesis experiments mentioned above were carried out in phosphate buffer (with a concentration in the order of 10 to 50 mM) at pH 7,³⁸ quite close to our experimental conditions. Hence the results reported here on the increased attraction of *S. oneidensis* cells to the CF UME polarized at high enough potential may be rationalized by a combination of three effects: single collisions of dead cells (in addition to living ones), electrostatic attraction and/or electrokinesis.

Concerning the chronoamperometric measurements recorded at -0.3 V vs. Ag/AgCl with $K_3Fe(CN)_6$ as aqueous redox probe in the presence of *S. oneidensis* bacteria (Fig. 2 Bottom, orange curve), the shape of associated single collisions is not a stair step but a current blip or spike. This trend was also observed for any applied potential corresponding to the steady-state current of $Fe(CN)_6^{3-}$ reduction ($E_{app.} \leq +0.1$ V vs. Ag/AgCl) at a CF UME in $K_3Fe(CN)_6$ aqueous solution containing bacteria (Fig. S2, orange curves). According to Ronspees and Thorgaard,¹² these blip-shaped current transients are associated to short bounce events onto CF UME surface as opposed to the sticking collisions and stair step current response discussed above. Indeed, in these experimental conditions, *S. oneidensis* bacteria single nano-impacts present spike-shaped current transients that return to the baseline current after 0.3-0.8 s. This behaviour can be explained by the inhibition of $Fe(CN)_6^{3-}$ diffusive flux during the short bounce events of the bacteria at the CF UME surface.¹² Compared with the chronoamperometry experiments recorded during $K_4Fe(CN)_6$ reduction and discussed above, only the polarization potential of the CF UME and the redox probe oxidation state were changed. The difference in the current transient response for *S. oneidensis* single collisions is most likely related to the potential applied at the CF UME rather than to the type of aqueous redox probe or to a specific intrinsic response from bacteria cells (as discussed in the next paragraph).^{13,14,23,39} Gao *et al.*¹³ suggested that negatively charged bacteria such as *E. coli* detach from the UME surface when the potential is more negative than the potential of zero charge. This suggestion is fully relevant to explain the bounce events (current spikes)

observed for single nano-impacts of *S. oneidensis* onto CF UME polarized at -0.3 V vs. Ag/AgCl in potassium ferricyanide aqueous solution. Indeed, it has been reported that *Shewanella* strains are highly negatively charged at neutral pH and hence are electrostatically repulsed by negatively charged electrode surfaces.⁴⁰ Here we find that the negatively charged *S. oneidensis* bacteria are repelled by the CF UME surface at applied potentials $E_{\text{app}} \leq +0.1$ V vs. Ag/AgCl. This hypothesis is also confirmed by the lower and poorly reproducible frequency of current spikes (ranging from 0.005 to 0.075 Hz) in comparison with the frequency of current steps observed in Fig. 2 and S2 for the same *S. oneidensis* cells concentration. Indeed, the detection of current spikes is more difficult to observe and the frequency of events is highly variable over this time range for the same bacteria concentration in solution, in agreement with the repulsing effect of the CF UME surface on the single cells. Hence the frequency of current spikes cannot be confidently related to the bacteria concentration in these experimental conditions.

Although it is well known that ferricyanide can be directly reduced by some bacteria¹³ and that it enhances the electricity generation by *S. oneidensis* in microbial fuel cells³⁹ we find no significant intrinsic redox activity from single *S. oneidensis* cells toward the aqueous redox probe in control chronoamperometry experiments (Fig. S3). This is in contrast with results reported by Gao *et al.* with *E. coli* (in 100 mM $\text{K}_3\text{Fe}(\text{CN})_6$ at +0.5 V vs. Ag/AgCl).¹³ Our observations point to the attraction/repulsion of negatively charged single *S. oneidensis* bacteria by the polarized CF UME surface as a function of the applied potential and to an electrokinesis of the cells toward the electrode when polarized as an electron acceptor for the electroactive bacteria.

