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# Electrophoresis as a simple method to detect deleterious actions of engineered nanoparticles on living cells

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## Abstract:

The release of engineered nanoparticles (NPs) in the environment may have profound implications for the health of aquatic biota. In this study, we evidence that the initial stage of NPs action on bacteria can be detected upon measurement of the electrophoretic fingerprints of mixed NPs-cells dispersions. Such electrokinetic signatures reflect a modification of the physicochemical cells and NPs surface properties following changes in cell envelope organization, subsequent intracellular material release and/or biomolecules cell excretion. The demonstration is based on a thorough investigation of the electrohydrodynamic features of genetically engineered *Escherichia coli* bacteria exhibiting distinct surface phenotypes (presence of adhesive YeeJ large proteins or F-pili proteinaceous filaments) exposed to silica NPs (65 nm radius) functionalized by -NH<sub>2</sub> terminal groups. At pH 7, electrostatics prevents interactions between bacteria and SiNH<sub>2</sub> NPs, regardless of the NPs concentration considered (0-10<sup>-2</sup> g L<sup>-1</sup> range). At pH 3, electrostatically-driven interactions allow intimate contacts between NPs and bacteria. In turn, significant modulation of the electrophoretic determinants of cells and NPs are generated due to cells envelope alteration and bio-corona acquisition by NPs. Differentiated roles of the cell surface appendages in the mediation of NPs impacts are evidenced by the measured dependence of electropherograms on cell surface phenotype and NPs concentration. Cell morphology and surface roughness, evaluated by Atomic Force Microscopy (AFM) in liquid, confirm the pH and NPs concentration conditions where NPs-cells interactions are operational. Combination of electrokinetics and AFM further pinpoints heterogeneities in cells response at the single cell and population scales. Altogether, results show that electrokinetics is suitable to detect the preliminary stage of events leading to NPs toxicity toward microorganisms.

## Environmental context:

Attractive interactions and subsequent contacts between NPs and microorganisms are the first steps of a chain of events leading to adverse NPs effects toward cells. Herein we show that the electrophoretic response of complex mixtures of engineered NPs (silica NPs) and bacteria (*E. coli*)

43 reflects initial NPs-mediated cell surface damages. We thus provide a promising option for a rapid  
44 electrokinetics-based detection of deleterious actions of NPs on biological cells.

## 45 **1. Introduction**

46 Over the past decades, nanomaterials and related products have gained a prominent importance  
47 in our everyday life. In particular, engineered nanoparticles (NPs) are now commonly used in various  
48 industries such as food-processing, electronics or cosmetics as well as in numerous pharmaceutical  
49 and biomedical applications (Ju-Nam and Lead 2008; Svenson 2009). NPs are released in the  
50 environment and their dissemination may severely impact on the proper functioning of ecosystems.  
51 For the sake of illustration, NPs are known to affect the speciation of essential trace metal elements  
52 (Duval 2017), to disturb their biogeochemical cycling (Chai et al. 2015), to modify microbial diversity  
53 (Yang et al. 2013), or to affect cells growth (El Badawy et al. 2011), cells viability (Choi et al. 2010;  
54 Stoimenov et al. 2002) and cells ability to form biofilms (Ikuma et al. 2015; Neal 2008; Peulen and  
55 Wilkinson 2011). These issues have motivated the scientific community to deploy important efforts  
56 for investigating the mechanisms governing the interactions between NPs and bacteria at various  
57 spatial scales (Beddoes et al. 2015; Choi et al. 2010; Shang et al. 2014), and for identifying  
58 implications in terms of adverse outcome pathways and toxicity (Colvin 2004; Kleinstreuer et al.  
59 2016; Moore 2006; Velzeboer et al. 2008). Acquiring such basic knowledge is a mandatory condition  
60 for the elaboration of environmental remediation strategies or the development of new industrial  
61 practice, *e.g.* the eco-friendly design of novel nanoparticulate products (Ingale and Chaudhari 2013).

62 The magnitude and nature of the interactions operational between bacterial cells and NPs (steric,  
63 electrostatic, van der Waals, hydrophobic and/or lock-and-key) are intimately connected to the  
64 physicochemical composition and spatial organization of the cell envelope (Beaussart et al. 2018a;  
65 Jacobson et al. 2015; Jiang et al. 2010; Monopoli et al. 2012). The latter include differences in cell  
66 wall structures (*e.g.* Gram+ vs. Gram- bacteria) (Beveridge and Graham 1991) or the presence of  
67 peripheral appendages, *e.g.* fimbriae, pili, lipopolysaccharides or flagella that mediate cell resistance  
68 and binding to NPs (Beaussart et al. 2018a; Jacobson et al. 2015; Jiang et al. 2010; Mathelié-Guinlet  
69 et al. 2018). Together with the defining physicochemical properties of NPs (*i.e.* NP size, charge, or  
70 hydrophobic/hydrophilic balance), the physicochemical functionalities of cell envelopes determine  
71 whether or not contact between cells and NPs takes place (Beaussart et al. 2018a; Beddoes et al.  
72 2015; Phillips et al. 2008; Thill et al. 2006). As such, the existence of NPs-cells attractive interactions  
73 is the required condition for NPs to induce possible cell surface alterations, cell dysfunction and  
74 toxicity effects (Choi et al. 2010; Hayden et al. 2012; Planchon et al. 2013). This situation obviously  
75 excludes NPs whose action mode does not require any contact with- or binding to the biological  
76 target, *e.g.* metallic NPs whose dissolution in aqueous media releases metal ions at the origin of  
77 adverse effects on biota (Choi et al. 2010).

78  
79 Various techniques may be employed for measuring the interactions between biological cells and  
80 NPs, and/or evaluating their respective electrostatic surface properties. Among them, protolytic  
81 titrations allow the determination of surface charges of biotic and abiotic colloids (Van Der Wal et al.  
82 1997). Though robust for abiotic systems like NPs, in case of *e.g.* bacteria the method suffers from  
83 the difficulty to discriminate between protons acting as charge determining-ions and protons that  
84 are released or uptaken by cells for metabolic purpose. As an alternative, Atomic Force Microscopy  
85 (AFM) now makes it possible to address the repartition of cell surface charges at the single cell level,  
86 or even measure the governing interactions between a given bacterium and a single NP with  
87 diameter less than 10 nm (Beaussart et al. 2018a). However, the inherent and obvious limit of such  
88 spatially-resolved AFM-based studies is the possible analysis of a restricted number of cells only.  
89 Besides, in some cases, the AFM probe jumps in contact with the investigated biosurface at

90 sufficiently low separation distances, which may render difficult any quantitative detection of short  
91 range electrostatic attraction events. Electrophoresis probably belongs to the most commonly  
92 adopted methods for addressing the electrostatic properties of cells surface or NPs. It consists in the  
93 application of a direct current electric field between two electrodes positioned in a solution  
94 containing the (bio)colloids of interest. Field-induced migration of the latter is tracked by dedicated  
95 optical or light-scattering systems depending on (bio)particle size. The so-evaluated (bio)particle  
96 electrophoretic mobility is then commonly converted into a zeta potential value and a surface charge  
97 density using classical electrokinetic and double layer theories (Planchon et al. 2013; Rowenczyk et  
98 al. 2017; Silva et al. 2014). Application of the latter is however strictly valid for hard particles (*i.e.*  
99 particles impermeable to ions and fluid flow) at the surface of which a slip plane can be  
100 unambiguously identified (Delgado et al. 2005), and the concept of zeta potential is physically  
101 meaningless for soft (*i.e.* ion- and flow-permeable) particles like bacteria or NPs decorated by  
102 polymeric coatings (Beaussart et al. 2018a; Dague et al. 2006; Duval and Gaboriaud 2010; Duval and  
103 Ohshima 2006; Li et al. 2010; López-Viota et al. 2009; Ohshima 1995). The electrophoretic mobility of  
104 soft NPs and bacteria is a measurable quantity that depends not only on the density of charges they  
105 carry, but also on the spatial distributions of the charges in the directions parallel and perpendicular  
106 to the interface they form with the outer solution (Duval and Gaboriaud 2010; Hill et al. 2003;  
107 Moussa et al. 2015), on their dimension (Duval and Ohshima 2006; Hill et al. 2003; Ohshima 1995),  
108 on their concentration in solution pending overlap of double layers and/or of hydrodynamic spheres  
109 of action between neighboring particles (Maurya et al. 2018), on their zwitterionic character if  
110 applicable (Beaussart et al. 2018b, Moussa et al. 2015), on the length and density of their  
111 (bio)polymeric fringe (Duval and Gaboriaud 2010; Francius et al. 2011), and on the physicochemical  
112 composition of the dispersing medium (pH, ionic strength, nature of the electrolyte) (Duval and  
113 Gaboriaud 2010; López-León et al. 2005; Ohshima 1995; Pagnout et al. 2012). Electrophoresis and  
114 analysis of so-called electropherograms may further serve to detect -within *e.g.* a given bacteria  
115 dispersion- the presence of subpopulations of cells defined by distinct electrostatic surface  
116 properties (Dague et al. 2006).

117

118 To the best of our knowledge, there has been no method reported so far for achieving a rapid *in*  
119 *situ* detection of attractive interactions between NPs and bacteria, and for analyzing heterogeneity in  
120 cells response to NPs. Such a method would however add value to preliminary and rapid-screening  
121 risk assessment procedures. The elements above indicate that electrophoresis could be used for that  
122 purpose. Indeed, NPs generally lead to a modification of cell surface features either as a result of NPs  
123 ad-/ab-sorptions (Thill et al. 2006; Zeyons et al. 2009), NPs-mediated cell abrasion and cell wall  
124 integrity loss (Brayner et al. 2006; Hayden et al. 2012; Huang et al. 2008; Mathelié-Guinlet et al.  
125 2017; Mecke et al. 2005; Shrivastava et al. 2007), and subsequent release of intracellular material  
126 (Hayden et al. 2012; Mathelié-guinlet 2017) that potentially decorates or aggregates NPs in solution.  
127 In turn, the electrophoretic cells surface properties and possibly those of the NPs are expected to  
128 differ from their pristine values. The purpose of the current study is to explore the potentialities of  
129 electrophoresis to discriminate between cells affected or not by NPs action. This requires the analysis  
130 of the electrophoretic behavior of NPs-cells mixtures, which is so far missing from nanotoxicology  
131 literature that mainly reports zeta potential-based assignment of (bio)particulate monospecies. To  
132 reach the aforementioned objective, the electrokinetic fingerprints of mixed suspensions of model  
133 NPs (silica NPs decorated by -NH<sub>2</sub> groups, 130 nm in diameter (Ribeiro et al. 2017, 2014)) and  
134 *Escherichia coli* (*E. coli*) cells harboring surface appendages (F-pili (Beloin et al. 2010) or YeeJ surface  
135 proteins (Martinez-Gil et al. 2017)) are analyzed as a function of NPs concentration and solution pH.  
136 Results are further complemented by AFM images of bacterial cells in liquid medium in order to  
137 visualize cell surface state and NP-mediated cell damage. Overall, the conclusions comfort the

138 potentialities of electrophoresis for assessing the first stage of NPs action toward the surface of  
139 biological cells.

140

## 141 **2. Material and Methods**

### 142 *2.1 Bacteria*

143 The *Escherichia coli* K12 mutant strains adopted in this work are listed in **Table 1** where relevant  
144 information on their expressed surface appendages, genotype and antibiotic resistance can be found.  
145 The genetically modified strains were kindly provided by the Institut Pasteur of Paris (Department of  
146 Microbiology, Unité de Génétique des Biofilms). The here-used isogenic strains were generated from  
147 *E. coli* MG1655 by construction of linear DNA fragments using Polymerase Chain Reaction (PCR) and  
148 the constructions were inserted in *E. coli* genome by  $\lambda$  red recombination (Chaveroche et al. 2000).  
149 Two cell surface phenotypes were considered: cell membranes exclusively decorated by either F-pili  
150 (E2302) appendages (Beloin et al. 2010) or by YeeJ large surface proteins (E2551) (Martinez-Gil et al.  
151 2017). Nude bacteria (E2152), devoid of any of these two surface appendages, served as a control  
152 strain. The cells strains E2302, E2551 and E2152 adopted here did not further harbor Ag43 adhesins  
153 nor type 1 fimbriae at their surface, as further specified in **Table 1**. F-pili are long flexible  
154 proteinaceous filaments with diameter below 10 nm (Costa et al. 2016; Wang et al. 2009) and their  
155 length may range from 10  $\mu$ m to 100  $\mu$ m under fully stretched configuration. The number of F-pili  
156 typically found on bacterial cells is between 1 and 3 (Dubey and Maheshwari 1999; Reisner et al.  
157 2003; Wang et al. 2009). These cell surface components play a key role in biofilm formation as they  
158 promote initial cell adhesion to abiotic surfaces (Beloin et al. 2010; Flemming et al. 2016). In addition,  
159 the F-pili is well known as mediator of conjugative transfer of plasmids (Silverman 1997). As far as  
160 YeeJ proteins are concerned, they also promote biofilm adhesion on abiotic surfaces, their  
161 theoretical isoelectric point is 4.79 and their theoretical molecular weight is 244 kDa (Martinez-Gil et  
162 al. 2017).