Summary

The electrochemical nano-impacts experiments of single electroactive *Shewanella oneidensis* bacteria at a carbon fibre ultramicroelectrode have been successfully achieved in potassium phosphate buffer solution containing an aqueous redox probe: potassium ferro- or ferricyanide. A typical stair step current response has been detected for cell nano-impacts at the potential of steady-state current of $\text{Fe}(\text{CN})_6^{4-}$ redox probe ($E_{\text{app}} \geq +0.3$ V vs. Ag/AgCl), corresponding to the sticking of single bacteria on the ultramicroelectrode surface. In contrast,

we have observed current spikes for bacteria collisions in the chronoamperometric measurements recorded at the potential of steady-state current of $\text{Fe}(\text{CN})_6^{3-}$ redox probe ($E_{\text{app}} \leq +0.1 \text{ V vs. Ag/AgCl}$). This has been assigned to short bounce events due to electrostatic repulsion of negatively charged *Shewanella oneidensis* cells by the negatively charged ultramicroelectrode surface. These first results open the way to studies with a range of other aqueous redox probes (with different redox potentials) and bacteria species.

Acknowledgments

The authors acknowledge the France-Portugal PHC PESSOA program for support, project 40814ZE.

ORCID

Estelle Lebègue: <https://orcid.org/0000-0002-8610-0891>

Ricardo O. Louro: <https://orcid.org/0000-0002-2392-6450>

Frédéric Barrière: <https://orcid.org/0000-0001-5515-4080>

References

1. J. Q. Boedicker, M. E. Vincent, and R. F. Ismagilov, *Angew. Chem. Int. Ed.*, **48**, 5908 (2009).
2. G. Acharya, C.-L. Chang, and C. Savran, *J. Am. Chem. Soc.*, **128**, 3862 (2006).
3. L. Sepunaru, K. Tschulik, C. Batchelor-McAuley, R. Gavish, and R. G. Compton, *Biomater. Sci.*, **3**, 816 (2015).
4. J. Y. Lee, B.-K. Kim, M. Kang, and J. H. Park, *Sci. Rep.*, **6**, 30022 (2016).
5. S. Hannah, E. Blair, and D. K. Corrigan, *Curr. Opin. Electrochem.*, **23**, 7 (2020).
6. S. G. Lemay, S. Kang, K. Mathwig, and P. S. Singh, *Acc. Chem. Res.*, **46**, 369 (2013).
7. R. M. Crooks, *Faraday Discuss.*, **193**, 533 (2016).
8. L. A. Baker, *J. Am. Chem. Soc.*, **140**, 15549 (2018).
9. S. Goines and J. E. Dick, *J. Electrochem. Soc.*, **167**, 037505 (2019).
10. T. H. T. Nguyen, J. Lee, H.-Y. Kim, K. M. Nam, and B.-K. Kim, *Biosens. Bioelectron.*,

151, 111999 (2020).

11. J.-H. Zhang and Y.-G. Zhou, *TrAC Trends Anal. Chem.*, **123**, 115768 (2020).

12. A. T. Ronspees and S. N. Thorgaard, *Electrochim. Acta*, **278**, 412 (2018).

13. G. Gao, D. Wang, R. Brocenschi, J. Zhi, and M. V. Mirkin, *Anal. Chem.*, **90**, 12123 (2018).

14. R. A. S. Couto, L. Chen, S. Kuss, and R. G. Compton, *Analyst*, **143**, 4840 (2018).

15. A. Kumar, L. H.-H. Hsu, P. Kavanagh, F. Barrière, P. N. L. Lens, L. Lapinsonnière, J. H. Lienhard V, U. Schröder, X. Jiang, and D. Leech, *Nat. Rev. Chem.*, **1**, 0024 (2017).

16. C. Yang, H. Aslan, P. Zhang, S. Zhu, Y. Xiao, L. Chen, N. Khan, T. Boesen, Y. Wang, Y. Liu, L. Wang, Y. Sun, Y. Feng, F. Besenbacher, F. Zhao, and M. Yu, *Nat. Commun.*, **11**, 1 (2020).

17. N. L. Costa, T. A. Clarke, L.-A. Philipp, J. Gescher, R. O. Louro, and C. M. Paquete, *Bioresour. Technol.*, **255**, 308 (2018).

18. E. Lebègue, N. L. Costa, B. M. Fonseca, R. O. Louro, and F. Barrière, *J. Electroanal. Chem.*, **847**, 113232 (2019).

19. E. Marsili, D. B. Baron, I. D. Shikhare, D. Coursolle, J. A. Gralnick, and D. R. Bond, *Proc. Natl. Acad. Sci.*, **105**, 3968 (2008).

20. D. Baron, E. LaBelle, D. Coursolle, J. A. Gralnick, and D. R. Bond, *J. Biol. Chem.*, **284**, 28865 (2009).

21. A. A. Carmona-Martinez, F. Harnisch, L. A. Fitzgerald, J. C. Biffinger, B. R. Ringeisen, and U. Schröder, *Bioelectrochemistry*, **81**, 74 (2011).

22. S. Moussa and J. Mauzeroll, *J. Electrochem. Soc.*, **166**, G25 (2019).

23. H. Liu, G. J. Newton, R. Nakamura, K. Hashimoto, and S. Nakanishi, *Angew. Chem. Int. Ed.*, **49**, 6596 (2010).

24. E. Lebègue, C. M. Anderson, J. E. Dick, L. J. Webb, and A. J. Bard, *Langmuir*, **31**, 11734 (2015).

25. J. E. Dick, E. Lebègue, L. M. Strawsine, and A. J. Bard, *Electroanalysis*, **28**, 2320 (2016).

26. E. Lebègue, F. Barrière, and A. J. Bard, *Anal. Chem.*, **92**, 2401 (2020).

27. H. Smida, E. Lebègue, J.-F. Bergamini, F. Barrière, and C. Lagrost, *Bioelectrochemistry*, **120**, 157 (4).

28. H. Smida, E. Lebègue, M. Cortes, J.-F. Bergamini, F. Barrière, and C. Lagrost, *Bioelectrochemistry*, **125**, 70 (2019).
29. Y.-S. Xu, T. Zheng, X.-Y. Yong, D.-D. Zhai, R.-W. Si, B. Li, Y.-Y. Yu, and Y.-C. Yong, *Bioresour. Technol.*, **211**, 542 (2016).
30. A. D. Corts, L. C. Thomason, R. T. Gill, and J. A. Gralnick, *Sci. Rep.*, **9**, 1 (2019).
31. D. Bray, *Cell movements : from molecules to motility*, 2nd ed., New York (N.Y.) : Garland, (2001).
32. K. Guo, A. PrévotEAU, S. A. Patil, and K. Rabaey, *Curr. Opin. Biotechnol.*, **33**, 149 (2015).
33. K. Guo, S. Freguia, P. G. Dennis, X. Chen, B. C. Donose, J. Keller, J. J. Gooding, and K. Rabaey, *Environ. Sci. Technol.*, **47**, 7563 (2013).
34. M. Picot, L. Lapinsonnière, M. Rothballer, and F. Barrière, *Biosens. Bioelectron.*, **28**, 181 (2011).
35. K. H. Nealson, D. P. Moser, and D. A. Saffarini, *Appl. Environ. Microbiol.*, **61**, 1551 (1995).
36. H. W. Harris, M. Y. El-Naggar, O. Bretschger, M. J. Ward, M. F. Romine, A. Y. Obraztsova, and K. H. Nealson, *Proc. Natl. Acad. Sci.*, **107**, 326 (2010).
37. K. Venkateswaran, D. P. Moser, M. E. Dollhopf, D. P. Lies, D. A. Saffarini, B. J. MacGregor, D. B. Ringelberg, D. C. White, M. Nishijima, H. Sano, J. Burghardt, E. Stackebrandt, and K. H. Nealson, *Int. J. Syst. Bacteriol.*, **49 Pt 2**, 705 (1999).
38. K. H. Nealson and J. Scott, in *The Prokaryotes: Volume 6: Proteobacteria: Gamma Subclass*, M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, Editors, p. 1133–1151, Springer New York, New York, NY (2006) https://doi.org/10.1007/0-387-30746-X_45.
39. D. Wu, D. Xing, L. Lu, M. Wei, B. Liu, and N. Ren, *Bioresour. Technol.*, **135**, 630 (2013).
40. A. Korenevsky and T. J. Beveridge, *Microbiology*, **153**, 1872 (2007).