163 Using the relevant antibiotics listed in **Table 1**, bacteria were pre-grown in Lysogeny Broth (LB)  
164 medium (BD Difco®) for 10 h at 37°C under 160 rpm agitation and aerobic conditions. The pre-culture  
165 was then diluted to a 1:50 ratio in minimum M63B1 medium containing 0.4% w/w glucose and  
166 appropriate antibiotics (hereafter called M63B1glu medium). In details, this M63B1glu medium  
167 contained 13.6 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub> (Sigma-Aldrich®), 2 g L<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (Emsure®), 0.1 g L<sup>-1</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O  
168 (Emsure®), 5·10<sup>-4</sup> g L<sup>-1</sup> FeSO<sub>4</sub>·7H<sub>2</sub>O (Sigma-Aldrich®), 1·10<sup>-3</sup> g L<sup>-1</sup> thiamine (Roth®) and 4 g L<sup>-1</sup> glucose  
169 (Sigma®). The pre-culture was further grown for 12 h at 37°C, 160 rpm agitation under aerobic  
170 conditions. Then, cells culture was re-suspended in fresh M61B1glu, without antibiotics, at an OD<sub>600</sub>  
171 of 0.05, and finally cultured at 37°C, 160 rpm until an OD<sub>600</sub> value of 0.5 was reached.

172 Preparation of cells suspensions for electrophoresis measurements on bacteria in the absence of NPs  
173 (results given in **Figure 3**). Bacteria cultured as detailed above were first centrifuged for 5 min at  
174 5000×g and then re-suspended in 10 mM KNO<sub>3</sub> (Sigma-Aldrich®, purity > 99%) so as to reach an OD<sub>600</sub>  
175 of 0.2. Then, batches of cells suspended in 1 mM to 250 mM KNO<sub>3</sub> final electrolyte concentration  
176 were prepared in such a way that a final OD<sub>600</sub> of 0.02 was reached. For that purpose, appropriate  
177 volumes of 2M KNO<sub>3</sub> electrolyte, of ultrapure water (milli-Q® water) and of the aforementioned cells  
178 suspensions (OD<sub>600</sub>=0.2, 10 mM KNO<sub>3</sub>) were added to get a total final suspension volume of 80 ml.  
179 The pH of the resulting cell suspension was then adjusted to the desired value upon addition of HNO<sub>3</sub>  
180 and KOH (10<sup>-2</sup>-10<sup>-1</sup> M, Fluka®) under agitation conditions (500-700 rpm) and the added acid/base  
181 volume did not correspond to more than 1% of the total suspension volume.

182 Preparation of mixed NPs-cells suspensions for electrophoretic mobility measurements on bacteria in  
183 the presence of NPs (Figure 4). For the electrokinetic experiments conducted on suspensions  
184 containing both bacterial cells and NPs (binary systems), bacteria were first centrifuged for 5 min at  
185 5000×g and then re-suspended in 1 mM KNO<sub>3</sub> (Sigma-Aldrich®, purity > 99%) so as to reach an OD<sub>600</sub>  
186 of 0.4. Appropriate volumes of (i) separately prepared 1 mM KNO<sub>3</sub> NPs suspension at 10<sup>-1</sup> g L<sup>-1</sup> (pH 3  
187 or 7) obtained after 20 min sonication, of (ii) bacteria suspension (OD<sub>600</sub>=0.4, 1 mM KNO<sub>3</sub>) and of (iii)  
188 1 mM KNO<sub>3</sub> solution (pH 3 or 7) were then mixed under agitation conditions (500-700 rpm) so as to  
189 achieve a final solution composition (28 ml total volume) with NPs concentration in the range 0 g L<sup>-1</sup>-  
190 10<sup>-2</sup> g L<sup>-1</sup>, and an OD<sub>600</sub> of 0.02. The suspension pH was controlled and readjusted if necessary to pH 3  
191 or 7. Again, the added acid/base volume did not correspond to more than 1% of the total suspension  
192 volume. Mobility measurements in such NPs-cells mixtures were conducted 15 minutes after  
193 introduction of the NPs. This time delay thus defines here the duration of the cells exposition to  
194 SiNH<sub>2</sub> NPs. Experiments were carried out twice on freshly prepared samples. Under the conditions  
195 applicable in **Figure 4**, the OD<sub>600nm</sub> is 0.02 and the maximum NPs concentration tested is 10<sup>-2</sup> g/L,  
196 which makes a ratio 'mass of NPs per cell' of about 0.5 pg/cell. This order of magnitude is well in  
197 agreement with that considered in other studies (Mathelié-Guinlet et al. 2018).

## 198 2.2 Silica nanoparticles (SiNPs in short)

199 SiNPs were kindly provided by Instituto Superior Técnico, University of Lisboa, Portugal. They were  
200 synthesized according to modified Stöber protocol as described elsewhere (Crucho et al. 2017;  
201 Ribeiro et al. 2017, 2014; Stöber et al. 1968). Briefly, silica NPs were generated upon hydrolysis and  
202 condensation of tetraethoxysilane (TEOS) in ethanol in the presence of ammonia as catalyst. The  
203 modification of the SiNPs surface was performed in dry toluene under argon atmosphere (Crucho et  
204 al. 2017; Ribeiro et al. 2014), which results in SiNPs decorated by 2 NH<sub>2</sub> surface groups per nm<sup>2</sup>, as  
205 determined by nuclear magnetic resonance (Crucho et al. 2017). The diameter of the synthesized  
206 particles is 130 nm ± 10 nm, as inferred from Transmission Electronic Microscopy (TEM)  
207 measurements (Supplementary Material, **Figure S1**). Dried SiNH<sub>2</sub> NPs were dispersed in 0.1 mM or 1  
208 mM KNO<sub>3</sub> electrolyte to obtain a final 0.1 g L<sup>-1</sup> NPs concentration. For that purpose, the appropriate  
209 weight of NPs was added to half of the desired volume of KNO<sub>3</sub> solution under 1000 rpm agitation  
210 conditions for 1 min, and the volume was filled with 0.1 mM or 1 mM KNO<sub>3</sub> to reach the final  
211 targeted volume. The so-obtained NPs suspension (pH 7) was finally stirred for 24 h at 1000 rpm.  
212 Prior to use of NPs suspensions, the latter were systematically sonicated for 20 min and then left at  
213 rest for 1 h.

## 214 2.3 Size and electrophoretic mobility measurements

215 Electrophoretic mobilities ( $\mu$ ) of NPs and bacteria considered separately were measured using  
216 Zetasizer NanoZS (Malvern Instruments®, UK) and Zetaphoremeter IV (CAD Instrumentations®, Les  
217 Essarts le Roi, France), respectively. The former apparatus is well suited for particles with diameter in  
218 the range 1 nm to 6  $\mu$ m (size measurements) and 5 nm to 10  $\mu$ m (electrophoretic mobility  
219 measurements), and the latter for larger systems with size in the range 0.1  $\mu$ m to 50  $\mu$ m (mobility  
220 measurements). For mobility measurements performed in the NPs-cells binary systems, the  
221 Zetaphoremeter equipment was adopted because our primary interest is the detection of changes in  
222 the electrophoretic signature of cells following their interaction with NPs.

### 223 2.3.1 NPs electrophoretic mobility and size measurements

#### 224 Size measurements

225 The hydrodynamic diameter of SiNH<sub>2</sub> NPs was measured at solution pH 3 and 7 as a function of KNO<sub>3</sub>  
226 salt concentration (0.1-250 mM) using Dynamic Light Scattering (DLS) (Zetasizer NanoZS, Malvern®)

227 with a 633 nm laser. The particle diffusion coefficient was obtained from the correlation of the  
228 measured scattered intensity and converted to hydrodynamic diameter using Stokes-Einstein  
229 equation. Results are reported here in the form of particle number-size distributions. The NPs  
230 suspensions used for size measurements were prepared from the NPs suspensions obtained in §2.2  
231 after proper dilution or addition of KNO<sub>3</sub> salt and pH adjustment using HNO<sub>3</sub> (10<sup>-1</sup>-10<sup>-2</sup>M Fluka®) or  
232 KOH (10<sup>-1</sup>-10<sup>-2</sup>M, Fluka®).

### 233 **Electrophoretic mobility measurements**

234 The electrophoretic mobility of SiNH<sub>2</sub> NPs was measured as a function of pH (3 to 9) and KNO<sub>3</sub> salt  
235 concentration in the range 0.1 mM to 250 mM at 25°C using Zetasizer NanoZS. A constant direct  
236 current electric field (25 V cm<sup>-1</sup>) applied between two electrodes immersed in the NPs-containing  
237 solution imposes a displacement of the particles. Electrophoretic mobility was then determined using  
238 Phase Analysis Light Scattering (PALS) technology (Malvern Instruments). NPs suspensions were  
239 prepared in a way similar to that adopted for size measurements. Experiments were carried out 3 to  
240 5 times on freshly prepared samples.

#### 241 2.3.2 Cells electrophoretic mobility measurements and electrokinetics of mixed NPs- 242 bacteria suspensions

243 The Zetaphoremeter was used for electrophoretic mobility measurements of bacteria and of binary  
244 NPs-bacteria systems. The instrument consists of a quartz suprasil cell, a laser and a charge-coupled  
245 device camera connected to a computer. The camera tracks the positions of the bacteria *via* a laser,  
246 and a software processes the obtained images in real time. From the displacement of the tracked  
247 cells, subjected to a constant direct-current electric field (800 V m<sup>-1</sup>), the electrophoretic velocity and  
248 electrophoretic mobility were evaluated (Francius et al. 2011; Pagnout et al. 2012). The  
249 electrophoretic mobility of bacteria was measured at pH 3 and 7 as a function of KNO<sub>3</sub> electrolyte  
250 concentration (1-250 mM). Cells suspensions and mixed NPs-cell suspensions were prepared along  
251 the lines detailed in §2.1.

#### 252 *2.4 Atomic Force Microscopy (AFM) measurements*

253 AFM imaging of bacterial cells was performed under liquid conditions using a Bruker FastScan  
254 Dimension Icon with Nanoscope V controller operated in Peak Force tapping mode at 1.0 Hz scan  
255 rate (Bruker® corporation, Palaiseau, France). This mode consists in monitoring the intermittent  
256 contact between tip and sample surface with application of a 7 nN force, at most (except 12 nN for  
257 imaging of E2302 cells at pH 3), which limits possible samples damage by the tip (NPG tips, nominal  
258 spring constant of 0.12 N m<sup>-1</sup>, Bruker, AXS®, Palaiseau, France). Prior to experiments, glass slides  
259 were coated with a polycationic polymer (polyethylenimine, PEI in short) to promote electrostatic  
260 immobilization of bacterial cells. In detail, the glass slides were cleaned with 1 g L<sup>-1</sup> RBS 25 solution  
261 (Sigma-Aldrich®) at 60°C for 30 min, then rinsed with milli-Q® water, subsequently immersed in a  
262 1 g L<sup>-1</sup> PEI solution (Sigma-Aldrich®, 7.5 10<sup>6</sup> Mw, 50 wt. % in H<sub>2</sub>O) for 30 min and finally rinsed with  
263 milli-Q® water. 1.4 mL of bacterial suspension (of pH 3 or 7, and OD<sub>600</sub> = 0.4) was deposited onto the  
264 PEI-coated slides. After 30 min, the sample was extensively rinsed with 1 mM KNO<sub>3</sub> solution (pH 3 or  
265 7) and the slide was then fixed to a magnetic puck and placed into the AFM fluid-cell containing 2 mL  
266 of 1 mM KNO<sub>3</sub> solution at the sample pH (3 or 7). For AFM imaging of binary systems, bacteria  
267 suspensions (OD<sub>600</sub> = 0.4) were first mixed with 1 mM KNO<sub>3</sub> NPs solution of final concentration 10<sup>-3</sup>  
268 g L<sup>-1</sup> under 15 min agitation conditions, similar to those adopted for preparing the mixed cells-NPs  
269 suspensions analysed by electrokinetics (§2.1). The suspensions were then deposited onto the PEI-  
270 coated slides for 30 min and rinsed with 1 mM KNO<sub>3</sub> solution, pH 3 or 7. For each tested condition  
271 (pH 3 or 7, presence/absence of SiNH<sub>2</sub> NPs), AFM images (256×256 pixels, 3.5 μm×3.5 μm, pixel

272 size=13.7 nm) were recorded on *ca.* 3 to 7 cells per strain originating from different cell cultures. Cell  
273 surface roughness features (mean value and standard deviation) were evaluated from analysis of  
274 areas (400 nm×400 nm) of 3 to 7 distinct cells imaged as detailed above.