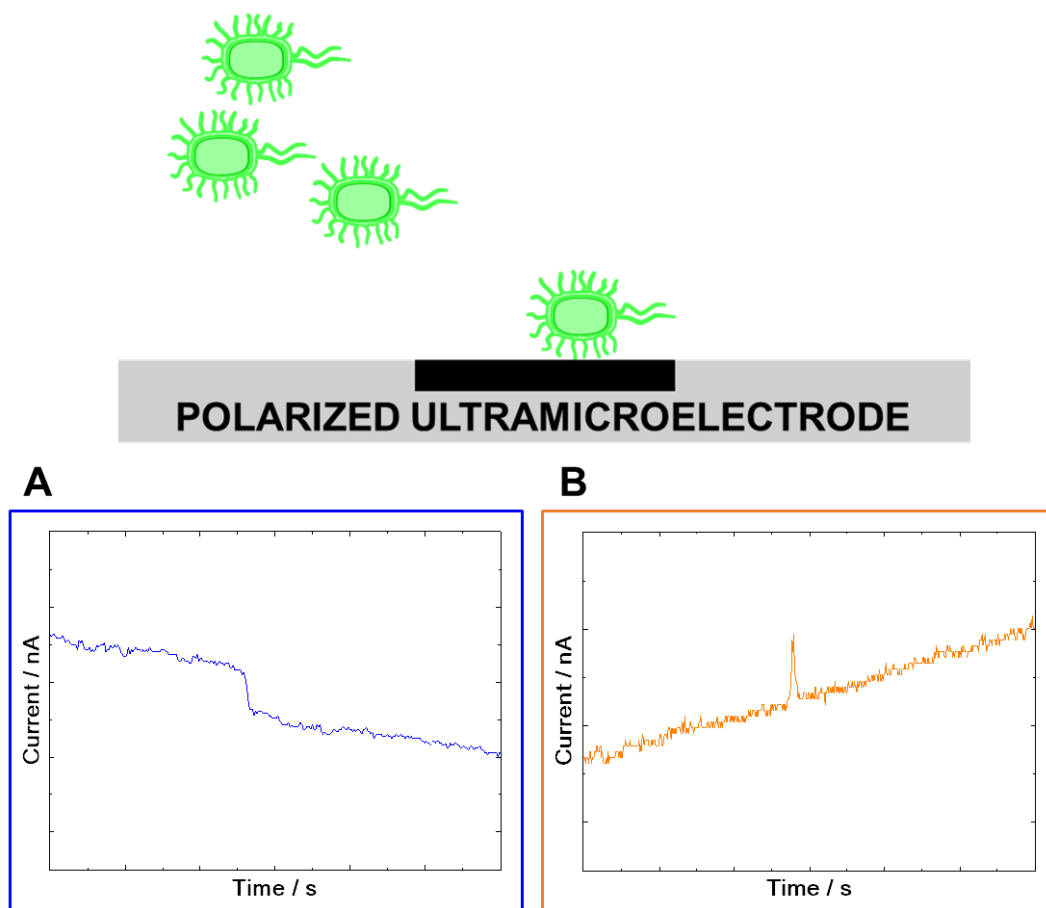


Figure 1. Schematic representation and typical electrochemical signal for single nano-impact experiments of *Shewanella oneidensis* bacteria recorded at a 7 μm carbon ultramicroelectrode polarized at **(A)** +0.6 V vs. Ag/AgCl in 20 mM potassium ferrocyanide and **(B)** -0.3 V vs. Ag/AgCl in 20 mM potassium ferricyanide with 20 mM potassium phosphate buffer at pH 7.2 as aqueous electrolyte.

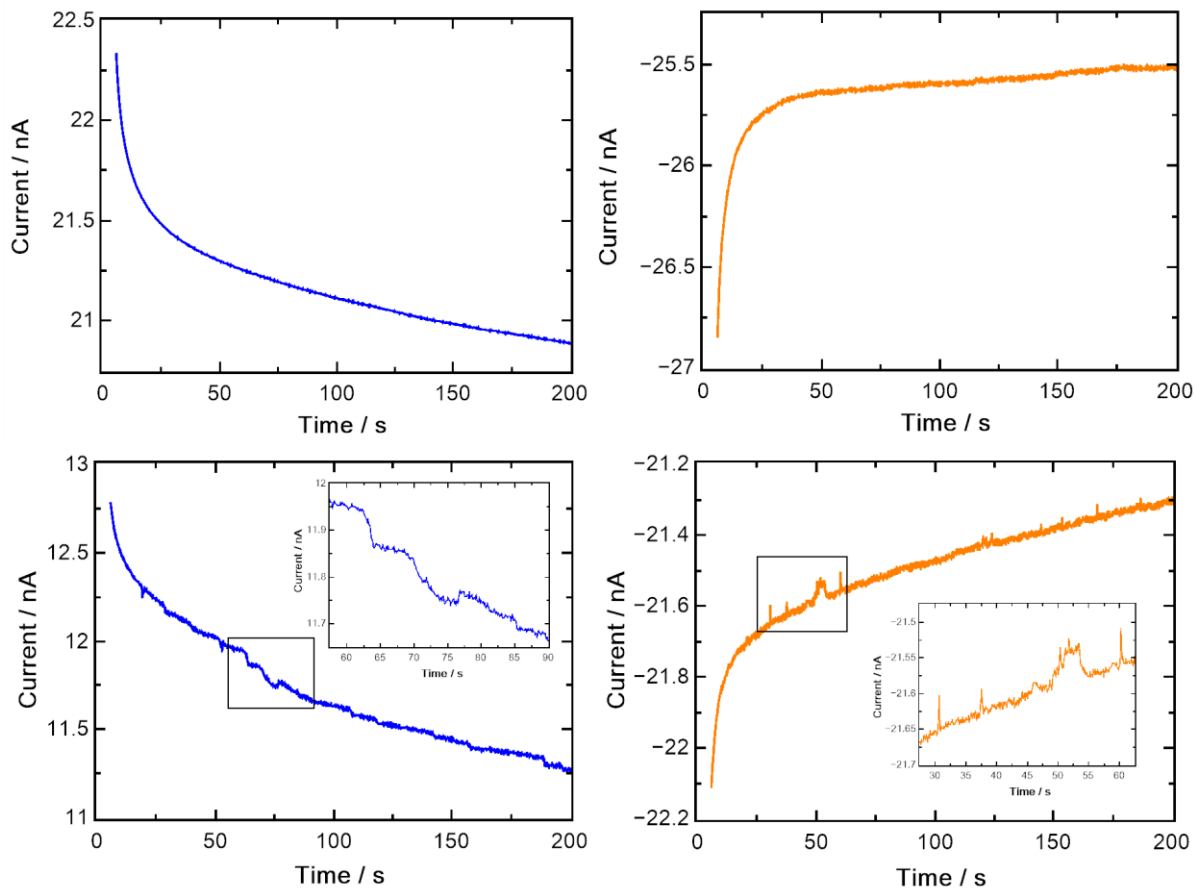


Figure 2. Chronoamperometric measurements recorded on 7 μm carbon ultramicroelectrode at +0.6 V vs. Ag/AgCl in 20 mM $\text{K}_4\text{Fe}(\text{CN})_6$ (LEFT, blue curves) and -0.3 V vs. Ag/AgCl in 20 mM $\text{K}_3\text{Fe}(\text{CN})_6$ (RIGHT, orange curves) in 20 mM potassium phosphate buffer at pH 7.2 as aqueous electrolyte under inert atmosphere (Ar) in (TOP) the absence and (BOTTOM) the presence of *Shewanella oneidensis* bacteria in solution.

Supplementary Material

Electrochemical Single Nano-Impacts of Electroactive *Shewanella Oneidensis* Bacteria Onto Carbon Ultramicroelectrode

Estelle Lebègue,^{1,z} Nazua L. Costa,² Ricardo O. Louro,² and Frédéric Barrière^{3,z}

¹ Université de Nantes, CNRS, CEISAM UMR 6230, F-44000, Nantes, France

² Universidade NOVA de Lisboa, Instituto de Tecnologia Química e Biológica, António Xavier, 2780-157 Oeiras, Portugal

³ Univ Rennes, CNRS, Institut des Sciences Chimiques de Rennes – UMR 6226, F-35000, Rennes, France