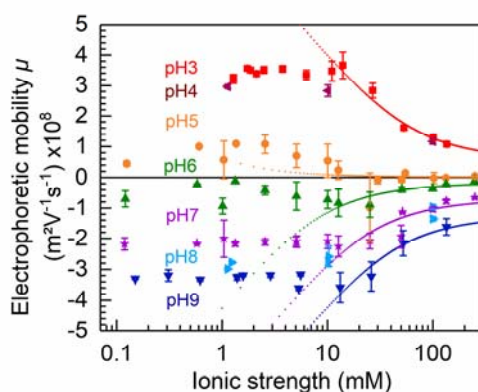
275

### 276 3. Results and Discussions

277 Before analyzing the modulations of the electrokinetic properties of bacterial cells as a result of  
278 their interactions with SiNH<sub>2</sub> NPs, we discuss below the electrophoretic mobility properties of SiNH<sub>2</sub>  
279 NPs and of the adopted *E. coli* strains, taken separately.

#### 280 3.1 Electrokinetics of SiNH<sub>2</sub> NPs and of bacteria

281 The dependence of the electrophoretic mobility  $\mu$  of SiNH<sub>2</sub> NPs on KNO<sub>3</sub> electrolyte concentration  
282 and pH is reported in **Figure 1**.



283

284 **Figure 1: Electrophoretic mobility of SiNH<sub>2</sub> NPs as a function of pH (indicated) and solution ionic**  
285 **strength** (changed upon modification of KNO<sub>3</sub> electrolyte concentration in solution). Colored  
286 symbols: experimental data. Lines: data reconstruction by means of Ohshima's theory (eqs 1-4).  
287 Measurements, performed with Zetasizer NanoZS equipment (Malvern Instruments®, UK), were  
288 carried out on 2 to 5 freshly prepared SiNH<sub>2</sub> NPs suspensions, and each of these measurements was  
289 done in triplicate. Accordingly, each point in Figure 1 is the average of 6 to 15 measurements.

290 The mobility  $\mu$  of SiNH<sub>2</sub> NPs is negative over the whole range of tested KNO<sub>3</sub> concentrations at pH>6  
291 and it is positive for pH values lower than 5-6. The underlying reversal of SiNH<sub>2</sub> NPs surface charge is  
292 surprising in view of the expected presence of amine surface groups that would lead -if solely  
293 considered- to positive NPs mobility regardless of pH and salinity conditions. The reason for this  
294 charge reversal stems from the NPs surface functionalization efficiency (2 NH<sub>2</sub> surface groups per  
295 nm<sup>2</sup>), leaving a considerable amount of hydroxyl groups from the silica structure exposed to the  
296 outer aqueous solution (Crucho et al. 2017). As a result, the positive charge of the NPs stems from  
297 the protonation of -NH<sub>2</sub> groups that becomes significant with decreasing pH below *ca.* 6 (**Figure 1**),  
298 which is in agreement with typical pK values reported for amine groups (Diallo et al. 2004; Tajarobi et  
299 al. 2001). For pH values larger than 6, the negative charge of NPs originates from the dissociation of  
300 the surface hydroxyl groups whose contribution overwhelms that of the poorly protonated -NH<sub>2</sub>  
301 groups under such pH conditions. The comparison between  $|\mu|$  reached at pH 9 and pH 3 in *e.g.*  
302 10 mM KNO<sub>3</sub> suggests that the NPs surface concentration of -NH<sub>2</sub> and -OH groups is similar. With  
303 decreasing salt concentration from 100 mM to 10 mM at fixed pH,  $|\mu|$  increases due to reduction of  
304 NPs surface charge screening by ions from the background electrolyte. Further decrease in  
305 electrolyte concentration results in a mobility that becomes -within experimental error- basically



306 independent of salt content in solution. Two reasons may be invoked for qualitatively explaining the  
 307 behavior of  $\mu$  at such low  $\text{KNO}_3$  concentrations (here, below 10 mM): (i) double layer polarization  
 308 effects, that tend to reduce the dependence of  $\mu$  on salt concentration, as demonstrated elsewhere  
 309 for highly charged polymer NPs (Duval et al. 2013), and/or (ii) changes in the spatial organization of  
 310 the cationic and anionic surface charges within the thin polyelectrolytic like-layer surrounding the  
 311 NPs. These latter changes also lead to moderate variations of  $\mu$  on electrolyte concentration for the  
 312 reasons detailed by (Moussa et al. 2015) in their electrophoresis analysis of zwitterionic carboxylate-  
 313 terminated poly(amidoamine) dendrimers. In the following developments, we restrict our  
 314 quantitative analysis of the electrophoretic properties of  $\text{SiNH}_2$  NPs to  $\text{KNO}_3$  concentrations larger  
 315 than 10 mM with adopting the analytical model developed by Ohshima for soft particle  
 316 electrokinetics (Ohshima 1995). Soft particles correspond to porous or core/shell particles whose  
 317 peripheral polyelectrolyte structure is permeable to ions and fluid flow and for which the concept of  
 318 zeta potential is meaningless (Duval and Gaboriaud 2010). Unlike standard zeta potential-based  
 319 models strictly applicable to hard particles that are impermeable to ions and flow, soft particle  
 320 representation was shown to correctly apply to silica NPs (Škvarla 2007) or to latex particles with  
 321 polymeric surface layer as thin as 1 nm (Duval et al. 2013). According to Ohshima (Ohshima 1995),  
 322 the mobility  $\mu$  of a soft particle reads as

$$323 \quad \mu = \frac{\rho_0}{\eta \lambda_0^2} + \frac{\varepsilon \psi^0 / \kappa_m + \psi^D / \lambda_0}{\eta (1 / \kappa_m + 1 / \lambda_0)} \quad (1)$$

324 , where  $\rho_0$  corresponds to the effective charge density of the soft surface layer and  $1/\lambda_0$  to the so-  
 325 called hydrodynamic penetration length, *i.e.* the extent by which the electroosmotic flow developed  
 326 under electrokinetic conditions penetrates the soft surface layer.  $\eta$  and  $\varepsilon$  are the dynamic viscosity  
 327 and dielectric permittivity of the medium, respectively,  $\kappa_m$  the reciprocal Debye layer thickness in the  
 328 soft surface layer,  $\psi^0$  the surface potential located at the edge of the soft layer, and  $\psi^D$  the Donnan  
 329 potential. Equation 1 holds under conditions where the particle hard core radius  $a$  and the soft  
 330 surface layer thickness  $d$  well exceed the thickness of the double layer at the fluid side of the  
 331 particle/solution interface, and the hydrodynamic penetration length  $1/\lambda_0$  is significantly lower than  
 332  $d$ , *i.e.*  $\kappa a \gg 1$ ,  $\kappa d \gg 1$ ,  $\lambda_0 d \gg 1$ . These conditions are satisfied at sufficiently large electrolyte  
 333 concentrations where polarization of electric double layers is insignificant and interfacial potentials  
 334 are in line with applicability of the Debye Hückel approximation (Duval and Gaboriaud 2010; Duval  
 335 and Ohshima 2006; Ohshima 1995). The quantities  $\psi^D$ ,  $\psi^0$  and  $\kappa_m$  all depend on charge density  $\rho_0$  and  
 336 electrolyte concentration  $c^\infty$  (1:1 symmetrical electrolyte is considered here) according to (Ohshima  
 337 1995)

$$338 \quad \psi^D = \frac{RT}{F} \sinh^{-1} \left( \frac{\rho_0}{2Fc^\infty} \right) \quad (2)$$

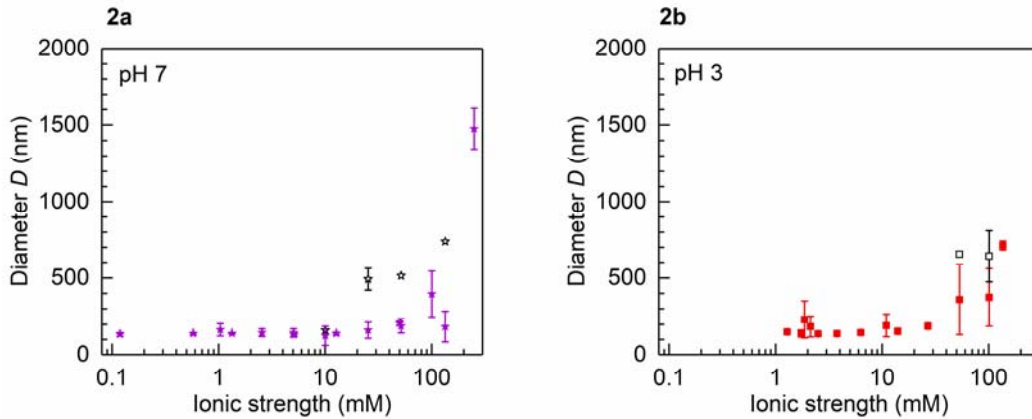
$$339 \quad \psi^0 = \psi^D - \frac{RT}{F} \tanh \left( \frac{F\psi^D}{2RT} \right) \quad (3)$$

$$340 \quad \kappa_m = \kappa \left\{ \cosh \left( \frac{F\psi^D}{RT} \right) \right\}^{1/2} \quad (4)$$

341 , where  $R$  is the gas constant,  $T$  the absolute temperature,  $F$  the Faraday number and  $\kappa$  the salinity-  
 342 dependent reciprocal Debye layer thickness. **Equations 1-4** successfully fitted the experimental data  
 343 above 10 mM  $\text{KNO}_3$  concentration in **Figure 1** upon adjustment of  $\rho_0$  and  $1/\lambda_0$  *via* Levenberg-  
 344 Marquardt procedure. Results are given in **Table 2** for the various tested pH conditions with  $\rho_0$   
 345 expressed in molar concentration of equivalent monovalent charges. In agreement with conclusions  
 346 from previous work (Škvarla 2007),  $\text{SiNH}_2$  NPs may be represented by Ohshima's soft (core/shell)

347 particle representation as  $1/\lambda_0$  significantly deviates from 0 value (the latter strictly pertains to the  
348 limit of hard impermeable particles), in line with the presence of a thin, fuzzy polyelectrolyte layer  
349 hosting the -OH and -NH<sub>2</sub> surface groups. In detail, the hydrodynamic softness of SiNH<sub>2</sub> NPs stems  
350 from the non-ideality of their surface *at the molecular scale*, materialized by the presence of  
351 asperities, depressions and non-homogeneous distributions of functional groups along- and  
352 perpendicular to- their surface. In turn, it is impossible to locate a slip plane for such particulate  
353 systems and to define rigorously a common planar location for all ionogenic groups (and therewith  
354 defined a *surface charge* density), as traditionally done for the ideal case of hard particles. A way to  
355 apprehend these deviations is to reason in terms of volume charge density and to consider that the  
356 electroosmotic flow profile *gradually* (and not abruptly as for hard particles) drops to zero from the  
357 bulk solution to the particle phase with a decay length scale given by  $1/\lambda_0$ . These elements explain  
358 why particles like SiNH<sub>2</sub> NPs behave as soft particles. Ohshima's model overestimates the magnitude  
359 of  $\mu$  for all pH conditions at KNO<sub>3</sub> concentrations below 10 mM where possible double layer  
360 polarization, structural modulation of the polyelectrolyte surface layer and deviation from linearized  
361 Poisson-Boltzmann and Donnan electrostatics come into play, all these features being discarded in  
362 **eqs 1-4** (Duval and Gaboriaud 2010; Moussa et al. 2015). Conformably to the above qualitative  
363 analysis of **Figure 1**, the isoelectric point of SiNH<sub>2</sub> NPs is located at pH~5. The nanometric length scale  
364  $1/\lambda_0 (= 1 \pm 0.3 \text{ nm})$  compares well with results obtained for latex particles of similar size (Duval et al.  
365 2013). The absence of marked maximum for  $|\mu|$  *versus* salt concentration in **Figure 1**, which  
366 contrasts with that evidenced for latex NPs (Duval et al. 2013), indicates that double layer  
367 polarization effects for SiNH<sub>2</sub> NPs are probably less significant than for latex NPs, which qualitatively  
368 agrees with the  $\rho_0$  values of the former particle type at pH 7 that are *ca.* 10 times lower in magnitude  
369 than those derived for the latex NPs (Duval et al. 2013). Finally, the increase in  $1/\lambda_0$  with pH suggests  
370 that the thickness and/or structure of the thin polyelectrolyte-like layer slightly changes with pH as a  
371 result of modification of the electrostatic interactions between neighboring -O<sup>-</sup> or -NH<sub>3</sub><sup>+</sup> ionogenic  
372 surface groups, a feature evidenced for nanoparticulate dendrimers (Moussa et al. 2015). Following  
373 these conclusions, and exploiting the unexpected reversal in SiNH<sub>2</sub> NPs mobility at pH 5, the pH 7 and  
374 pH 3 conditions were selected for deciphering below the impacts of SiNH<sub>2</sub> NPs on cell electrokinetic  
375 properties (§3.2).

376 In order to ascertain that suspensions of SiNH<sub>2</sub> NPs remain stable against aggregation under  
377 conditions adopted for the experiments carried out in the presence of bacterial cells (*i.e.* pH 3 or 7,  
378 and 1 mM solution ionic strength), particle size measurements were performed by DLS at pH 3 and 7  
379 as a function of salt concentration (**Figure 2**), as detailed in §2.3.1. In good agreement with  
380 independent TEM measurements (**Figure S1**), SiNH<sub>2</sub> NPs hydrodynamic diameter is  $140 \pm 10 \text{ nm}$  at  
381 ionic strengths lower than 10 mM, regardless of pH. The NPs suspensions stability at pH 7 confirms  
382 the existence of (negative) electrostatic charges at the surface of SiNH<sub>2</sub> NPs (as independently  
383 evidenced by electrokinetics), which ensures the operability of repulsive interactions between  
384 particles and the establishment of a significant energy barrier they must overcome to form  
385 aggregates at such a pH. At larger solution ionic strengths (25 mM-150 mM) and pH 7, a bimodal  
386 distribution in particle size is obtained, which pinpoints the presence of particle aggregates with  
387 broad size distribution. The respective magnitude of the detected intensity peaks still denotes a  
388 predominant proportion of individual particles over that of aggregates (500 nm-700 nm in diameter).  
389 At 250 mM solution ionic strength, particles suspensions become unstable as particle size may then  
390 exceed micrometer scale and the contributions of aggregates to the overall size distributions  
391 becomes significant. Similar features are observed at pH 3. Given these elements, the stability of the  
392 SiNH<sub>2</sub> NPs suspensions against aggregation is ensured in 1 mM solution ionic strength both at pH 3  
393 and 7 (the conditions adopted for electrokinetic analysis of binary NPs-cells system).



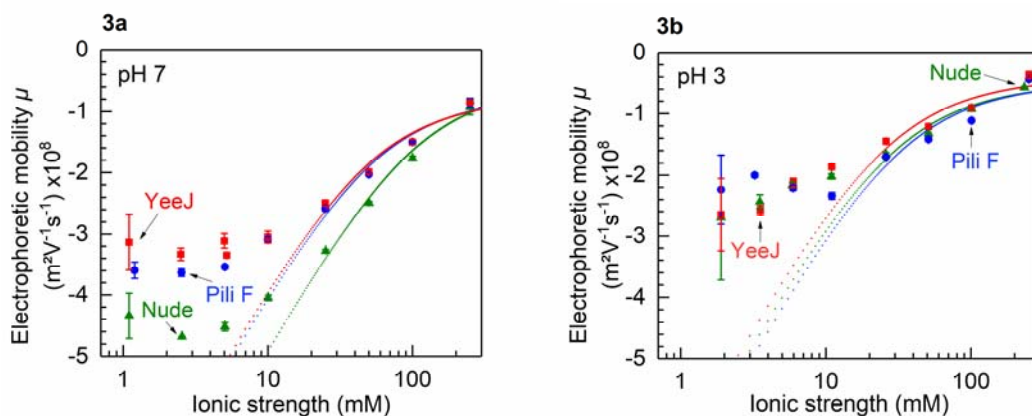
394

395 **Figure 2: SiNH<sub>2</sub> NPs hydrodynamic diameter *D* at pH 7 (2a) and pH 3 (2b) as a function of solution**  
 396 **ionic strength.** Filled symbols correspond to the first mode detected in the number-size distributions  
 397 obtained by DLS. Empty bullet points correspond to the second mode if applicable. Due to the  
 398 contribution of protons to the overall solution ionic strength, there are no data points in panel b at 1  
 399 mM ionic strength after addition of KNO<sub>3</sub> electrolyte.

400 As our intention is to assess the electrokinetic cell features (in mixed cell-SiNH<sub>2</sub> NPs suspensions)  
 401 as a function of NPs concentrations in solution, we further identified the range of SiNH<sub>2</sub> NPs  
 402 concentration in line with constant SiNH<sub>2</sub> NPs electrophoretic mobility. Respecting the NPs  
 403 concentrations window recommended by Malvern (NanoZS) and CAD (Zetaphoremeter IV apparatus)  
 404 manufacturers, we found that the mobility of SiNH<sub>2</sub> NPs at pH 3 (pH 7, respectively) in 1 mM solution  
 405 ionic strength is  $\mu=2.1\pm0.6\times10^{-8}$  m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup> at 10<sup>-3</sup> g L<sup>-1</sup> ( $\mu=-1.8\pm0.1\times10^{-8}$  m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup>, respectively) and  
 406  $\mu=2.5\pm0.5\times10^{-8}$  m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup> at 10<sup>-2</sup> g L<sup>-1</sup> ( $\mu=-2.0\pm0.4\times10^{-8}$  m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup>, respectively), as obtained with  
 407 Zetaphoremeter IV equipment (CAD Instrumentations®). Measurements with Zetasizer NanoZS  
 408 (Malvern) lead at pH 3 (pH 7, respectively) to  $\mu=2.4\pm0.6\times10^{-8}$  m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup> at 10<sup>-2</sup> g L<sup>-1</sup> ( $\mu=-1.9\pm0.7\times10^{-8}$   
 409 m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup>, respectively) and  $\mu=3.0\pm0.7\times10^{-8}$  m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup> at 10<sup>-1</sup> g L<sup>-1</sup> ( $\mu=-1.7\pm0.9\times10^{-8}$  m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup>, respectively).  
 410 At fixed pH (3 or 7), within experimental error  $\mu$  values therefore remain constant in the 10<sup>-3</sup> g L<sup>-1</sup> (or  
 411 lower) to 10<sup>-2</sup> g L<sup>-1</sup> NPs concentration range: this NP concentration range is therefore adopted in §3.2  
 412 dedicated to the electrokinetic analysis of mixed NPs-bacteria suspensions using Zetaphoremeter IV  
 413 equipment.

414 The electrophoretic mobility of the 3 different bacterial strains (nude, YeeJ or F-pili decorated  
 415 cells, see §2.1) was measured as a function of solution ionic strength (1 mM to 250 mM at pH 3 and  
 416 7). Results are reported in **Figure 3**. As expected for bacterial cells,  $\mu$  is negative for all strains at both  
 417 pH conditions and the observed classical decrease of  $|\mu|$  with increasing salt concentration refers to  
 418 screening of the charges carried by cells envelopes (Duval and Gaboriaud 2010). Distinct  
 419 electrokinetic cells responses are further observed depending on their respective surface phenotype.  
 420 In detail, at pH 7 (**Figure 3a**)  $|\mu|$  of the nude cells (E2152) is systematically higher than that measured  
 421 for the F-pili- and YeeJ-decorated bacteria (E2302 and E2551, respectively). In addition,  $|\mu|$  of E2302  
 422 (F-pili) and of E2551 (YeeJ) cells exhibit -within experimental error- identical electrokinetic behavior  
 423 over the range of ionic strengths tested. The results displayed for E2152 (nude cells) and E2302 (F-pili  
 424 bacteria) compare well with those previously reported by Francius et al. (2011), to which the reader  
 425 is referred for details. The differences in mobility between the three strains primarily originate from  
 426 modifications of the electrostatic cell surface features and cell drag forces as a result of the selective  
 427 expressions of YeeJ and of F-pili protruding surface appendages. These modifications are addressed  
 428 here from analysis of the data in **Figure 3** on the basis of eqs 1-4 along the lines detailed above for

429 investigation of NPs electrokinetics (**Figure 1**), recalling that bacteria are paradigms of soft  
 430 (bio)colloids (Duval and Gaboriaud 2010). The results provided in **Table 3** highlight that the density of  
 431 charges distributed within the electrokinetically active peripheral zone of the nude bacteria at pH 7 is  
 432 *ca.* two times higher than that pertaining to E2302 and E2551 cells. While the latter two bacterial  
 433 strains are defined by similar  $\rho_o$  ( $\sim 97 \pm 4$  mM) and similar Brinkman lengths ( $1/\lambda_o \sim 0.85$  nm), their  
 434 permeability to electroosmotic flow appears to be somewhat larger than that for nude cells ( $1/\lambda_o \sim 0.5$   
 435 nm). Surface appendages thus contribute here to slightly increase flow permeability (*i.e.* increase of  
 436  $1/\lambda_o$ ) and to hydrodynamically screen the charges carried by the underlying supporting membrane,  
 437 thereby reducing the density of the charges that are effectively probed by the tangential  
 438 electroosmotic flow developed under electrophoretic conditions. Remarkably, within experimental  
 439 error the electrokinetic properties of the three strains of interest are similar at pH 3 over the whole  
 440 range of solution ionic strengths tested (**Figure 3b**), and the corresponding  $|\mu|$  is systematically  
 441 smaller than that measured at pH 7 due to expected protonation of the proteins and other moieties  
 442 located at the bacterial cell surfaces. Overall, the respective magnitudes of  $1/\lambda_o$  ( $= 0.7 \pm 0.2$  nm, with  
 443 no marked dependence on pH) and  $\rho_o$  values derived for the cells of interest at pH 7 and 3 correctly  
 444 fit into the data compilation done by Duval and Gaboriaud from electrokinetic literature on Gram-  
 445 negative bacteria (Duval and Gaboriaud 2010).



446

447 **Figure 3: Electrophoretic mobility of the adopted bacterial strains** (nude (E2152), F-pili (E2302) and  
 448 YeeJ (E2551) cells, indicated) **at pH 7 (3a) and pH 3 (3b) as a function of solution ionic strength.**  
 449 Symbols: experimental data. Dotted lines: theory (eqs 1-4). Due to the contribution of protons to the  
 450 overall solution ionic strength, there are no data points in panel b at 1 mM ionic strength after  
 451 addition of  $\text{KNO}_3$  electrolyte.

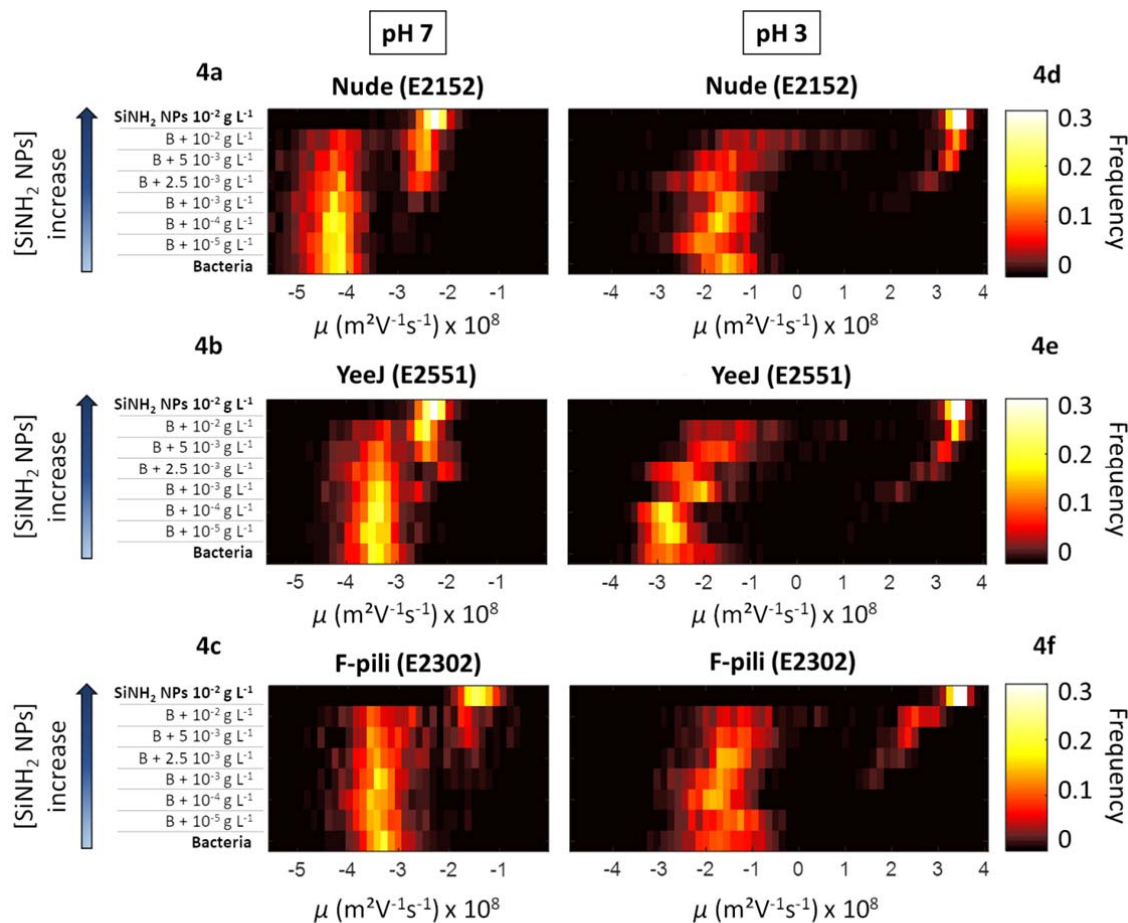
452

### 453 3.2 Electrokinetics of mixed bacteria-NPs suspensions

454 The electrophoretic properties of the mixed bacteria-NPs suspensions (see methodology detailed  
 455 in §2) are given in **Figure 4** in the forms of electrophoretic mobility distributions reported as a  
 456 function of concentration of  $\text{SiNH}_2$  NPs in solution at pH 7 and pH 3 for the different cell surface  
 457 phenotypes considered in this work. For the sake of comparison, the mobility distributions featuring  
 458 the electrokinetic properties of cells and NPs taken separately are further displayed in **Figure 4**.  
 459 Accordingly, the first and last lines in each of the diagrams of **Figure 4** refers to the electrokinetic  
 460 responses of  $\text{SiNH}_2$  NPs and bacteria, respectively. The electrophoretic fingerprints of the cells and  
 461 those of the  $\text{SiNH}_2$  NPs in mixed NP-cells suspensions correspond to the mobility-plumes located at  
 462 the left and right side of the diagrams in **Figure 4**, respectively. In **Figure 4**, the frequency reported

463 for a given class of electrophoretic mobility values  $\mu$  (the width of each class is set here to  $0.15 \times 10^{-8}$   
464  $\text{m}^2\text{V}^{-1}\text{s}^{-1}$  increment in  $\mu$ ) corresponds to the ratio between the number of particle trajectories  
465 detected at these selected  $\mu$  and the total number of trajectories measured over the whole range of  
466 measured mobility values. As such, this frequency is systematically lower than unity. Traditionally,  
467 electropherograms display distributions of electrophoretic mobility in the form of frequency (of  
468 detected trajectories) *versus*  $\mu$  (see *e.g.* Dague et al. 2006). The representation in **Figure 4** is nothing  
469 else than this, except that distributions are here reported as a function of NPs concentration in  
470 solution (*y*-axis) and that frequencies are scaled according to the color code detailed in **Figure 4**. The  
471 result is the establishment of 2D electrophoretic plumes of the cells and of the NPs whose  
472 deformation along the *y*-axis informs about NPs actions on microorganisms surface, as extensively  
473 discussed below.