^z estelle.lebegue@univ-nantes.fr and frederic.barriere@univ-rennes1.fr

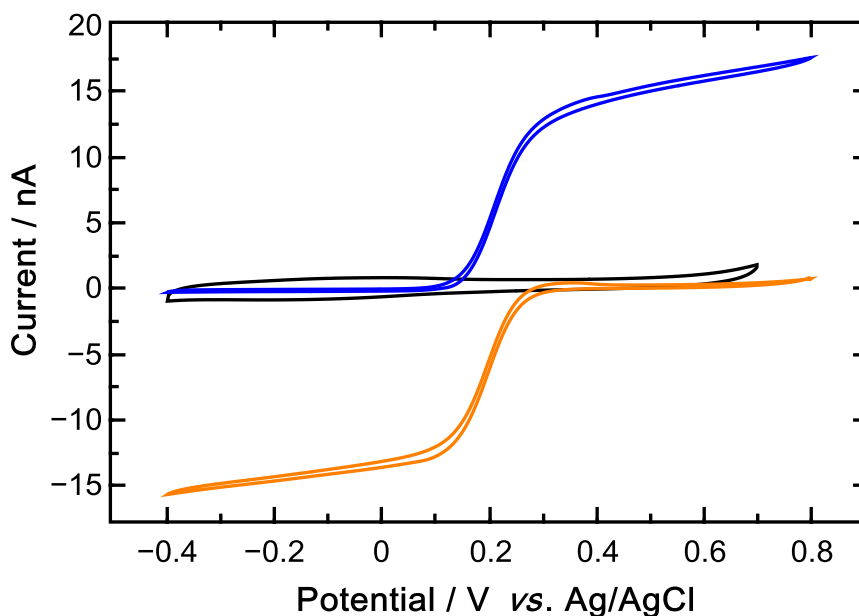


Figure S1. Cyclic voltammograms recorded at 20 mVs^{-1} at a $7 \mu\text{m}$ carbon ultramicroelectrode in 20 mM potassium phosphate buffer at pH 7.2 as aqueous electrolyte (black) with 20 mM $\text{K}_4\text{Fe}(\text{CN})_6$ (blue) and $\text{K}_3\text{Fe}(\text{CN})_6$ (orange) under inert atmosphere (Ar).