474 At pH 7, in line with data in **Figure 3**, cells electrophoretic mobility ranges from  $-5$  to  $-4 \times 10^{-8}$   $\text{m}^2\text{V}^{-1}\text{s}^{-1}$   
475  $\text{m}^2\text{V}^{-1}\text{s}^{-1}$  (E2152, nude cells), from  $-4$  to  $-3 \times 10^{-8}$   $\text{m}^2\text{V}^{-1}\text{s}^{-1}$  (E2551, YeeJ) and from  $-4$  to  $-2.5 \times 10^{-8}$   $\text{m}^2\text{V}^{-1}\text{s}^{-1}$   
476 (E2302, F-pili decorated). The width and frequencies defining the mobility distributions of E2152 and  
477 E2551 cells remain basically unchanged upon increase of  $\text{SiNH}_2$  NPs concentration up to  $10^{-3}$   $\text{g L}^{-1}$  (see  
478 the yellow core of the cells-associated plumes). With further increasing NPs concentration in  
479 solution, electrophoretic detection of the NPs becomes possible and their frequency of electrokinetic  
480 apparition increases noticeably, as judged from the color gradient in the down-to-top direction along  
481 the NPs-associated plumes in **Figures 4a** and **4b**. Concomitantly, there are lesser bacterial cells  
482 detected and their maximum frequency of electrokinetic apparition decreases from 0.2 to 0.05 with  
483 increasing NP concentrations from  $10^{-3}$   $\text{g L}^{-1}$  to  $10^{-2}$   $\text{g L}^{-1}$ . The most important conclusion at this stage  
484 is the absence of deformation of the cells-electrokinetic plume with increasing  $\text{SiNH}_2$  NPs, which  
485 suggests that the electrohydrodynamic properties of E2152 and E2551 cells remain basically  
486 unaffected in the presence of  $\text{SiNH}_2$  NPs. This is in line with the repulsive electrostatic interactions  
487 expected on the basis of the electrostatic properties of the negatively charged E2152/E2551 cells and  
488  $\text{SiNH}_2$  NPs as pictured in **Figure 3a** and **Figure 1**, respectively. Similar conclusions may be formulated  
489 for E2302 cells even though, qualitatively, the width of their mobility distributions tends to slightly  
490 increase with increasing  $\text{SiNH}_2$  NPs from 0 to  $10^{-2}$   $\text{g L}^{-1}$ , resulting in an overlap of the cells- and NPs-  
491 plumes that is significantly more pronounced than for E2152 cells. This finding underpins that a  
492 fraction of E2302 cells are impacted *via e.g.* interactions of their long protruding pili with NPs, a  
493 mechanism that can bypass the repulsive energy barrier that is otherwise required for E2152 cells  
494 and NPs to get into contact (van Loosdrecht et al. 1990). This feature would obviously require further  
495 investigation at the proper F-pili spatial scale *via e.g.* dedicated AFM-based investigations. Within the  
496 limits of detection, there are no obvious sign of changes of the native NP electrokinetic signal in the  
497 presence of bacteria at pH 7, irrespective of the cells surface phenotype considered.  
498



499

500 **Figure 4: Electrophoretic fingerprints of mixed bacteria-SiNH<sub>2</sub> NPs suspensions at pH 7 and pH 3**  
 501 **(indicated) as a function of SiNH<sub>2</sub> NPs concentration in solution for the three cell surface**  
 502 **phenotypes of interest (indicated). Solution ionic strength: 1 mM at pH 7 and 2 mM at pH 3. See text**  
 503 **for details (§2 and §3.2). ‘B’ stands for bacteria.**

504 At pH 3 (**Figures 4d, e, f**), the situation dramatically differs from that discussed at pH 7. Indeed,  
 505 the mobility distributions pertaining to the bacterial cells and/or to the SiNH<sub>2</sub> NPs, set under mixed  
 506 conditions, are now significantly impacted with increasing NPs concentration in solution. The  
 507 associated deformations of the cells- and/or NPs-electrokinetic plumes further differ according to the  
 508 cell surface phenotype considered. In detail, starting with the case of E2551 bacteria (YeeJ-decorated  
 509 cells, **Figure 4e**), the signal stemming from bacteria is clearly shifted to lesser negative mobility  
 510 values in the presence of SiNH<sub>2</sub> NPs and the more so as the NPs concentration increases. The  
 511 corresponding width of mobility distribution further increases and its extremes are shifted to more  
 512 positive values. This distortion of the cells-electrokinetic plume compounds the shift of the NPs-  
 513 associated electrokinetic fingerprints to more positive values with increasing NPs concentration in  
 514 solution. These results indicate that electrostatic attractions operating between positively charged  
 515 SiNH<sub>2</sub> NPs (**Figure 1**) and negatively charged E2551 cells (**Figure 3b**) at pH 3 induce significant changes  
 516 of the surface features of **both** bacteria and NPs. More specifically, a fraction of NPs likely adsorbs at  
 517 the cells surface to an extent that depends on electrostatics (favourable at pH 3) and on their  
 518 chemical affinity to cell surface biocompounds (YeeJ proteins or other molecules like  
 519 lipopolysaccharides). Other possible NPs action mode includes cell surface abrasion and subsequent

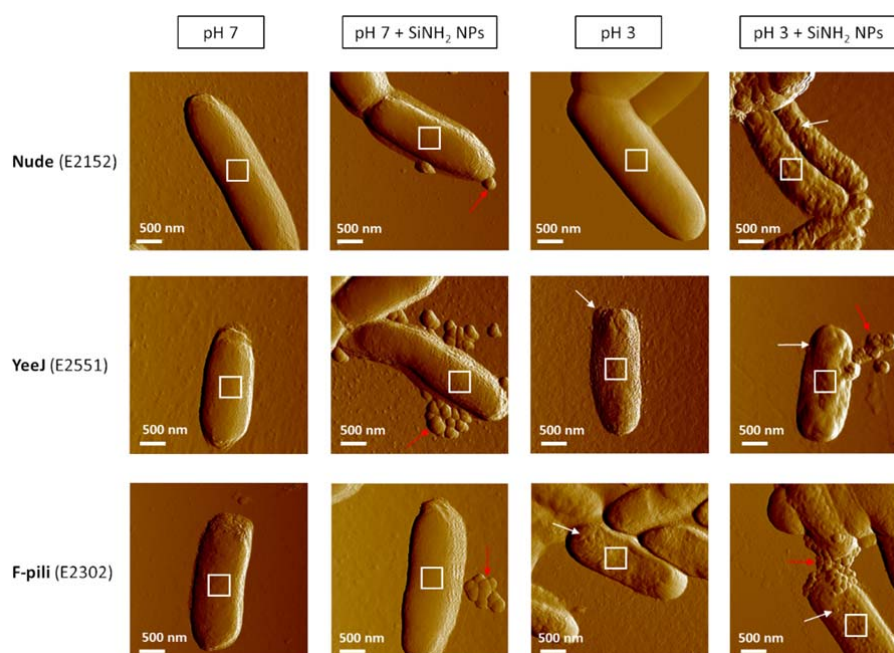
520 removal of charged bio-residues originally anchored at the cell membrane (Shrivastava et al. 2007).  
521 As a result, the effective charge density of the so-modified cell envelope probed by electrokinetics  
522 becomes less negative, in line with the cells-plume features given in **Figures 4e**. In addition, bacterial  
523 cells under stress conditions may excrete biomolecules (*e.g.* proteins, metabolites,  
524 exopolysaccharides). The latter in turn can modify the surface properties of the external agents at  
525 the origin of the stress (here the SiNH<sub>2</sub> NPs) (Kimkes and Heinemann 2018; Majdalani et al. 2005),  
526 minimize the impacts cells undergo and/or diminish the long-scale interactions that cells experience  
527 with these stressors (Zeyons et al. 2009). These biomolecules may further be released following NP-  
528 mediated cell surface abrasion invoked above and/or cleavage of cellular surface material following  
529 holes formation at the membrane. Such biotic retroaction processes would lead to a (negatively  
530 charged) bio-corona acquisition by NPs, and thus to a decrease of the net surface charge of the SiNH<sub>2</sub>  
531 NPs (Sheng et al. 2005; Zeyons et al. 2009). This expectation agrees with the trend in **Figure 4e** and  
532 with the aforementioned changes in SiNH<sub>2</sub> NPs electrokinetic plume. Obviously, the higher the SiNH<sub>2</sub>  
533 NPs content in solution (at fixed cells concentration), the lower the modification of their surface  
534 upon interaction with cells and/or with excreted cells biocomponents, the easier is their  
535 electrokinetic detection in mixed cell-NPs suspension and, concomitantly, the lesser efficient  
536 becomes the electrophoretic tracking of cells. This argument explains the decrease (increase,  
537 respectively) of the frequency maximum in mobility distributions pertaining to the cells (NPs,  
538 respectively) upon increasing SiNH<sub>2</sub> NPs concentration. This conclusion is supported by the colour  
539 scale gradient upon moving along the deformed cell- and NPs-electrokinetic plumes.

540 Qualitatively, the modulations discussed above for the E2551/SiNH<sub>2</sub> NPs electrokinetic diagram  
541 at pH 3 are also observed for the mixed E2152/SiNH<sub>2</sub> NPs at this pH (**Figure 4d**). However, the  
542 distortions of the SiNH<sub>2</sub> NPs- and E2152-plumes take place at larger NPs concentrations, *i.e.*  $2.5 \times 10^{-3}$   
543  $\text{g L}^{-1}$  and  $10^{-2} \text{ g L}^{-1}$ , respectively, instead of  $10^{-3} \text{ g L}^{-1}$  and  $5 \times 10^{-3} \text{ g L}^{-1}$  for the E2551/NPs system. This  
544 suggests that the resistance of the nude E2152 bacteria against NPs action is more efficient than that  
545 of YeeJ-decorated cells. Possibly, the protusion of YeeJ proteic islands over the cell surface facilitates  
546 non-specific electrostatic attractions with incoming NPs, as anticipated from theory by Duval *et al.* on  
547 interactions between rough surfaces (Duval et al. 2004). YeeJ proteins can also feature shorter-range  
548 specific interactions with SiNH<sub>2</sub> NPs. In both scenarios, YeeJ proteins promote contacts between cells  
549 and SiNH<sub>2</sub> NPs and, therewith, NPs-mediated modifications of the overall cell electro-surface  
550 characteristics. Finally, it is found that the situation for E2302/SiNH<sub>2</sub> NPs (**Figure 4f**) is intermediate  
551 between that for E2551/SiNH<sub>2</sub> NPs and E2152/SiNH<sub>2</sub> NPs systems. Indeed, while the deformation of  
552 the E2302 electrokinetic plume better compares with that obtained for the nude E2152 bacteria  
553 (**Figure 4d**), the marked modulation of the SiNH<sub>2</sub> NPs signal in the presence of E2302 cells is  
554 qualitatively more similar to that of SiNH<sub>2</sub> NPs in presence of E2551 cells (**Figure 4e**). Further  
555 comparison between **Figure 4e** and **Figure 4f** reveals that modifications of the SiNH<sub>2</sub> NPs surface  
556 properties with increasing SiNH<sub>2</sub> NPs concentration in NPs-cells mixtures are more significant in  
557 presence of E2302 cells than they are with E2551 cells. This conclusion is inferred from the  
558 corresponding shift in NPs electrophoretic mobility distribution to lesser positive values at fixed NPs  
559 concentration. Addressing the complex molecular processes that govern the modifications of the  
560 cells and NPs surface properties would obviously require reasoning beyond the electrokinetics  
561 framework and ask for integration of *e.g.* genomic/transcriptomic or micro-spectroscopy data in the  
562 analysis, which is not the scope of the current study. Instead, we demonstrate that simple  
563 electrophoretic methods may already help in detecting rapidly cells that are impacted by NPs, and  
564 identify some conditions where such effects are at stake. Altogether, **Figure 4** evidences (i) the role  
565 of electrostatics in mediating NPs-cells interactions, (ii) the heterogeneous response of the cells to  
566 NPs exposition (as reflected by changes in mobility distribution width), and (iii) the differentiated

567 roles played by cells surface bio-compounds in promoting or not NPs action. In the next section, we  
568 provide AFM images of individual cells exposed to SiNH<sub>2</sub> NPs as an additional support for the trends  
569 given in **Figure 4**, recalling that electrokinetic measurements refer to averages of  
570 electrohydrodynamic properties over the whole assembly of cells in solution.

### 571 *3.3 Bacterial cell surface imaging and roughness evaluation*

572 TEM is commonly adopted for imaging biological samples (El Badawy et al. 2011; Silva et al. 2014).  
573 However, the required sample preparation protocol, which involves sample drying, may lead to  
574 degradation of the native biosurface structures (Hardij et al. 2013). AFM measurements in liquid  
575 environment offer an option for avoiding such artefacts (Beaussart et al. 2018b; Bolshakova et al.  
576 2004; Dufrêne 2008; Jandt 2001). **Figure 5** collects typical AFM Peak Force images obtained for the  
577 three bacterial strains of interest, E2152, E2551 and E2302 before and after exposure to SiNH<sub>2</sub> NPs at  
578 pH 7 and pH 3.



579

580 **Figure 5: AFM peak force error images (3.5  $\mu\text{m} \times 3.5 \mu\text{m}$ , 256 pixels  $\times$  256 pixels) taken in liquid for**  
581 **E2152, E2551 and E2302 bacteria (indicated) at pH 7 and 3 (indicated) prior to and after incubation**  
582 **with NPs** (see details in §2). Red arrows correspond to NPs, white arrows to asperities or  
583 depressions, white squares define 400 nm  $\times$  400 nm spatial regions where cell surface roughness  
584 features were evaluated.

585 The AFM cell pictures obtained in the absence of SiNH<sub>2</sub> NPs at pH 7 (left column, **Figure 5**) display  
586 normal bacterial morphologies with aspects that agree well with those detailed in previous reports  
587 (Beloin et al. 2010; Francius et al. 2011). In particular, identification of YeeJ and pili structures at the  
588 surfaces of E2551 and E2302 is not possible from AFM imaging under liquid conditions as argued  
589 elsewhere (Francius et al. 2011). Cell surface imaging in air allows however visualization of the long  
590 surface appendages like pili (**Figure S2** in Supplementary Material). In the presence of SiNH<sub>2</sub> NPs (2<sup>nd</sup>  
591 column from the left, **Figure 5**), cell envelopes imaged at pH 7 do not suffer from marked asperities  
592 or depressions even though individual SiNH<sub>2</sub> NPs or aggregates may be found in the close vicinity of  
593 the cells and/or at their surface. The corresponding AFM images contrast with those obtained at pH 3  
594 in the presence of SiNH<sub>2</sub> NPs (right column in **Figure 5**). Under such conditions, nude E2152 cell

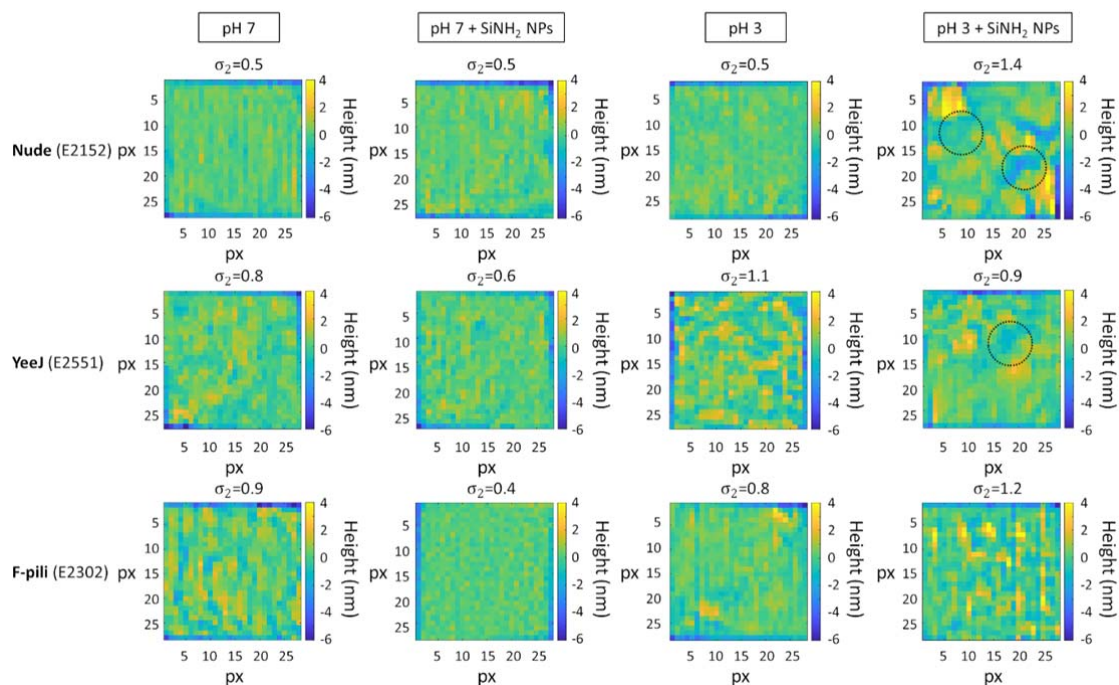


595 surfaces appear wrinkled, YeeJ-decorated E2551 cells anomalously disrupted with pronounced  
596 inflammatory points, and E2302 cells (with F-pili) significantly blistered all over their surface  
597 envelope that is in further intimate contact with apparently cohesive SiNH<sub>2</sub> NPs clusters.  
598 Modifications of E2302 and E2551 surface morphologies and topographies are also -to a lesser  
599 extent- caused by the acidic pH environment, as revealed by AFM images on selected cells obtained  
600 at pH 3 in the absence of SiNH<sub>2</sub> NPs (third column from left in **Figure 5**). Images of certain YeeJ-  
601 decorated cells collected at pH 3 without SiNH<sub>2</sub> NPs (not shown here) indicate the presence of  
602 spheroids with *ca.* 70 nm diameter in the vicinity of their surface. The origin and nature of these  
603 entities remain difficult to establish, *e.g.* they could identify with outer membrane vesicles (OMVs).  
604 The latter may originate from the constitutively expressed YeeJ, which slightly perturbs outer  
605 membrane structure and favours OMVs production (McBroom and Kuehn 2007) and/or acidic pH  
606 condition may induce outer membrane structure modifications and lead to a release of (aggregated)  
607 YeeJ proteins. Altogether, the SiNH<sub>2</sub> NPs-induced changes in cell surface integrity recall those  
608 identified on *E. coli* bacteria (MRE 162 strain) exposed to negatively-charged modified SiO<sub>2</sub> NPs  
609 (diameter < 80 nm) or to positively-charged functionalized SiO<sub>2</sub> NPs (diameter *ca.* 100 nm)  
610 synthesized according to Hartlen protocol (Hartlen et al. 2008; Mathelié-Guinlet et al. 2018).  
611 Qualitatively, the electrokinetic fingerprints of mixed NPs-cells suspensions displayed in **Figure 4** at  
612 pH 7 and 3 well conform to the qualitative presence or absence of marked cell surface alterations  
613 pictured in **Figure 5**. The highly heterogeneous cell surface properties identified by AFM at the single  
614 cell level in the presence of SiNH<sub>2</sub> NPs echo the dispersity of the mobility distributions symptomatic  
615 of the heterogeneous cells response examined at the population scale.

616 Various procedures are reported in the literature to quantify cells surface roughness, and they  
617 differ according to the mode of evaluation of the reference surface, *e.g.* using least-squares,  
618 multinomial or arithmetic mean regression (Chen et al. 1999). Once determined, this reference  
619 surface is subtracted from the initial raw image and the sample surface roughness can then be  
620 estimated on the basis of the distribution in local height variations. These methods however suffer  
621 from the non-unicity of the reference surface whose characteristics inherently depend on factors like  
622 sampling length and sampling location (Chen et al. 1999). It is therefore required to resort to  
623 frequency-based sampling of cells surface heterogeneities, as done within the framework of wavelet  
624 analysis (Chen et al. 1999) or Gaussian filtering (Geusebroek et al. 2003) of images. In this work, a  
625 Gaussian filter with standard deviation  $\sigma_1$  ranging from 0.4 to unity was applied to 400 nm × 400 nm  
626 AFM images of bacteria (**Figure 5**). The resulting images were subsequently subtracted from the  
627 initial AFM images, thus making possible the estimation of the relevant distribution of heights  
628 counted from the corresponding basal cell topography reference. Typical examples are given in  
629 **Figure 6**. The height distributions were then successfully fitted according to a normal distribution law  
630 (centered at the maximal height value) defined by a standard deviation, hereafter denoted as  $\sigma_2$ , that  
631 varies linearly with  $\sigma_1$ . The (dimensionless) slope (denoted as  $\omega$ ) of the obtained  $\sigma_2$  versus  $\sigma_1$  linear  
632 plots reflects the searched cell surface roughness, with a perfectly smooth surface corresponding to  
633  $\omega = 0$ . **Figures S3, S4** and **S5** in Supplementary Material illustrate the different steps of this procedure  
634 we followed for evaluation of cells surface roughness. The  $\omega$  proxies for cell surface roughness, as  
635 derived under the conditions of **Figure 5**, are reported in **Table 4**.

636

637



638

639 **Figure 6: Illustrative spatial mapping (400 nm × 400 nm, 28 pixels × 28 pixels) of sample heights**  
 640 **derived after application of a Gaussian filter (here, standard deviation  $\sigma_1=1$ ) and subtraction from**  
 641 **the corresponding raw images.** Locations of surface areas where roughness analysis was conducted  
 642 are provided in **Figure 5** for illustration (white squares therein). Bacterial surfaces were imaged at  
 643 pH 7 and 3 in the presence of  $10^{-3} \text{ g L}^{-1}$  SiNH<sub>2</sub> NPs (indicated).  $\sigma_2$  corresponds to the standard  
 644 deviation in height distribution successfully fitted according to a normal distribution law. The black  
 645 dotted circles are defined by a radius that is equivalent to that of a single NP, and they are positioned  
 646 at cell surface areas featuring lateral heterogeneities with dimensions reminiscent of NP fingerprints.  
 647 'px' stands for pixel. Lines of blue pixels positioned at the image borders correspond to side effects in  
 648 Gaussian filtration procedure and these pixels were thus not considered for evaluation of  $\sigma_2$ .

649 For the nude (E2152) strain imaged at pH 7 in the absence of NPs,  $\sigma_2$  evaluated at  $\sigma_1=1$  is 0.5 (**Figure**  
 650 **6**). For the YeeJ (E2551) and F-pili (E2302) strains,  $\sigma_2$  reaches larger values (0.8 or 0.9 at  $\sigma_1=1$ ), which  
 651 is in agreement with the presence of peripheral surface appendages and with the so-rendered  
 652 rougher cell walls. The presence of SiNH<sub>2</sub> NPs tends to 'smooth' the surface of YeeJ and F-pili bacteria  
 653 at pH 7 ( $\sigma_2 \sim 0.4-0.6$  at  $\sigma_1=1$ ) and the roughness associated with the presence of the parietal  
 654 structures then becomes less pronounced. Acidic pH environment does not seem to significantly  
 655 affect the roughness of bacterial surface evaluated at pH 7, except for YeeJ cell type. However, the  
 656 presence of SiNH<sub>2</sub> NPs at pH 3 leads to the formation of craters or depressions (-2 nm to 0 nm in  
 657 height) for the nude (E2152) and YeeJ (E2551) strains -which possibly corresponds to NP fingerprints  
 658 (**Figure 6**)- and to surface islands whose size is significantly larger than that observed in the absence  
 659 of NPs. Although distinguishable artefacts remain after application of the Gaussian filter to images of  
 660 F-pili (E2302) at pH 3 in the presence of NPs,  $\sigma_2$  estimated at  $\sigma_1=1$  under such conditions is  
 661 significantly larger than that obtained at pH 3 in the absence of NPs. The above observations are  
 662 based on height mappings collected on a given bacterial cell and discussed at a specific frequency-  
 663 filtration defined by  $\sigma_1=1$ . To provide a hint of the inherent heterogeneities in cell damages caused  
 664 by NPs, we discuss below the  $\omega$ -results obtained from analysis of 3 to 7 distinct cells (**Table 4**). These  
 665 results reflect the outcomes of the image filtration procedure over a larger range of selected  $\sigma_1$   
 666 values (0.4 to 1).