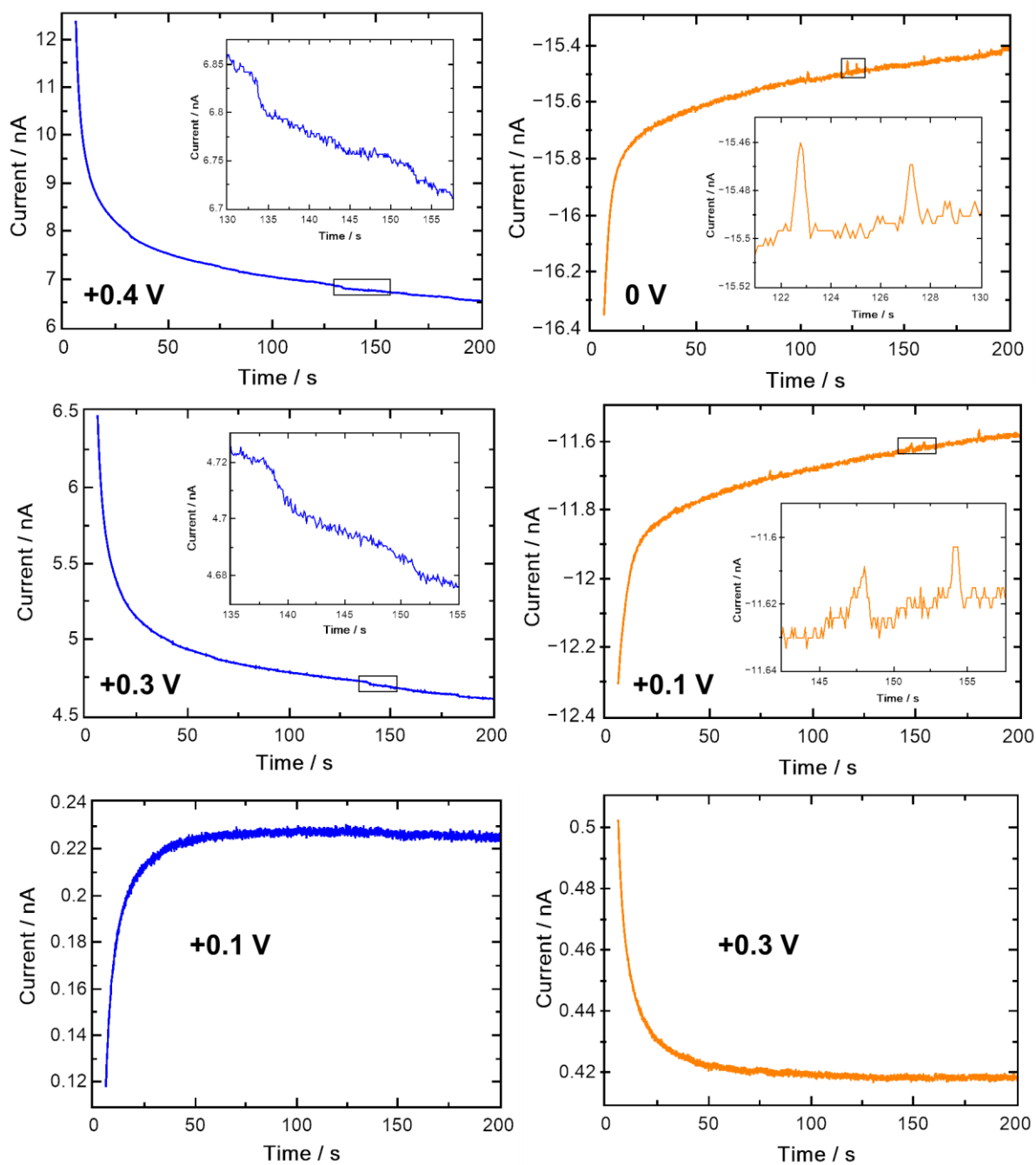


Figure S2. Chronoamperometric measurements recorded at different potentials (vs. Ag/AgCl) at a 7 μm carbon ultramicroelectrode in 20 mM $\text{K}_4\text{Fe}(\text{CN})_6$ (LEFT, blue curves) and $\text{K}_3\text{Fe}(\text{CN})_6$ (RIGHT, orange curves) in 20 mM potassium phosphate buffer at pH 7.2 as aqueous electrolyte under inert atmosphere (Ar) in the presence of *Shewanella oneidensis* bacteria in solution.

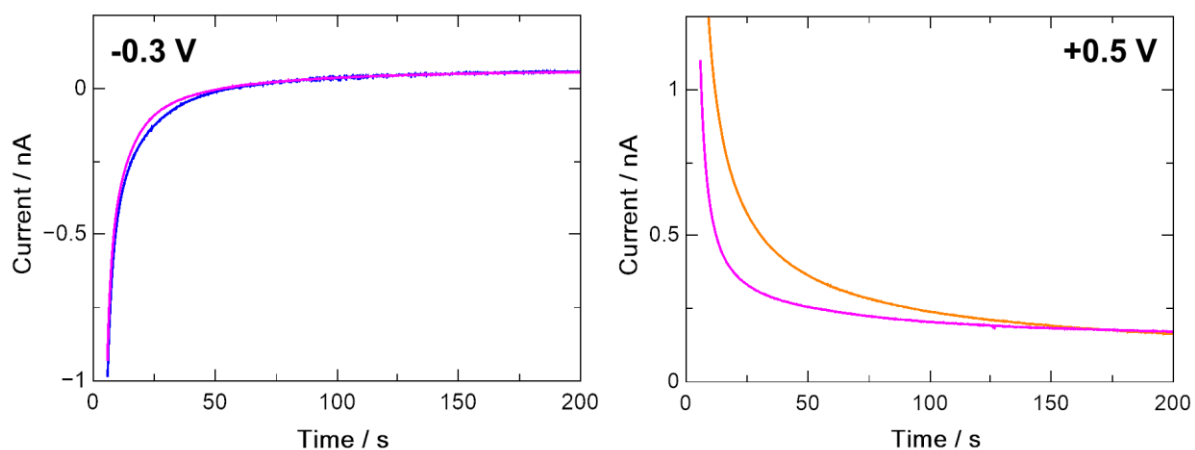


Figure S3. Chronoamperometric measurements recorded at two different potentials (vs. Ag/AgCl) at a 7 μm carbon ultramicroelectrode in 20 mM potassium phosphate buffer at pH 7.2 as aqueous electrolyte (pink curves) with 20 mM $\text{K}_4\text{Fe}(\text{CN})_6$ (blue curve) and $\text{K}_3\text{Fe}(\text{CN})_6$ (orange curve) under inert atmosphere (Ar) in the presence of *Shewanella oneidensis* bacteria in solution.