667 Concerning nude (E2152) bacteria,  $\omega$  is of the same order of magnitude at pH 7 and at pH 3 in  
668 the absence of SiNH<sub>2</sub> NPs, which is in line with qualitative observations of **Figure 5**. For the YeeJ  
669 (E2551) strain,  $\omega$  significantly increases from pH 7 (1.23) to pH 3 (1.96), thus highlighting the impact  
670 of an acidic environment on cell surface morphology, as invoked from analysis of **Figure 5**. For F-pili  
671 (E2302) bacteria, acidic pH condition seems to induce a ‘smoothing’ of the cells surface (1.15 instead  
672 of 1.43 at pH 7) but firm conclusions are difficult to derive due to lack of statistics (see reasons in  
673 caption **Table 4**). Furthermore, the presence of SiNH<sub>2</sub> NPs at pH 3 magnifies the roughness of F-pili  
674 (E2302) cells surface estimated in the absence of NPs. Unlike E2302 cells at pH 7,  $\omega$  pertaining to  
675 E2152 and E2551 cells is not significantly impacted by the presence of SiNH<sub>2</sub> NPs. At pH 3,  $\omega$  for  
676 E2152 cells is *ca.* two times higher in the presence of SiNH<sub>2</sub> NPs, which conforms to the disrupted cell  
677 surface featured in **Figure 5**. The pronounced depressions observed all over E2152 cell surfaces  
678 possibly result from contacts with SiNH<sub>2</sub> NPs as their diameter (*ca.* 150 nm) qualitatively compares  
679 with that of SiNH<sub>2</sub> NPs (**Figures 5, 6**). These lesions are the result of an alteration of the bacterial  
680 membrane integrity and potentially to a disruption of the peptidoglycan layer (Mathelié-Guinlet et al.  
681 2018). For the YeeJ (E2551) strain,  $\omega$  at pH 3 is lower in presence of NPs, consistent with the  
682 apparent cell surface flattening observed in **Figures 5 and 6**. This is possibly due to the presence of  
683 the spheroids with *ca.* 70 nm diameter observed at pH 3 in the absence of NPs by AFM for YeeJ  
684 (E2551) strain. These spheroids may correspond to outer membrane vesicles and their presence at  
685 the cell surface as well as their very production mechanism (budding of the outer membrane) likely  
686 result in the formation of membrane defects, reflected by an increase in surface roughness at pH 3 as  
687 compared to pH 7. In turn, such so-rendered fragile membrane could undergo an efficient abrasion  
688 by the NPs, leading to smoothing of the cell envelope. Another scenario can be proposed by  
689 examining the way YeeJ proteins are anchored at the outer membrane. Indeed, YeeJ proteins are  
690 composed of several domains: a LysM domain which is anchored in the peptidoglycan, a  $\beta$ -barrel  
691 domain embedded in the outer membrane and a passenger domain on the exposed C-terminal  
692 region of the protein (Martinez-Gil et al. 2017). The only passenger domain can be cleaved from the  
693 bacterial surface. Upon NPs action, YeeJ could undergo an abrasion of the exposed passenger  
694 domain, leading to a smoothing of the cell envelope, while the LyM and  $\beta$ -barrel domains would  
695 stabilise the cell wall, thereby preventing further damage of the outer membrane (such mechanism  
696 could hold both at pH 3 and 7). Obviously, analysis of such scenario would require further  
697 investigations with use of dedicated micro-spectroscopies. **Table 4** suggests that YeeJ-cells E2551 are  
698 the most affected strain when lowering pH from 7 to 3 in the absence of SiNH<sub>2</sub> NPs. In parallel, **Figure**  
699 **4** evidences that this strain is the one among the three investigated for which the shift in  
700 electrophoretic mobility is the most significant at pH 3 with increasing SiNH<sub>2</sub> NPs concentration.  
701 These observations underpin the pronounced sensitivity of E2551 to external stressors (pH and NPs  
702 conditions) and the propensity of these cells to be strongly affected when these stressors are  
703 combined (cocktail effect).

#### 704 **4. Conclusions**

705 In this study, we show that electrophoresis of mixed suspensions of bacteria and NPs is a suitable  
706 method for screening the impacts of NPs on cell charge density, cell envelope integrity and/or  
707 peripheral structure, which translates into detectable modifications of the electrohydrodynamic cell  
708 fingerprints. The existence of favorable attractive interactions between NPs and cells -and of the  
709 subsequent changes in the physicochemical properties of the cell surfaces following their contacts  
710 and collisions with NPs- can be rapidly ‘read’ from inspection of the cells and NPs mobility  
711 distributions, *e.g.* measured as a function of NPs concentration and pH. In particular, the associated  
712 deformation of the electrokinetic plumes pertaining to cells and NPs can provide information not  
713 only on the critical NPs concentration marking the onset of significant NPs action, but also on

714 possible biological retroaction processes leading to alterations of NPs surface properties. The  
715 demonstration is based on measurements performed on genetically engineered *E. coli* cells defined  
716 by distinct surface phenotypes and on functionalized silica NPs (65 nm radius) whose respective  
717 electrokinetic characterizations are given here in terms of relevant soft surface electrokinetic  
718 concepts instead of meaningless zeta potential parameter. The analysis clearly highlights the role of  
719 electrostatics and of cell surface bio-compounds in mediating cells-NPs interactions and ensuing NPs-  
720 mediated deleterious effects on bio-surface. Electrokinetic data are further qualitatively supported  
721 by AFM cell surface imaging, thereby featuring multiscale heterogeneities in cells response to NPs,  
722 from the cell (*via* AFM) to the population (*via* electrokinetics) organization levels. Altogether, this  
723 study demonstrates the potential of electrokinetics to probe the precursory NPs-cells interactions  
724 that initiate adverse impacts of NPs on living organisms. This work further paves the way for  
725 preliminary electrokinetics-based assessment of NPs toxicity to microorganisms and for rapid pre-  
726 screening of eco-friendly nanoparticulate materials.

## 727 **5. Supplementary material**

728 TEM picture of SiNH<sub>2</sub> NPs (**Figure S1**), AFM peak force error images, taken in air, for F-pili (E2302)  
729 bacteria at pH 7 (**Figure S2**), and illustration of the various steps followed for the evaluation of cells  
730 surface roughness for YeeJ (E2551) strain at pH 7, for nude (E2152) strain at pH 3 in the presence of  
731 SiNH<sub>2</sub> NPs (10<sup>-3</sup> g L<sup>-1</sup>) and for F-pili (E2302) strain at pH 3 in the presence of SiNH<sub>2</sub> NPs (10<sup>-3</sup> g L<sup>-1</sup>)  
732 (**Figures S3, S4 and S5**, respectively) are provided as supplementary material.  
733

## 734 **6. Conflicts of interest**

735 The authors declare no conflicts of interest.

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738

## 739 **References**

- 740 El Badawy, A.M., Silva, R.G., Morris, B., Scheckel, K.G., Suidan, M.T., Tolaymat, T.M., 2011. Surface  
741 charge-dependent toxicity of silver nanoparticles. *Environ. Sci. Technol.* **45**, 283–287.  
742 <https://doi.org/10.1021/es1034188>
- 743 Beaussart, A., Beloin, C., Ghigo, J.-M., Chapot-Chartier, M.-P., Kulakauskas, S., Duval, J.F.L., 2018a.  
744 Probing the influence of cell surface polysaccharides on nanodendrimer binding to Gram-  
745 negative and Gram-positive bacteria using single-nanoparticle force spectroscopy. *Nanoscale*  
746 **10**, 12743–12753. <https://doi.org/10.1039/c8nr01766b>
- 747 Beaussart, A., Caillet, C., Bihannic, I., Zimmermann, R., Duval, J.F.L., 2018b. Remarkable reversal of  
748 electrostatic interaction forces on zwitterionic soft nanointerfaces in a monovalent aqueous  
749 electrolyte: An AFM study at the single nanoparticle level. *Nanoscale* **10**, 3181–3190.  
750 <https://doi.org/10.1039/c7nr07976a>
- 751 Beddoes, C.M., Case, C.P., Briscoe, W.H., 2015. Understanding nanoparticle cellular entry: A  
752 physicochemical perspective. *Adv. Colloid Interface Sci.* **218**, 48–68.  
753 <https://doi.org/10.1016/j.cis.2015.01.007>
- 754 Beloin, C., Roux, A., Ghigo, J., 2010. Escherichia coli biofilms. *Curr Top Microbiol Immunol* **322**, 249–  
755 389. [https://doi.org/10.1007/978-3-540-75418-3\\_12](https://doi.org/10.1007/978-3-540-75418-3_12)
- 756 Beveridge, T.J., Graham, L.L., 1991. Surface layers of bacteria. *Microbiol. Rev.* **55**, 684–705.

- 757 Bolshakova, A. V., Kiselyova, O.I., Yaminsky, I. V., 2004. Microbial surfaces investigated using atomic  
758 force microscopy. *Biotechnol. Prog.* **20**, 1615–1622. <https://doi.org/10.1021/bp049742c>
- 759 Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M.F., Fiévet, F., 2006. Toxicological  
760 impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal  
761 medium. *Nano Lett.* **6**, 866–870. <https://doi.org/10.1021/nl052326h>
- 762 Chai, H., Yao, J., Sun, J., Zhang, C., Liu, W., Zhu, M., Ceccanti, B., 2015. The effect of metal oxide  
763 nanoparticles on functional bacteria and metabolic profiles in agricultural soil. *Bull. Environ.*  
764 *Contam. Toxicol.* **94**, 490–495. <https://doi.org/10.1007/s00128-015-1485-9>
- 765 Chaveroche, M.K., Ghigo, J.M., D'Enfert, C., 2000. A rapid method for efficient gene replacement in  
766 the filamentous fungus *Aspergillus nidulans*. *Nucleic Acids Res.* **28**.  
767 <https://doi.org/10.1093/nar/28.22.e97>
- 768 Chen, Q., Yang, S., Li, Z., 1999. Surface roughness evaluation by using wavelets analysis. *Precis. Eng.*  
769 **23**, 209–212. [https://doi.org/https://doi.org/10.1016/S0141-6359\(99\)00013-6](https://doi.org/https://doi.org/10.1016/S0141-6359(99)00013-6)
- 770 Choi, O., Yu, C.-P., Esteban Fernández, G., Hu, Z., 2010. Interactions of nanosilver with Escherichia coli  
771 cells in planktonic and biofilm cultures. *Water Res.* **44**, 6095–6103.  
772 <https://doi.org/10.1016/j.watres.2010.06.069>
- 773 Colvin, V.L., 2004. The potential environmental impact of engineered nanomaterials. *Nat. Biotechnol.*  
774 **22**, 760–760. <https://doi.org/10.1038/nbt0604-760c>
- 775 Costa, T.R.D., Ilangovan, A., Ukleja, M., Redzej, A., Santini, J.M., Smith, T.K., Egelman, E.H., Waksman,  
776 G., 2016. Structure of the bacterial sex F pilus reveals an assembly of a stoichiometric protein-  
777 phospholipid complex. *Cell* **166**, 1436–1444. <https://doi.org/10.1016/j.cell.2016.08.025>
- 778 Crucho, C.I.C., Baleizão, C., Farinha, J.P.S., 2017. Functional group coverage and conversion  
779 quantification in nanostructured silica by  $^1\text{H}$  NMR. *Anal. Chem.* **89**, 681–687.  
780 <https://doi.org/10.1021/acs.analchem.6b03117>
- 781 Dague, E., Duval, J., Jorand, F., Thomas, F., Gaboriaud, F., 2006. Probing surface structures of  
782 *Shewanella* spp. by microelectrophoresis. *Biophys. J.* **90**, 2612–2621.  
783 <https://doi.org/10.1529/biophysj.105.068205>
- 784 Delgado, A. V., González-Caballero, F., Hunter, R.J., Koopal, L.K., Lyklema, J., 2005. Measurement and  
785 interpretation of electrokinetic phenomena (IUPAC Technical Report). *Pure Appl. Chem.* **77**,  
786 1753–1805. <https://doi.org/10.1351/pac200577101753>
- 787 Diallo, M.S., Christie, S., Swaminathan, P., Balogh, L., Shi, X., Um, W., Papelis, C., Iii, W.A.G., Johnson,  
788 J.H., 2004. Dendritic chelating agents. 1. Cu(II) binding to ethylene diamine core  
789 Poly(amidoamine) dendrimers in aqueous solutions. *Langmuir* **20**, 2640–2651.  
790 <https://doi.org/10.1021/la036108k>
- 791 Dubey, R.V., Maheshwari, D.K., 1999. A textbook of microbiology. S. Chand Publishing.
- 792 Dufrêne, Y.F., 2008. Towards nanomicrobiology using atomic force microscopy. *Nat. Rev. Microbiol.*  
793 **6**, 674–680. <https://doi.org/10.1038/nrmicro1948>
- 794 Duval, J.F.L., 2017. Chemodynamics of metal ion complexation by charged nanoparticles: A  
795 dimensionless rationale for soft, core-shell and hard particle types. *Phys. Chem. Chem. Phys.* **19**,  
796 11802–11815. <https://doi.org/10.1039/c7cp01750b>
- 797 Duval, J.F.L., Farinha, J.P.S., Pinheiro, J.P., 2013. Impact of electrostatics on the chemodynamics of  
798 highly charged metal-polymer nanoparticle complexes. *Langmuir* **29**, 13821–13835.  
799 <https://doi.org/10.1021/la403106m>

- 800 Duval, J.F.L., Gaboriaud, F., 2010. Progress in electrohydrodynamics of soft microbial particle  
801 interphases. *Curr. Opin. Colloid Interface Sci.* **15**, 184–195.  
802 <https://doi.org/10.1016/j.cocis.2009.12.002>
- 803 Duval, J.F.L., Leermakers, F.A.M., Van Leeuwen, H.P., 2004. Electrostatic interactions between double  
804 layers: Influence of surface roughness, regulation, and chemical heterogeneities. *Langmuir* **20**,  
805 5052–5065. <https://doi.org/10.1021/la030404f>
- 806 Duval, J.F.L., Ohshima, H., 2006. Electrophoresis of diffuse soft particles. *Langmuir* **22**, 3533–3546.  
807 <https://doi.org/10.1021/la0528293>
- 808 Flemming, H.C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., Kjelleberg, S., 2016. Biofilms: An  
809 emergent form of bacterial life. *Nat. Rev. Microbiol.* **14**, 563–575.  
810 <https://doi.org/10.1038/nrmicro.2016.94>
- 811 Francius, G., Polyakov, P., Merlin, J., Abe, Y., Ghigo, J.M., Merlin, C., Beloin, C., Duval, J.F.L., 2011.  
812 Bacterial surface appendages strongly impact nanomechanical and electrokinetic properties of  
813 *Escherichia coli* cells subjected to osmotic stress. *PLoS One* **6**: e20066.  
814 <https://doi.org/10.1371/journal.pone.0020066>
- 815 Geusebroek, J., Smeulders, A.W.M., van de Weijer, J., 2003. Fast anisotropic gauss filtering **12**, 938–  
816 943.
- 817 Hardij, J., Cecchet, F., Berquand, A., Gheldof, D., Chatelain, C., Mullier, F., Chatelain, B., Hardij, J.,  
818 Cecchet, F., Berquand, A., Gheldof, D., Chatelain, C., 2013. Characterisation of tissue factor-  
819 bearing extracellular vesicles with AFM: Comparison of air-tapping-mode AFM and liquid Peak  
820 Force AFM. *J. Extracell. Vesicles* **3078**. <https://doi.org/10.3402/jev.v2i0.21045>
- 821 Hartlen, K.D., Athanasopoulos, A.P.T., Kitaev, V., 2008. Facile preparation of highly monodisperse  
822 small silica spheres (15 to >200 nm) suitable for colloidal templating and formation of ordered  
823 arrays. *Langmuir* **24**, 1714–1720. <https://doi.org/10.1021/la7025285>
- 824 Hayden, S.C., Zhao, G., Saha, K., Phillips, R.L., Li, X., Miranda, O.R., Rotello, V.M., El-Sayed, M.A.,  
825 Schmidt-Krey, I., Bunz, U.H.F., 2012. Aggregation and interaction of cationic nanoparticles on  
826 bacterial surfaces. *J. Am. Chem. Soc.* **134**, 6920–6923. <https://doi.org/10.1021/ja301167y>
- 827 Hill, R.J., Saville, D.A., Russel, W.B., 2003. Electrophoresis of spherical polymer-coated colloidal  
828 particles. *J. Colloid Interface Sci.* **258**, 56–74. [https://doi.org/10.1016/S0021-9797\(02\)00043-7](https://doi.org/10.1016/S0021-9797(02)00043-7)
- 829 Huang, Z., Zheng, X., Yan, D., Yin, G., Liao, X., Kang, Y., Yao, Y., Huang, D., Hao, B., 2008. Toxicological  
830 effect of ZnO nanoparticles based on bacteria. *Langmuir* **24**, 4140–4144.  
831 <https://doi.org/10.1021/la7035949>
- 832 Ikuma, K., Decho, A.W., Lau, B.L.T., 2015. When nanoparticles meet biofilms-interactions guiding the  
833 environmental fate and accumulation of nanoparticles. *Front. Microbiol.* **6**, 1–6.  
834 <https://doi.org/10.3389/fmicb.2015.00591>
- 835 Ingale, A.G., Chaudhari, A.N., 2013. Biogenic synthesis of nanoparticles and potential applications : an  
836 eco-friendly approach. *Nanomedicine Nanotechnol.* **4**. <https://doi.org/10.4172/2157-7439.1000165>
- 837
- 838 Jacobson, K.H., Gunsolus, I.L., Kuech, T.R., Troiano, J.M., Melby, E.S., Lohse, S.E., Hu, D., Chrisler,  
839 W.B., Murphy, C.J., Orr, G., Geiger, F.M., Haynes, C.L., Pedersen, J.A., 2015. Lipopolysaccharide  
840 density and structure govern the extent and distance of nanoparticle interaction with actual  
841 and model bacterial outer membranes. *Environ. Sci. Technol.* **49**, 10642–10650.  
842 <https://doi.org/10.1021/acs.est.5b01841>

- 843 Jandt, K.D., 2001. Atomic force microscopy of biomaterials surfaces and interfaces. *Surf. Sci.* **491**,  
844 303–332. [https://doi.org/10.1016/S0039-6028\(01\)01296-1](https://doi.org/10.1016/S0039-6028(01)01296-1)
- 845 Jiang, W., Yang, K., Vachet, R.W., Xing, B., 2010. Interaction between oxide nanoparticles and  
846 biomolecules of the bacterial cell envelope as examined by infrared spectroscopy. *Langmuir* **26**,  
847 18071–18077. <https://doi.org/10.1021/la103738e>
- 848 Ju-Nam, Y., Lead, J.R., 2008. Manufactured nanoparticles: An overview of their chemistry,  
849 interactions and potential environmental implications. *Sci. Total Environ.* **400**, 396–414.  
850 <https://doi.org/10.1016/j.scitotenv.2008.06.042>
- 851 Kimkes, T.E.P., Heinemann, M., 2018. Reassessing the role of the Escherichia coli CpxAR system in  
852 sensing surface contact. *PLoS One* **13**, 1–11. <https://doi.org/10.1371/journal.pone.0207181>
- 853 Kleinstreuer, N.C., Sullivan, K., Allen, D., Edwards, S., Mendrick, D.L., Embry, M., Matheson, J.,  
854 Rowlands, J.C., Munn, S., Maull, E., Casey, W., 2016. Adverse outcome pathways: From research  
855 to regulation scientific workshop report. *Regul. Toxicol. Pharmacol.* **76**, 39–50.  
856 <https://doi.org/10.1016/j.yrtph.2016.01.007>
- 857 Li, N., Zeng, S., He, L., Zhong, W., 2010. Probing nanoparticle-protein interaction by capillary  
858 electrophoresis. *Anal. Chem.* **82**, 7460–7466. <https://doi.org/10.1021/ac101627p>
- 859 van Loosdrecht, M.C.M., Lyklema, J., Norde, W., Zehnder, A.J.B., 1990. Influence of interfaces on  
860 microbial activity. *Microbiol. Rev.* **54**, 75–87.
- 861 López-León, T., Carvalho, E.L.S., Seijo, B., Ortega-Vinuesa, J.L., Bastos-González, D., 2005.  
862 Physicochemical characterization of chitosan nanoparticles: Electrokinetic and stability  
863 behavior. *J. Colloid Interface Sci.* **283**, 344–351. <https://doi.org/10.1016/j.jcis.2004.08.186>
- 864 López-Viota, J., Mandal, S., Delgado, A. V., Toca-Herrera, J.L., Möller, M., Zanuttin, F., Balestrino, M.,  
865 Krol, S., 2009. Electrophoretic characterization of gold nanoparticles functionalized with human  
866 serum albumin (HSA) and creatine. *J. Colloid Interface Sci.* **332**, 215–223.  
867 <https://doi.org/10.1016/j.jcis.2008.11.077>
- 868 Majdalani, N., Heck, M., Stout, V., Gottesman, S., 2005. Role of RcsF in signaling to the Rcs  
869 phosphorelay pathway in Escherichia coli. *J. Bacteriol.* **187**, 6770–6778.  
870 <https://doi.org/10.1128/JB.187.19.6770-6778.2005>
- 871 Malvern Instruments Limited, 2012. A basic guide to particle characterization. *White Pap.* 1–26.  
872 <https://doi.org/10.1021/ac00110a016>
- 873 Martinez-Gil, M., Goh, K.G.K., Rackaityte, E., Sakamoto, C., Audrain, B., Moriel, D.G., Totsika, M.,  
874 Ghigo, J.M., Schembri, M.A., Beloin, C., 2017. YeeJ is an inverse autotransporter from  
875 Escherichia coli that binds to peptidoglycan and promotes biofilm formation. *Sci. Rep.* **7**, 1–16.  
876 <https://doi.org/10.1038/s41598-017-10902-0>
- 877 Mathélié-guinlet, M., 2017. Etude de l'interaction nanoparticules-bactéries : application à  
878 l'élaboration d'un biocapteur. Université de Bordeaux.
- 879 Mathélié-Guinlet, M., Béven, L., Moroté, F., Moynet, D., Grauby-Heywang, C., Gammoudi, I., Delville,  
880 M.H., Cohen-Bouhacina, T., 2017. Probing the threshold of membrane damage and cytotoxicity  
881 effects induced by silica nanoparticles in Escherichia coli bacteria. *Adv. Colloid Interface Sci.* **245**,  
882 81–91. <https://doi.org/10.1016/j.jcis.2017.04.012>
- 883 Mathélié-Guinlet, M., Grauby-Heywang, C., Martin, A., Février, H., Moroté, F., Vilquin, A., Béven, L.,  
884 Delville, M.H., Cohen-Bouhacina, T., 2018. Detrimental impact of silica nanoparticles on the  
885 nanomechanical properties of Escherichia coli, studied by AFM. *J. Colloid Interface Sci.* **529**, 53–

- 886 64. <https://doi.org/10.1016/j.jcis.2018.05.098>
- 887 Maurya, S.K., Gopmandal, P.P., Ohshima, H., 2018. Electrophoresis of concentrated suspension of  
888 soft particles with volumetrically charged inner core. *Colloid Polym. Sci.* **296**, 721–732.  
889 <https://doi.org/10.1007/s00396-018-4292-0>
- 890 McBroom, A.J., Kuehn, M.J., 2007. Release of outer membrane vesicles by Gram-negative bacteria is  
891 a novel envelope stress response. *Mol. Microbiol.* **63**, 545–558. <https://doi.org/10.1111/j.1365-2958.2006.05522.x>  
892
- 893 Mecke, A., Majoros, I.J., Patri, A.K., Baker, J.R., Banaszak Holl, M.M., Orr, B.G., 2005. Lipid bilayer  
894 disruption by polycationic polymers: The roles of size and chemical functional group. *Langmuir*  
895 **21**, 10348–10354. <https://doi.org/10.1021/la050629l>
- 896 Monopoli, M.P., Åberg, C., Salvati, A., Dawson, K.A., 2012. Biomolecular coronas provide the  
897 biological identity of nanosized materials. *Nat. Nanotechnol.* **7**, 779–786.  
898 <https://doi.org/10.1038/nnano.2012.207>
- 899 Moore, M.N., 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic  
900 environment? *Environ. Int.* **32**, 967–976. <https://doi.org/10.1016/j.envint.2006.06.014>
- 901 Moussa, M., Caillet, C., Town, R.M., Duval, J.F.L., 2015. Remarkable electrokinetic features of charge-  
902 stratified soft nanoparticles: Mobility reversal in monovalent aqueous electrolyte. *Langmuir* **31**,  
903 5656–5666. <https://doi.org/10.1021/acs.langmuir.5b01241>
- 904 Neal, A.L., 2008. What can be inferred from bacterium-nanoparticle interactions about the potential  
905 consequences of environmental exposure to nanoparticles? *Ecotoxicology* **17**, 362–371.  
906 <https://doi.org/10.1007/s10646-008-0217-x>
- 907 Ohshima, H., 1995. Electrophoresis of soft particles. *Adv. Colloid Interface Sci.* **62**, 189–235.  
908 [https://doi.org/10.1016/0001-8686\(95\)00279-Y](https://doi.org/10.1016/0001-8686(95)00279-Y)
- 909 Pagnout, C., Jomini, S., Dadhwal, M., Caillet, C., Thomas, F., Bauda, P., 2012. Role of electrostatic  
910 interactions in the toxicity of titanium dioxide nanoparticles toward *Escherichia coli*. *Colloids*  
911 *Surfaces B Biointerfaces* **92**, 315–321. <https://doi.org/10.1016/j.colsurfb.2011.12.012>
- 912 Peulen, T.O., Wilkinson, K.J., 2011. Diffusion of nanoparticles in a biofilm. *Environ. Sci. Technol.* **45**,  
913 3367–3373. <https://doi.org/10.1021/es103450g>
- 914 Phillips, R.L., Miranda, O.R., You, C.C., Rotello, V.M., Bunz, U.H.F., 2008. Rapid and efficient  
915 identification of bacteria using gold-nanoparticle-poly(para-phenyleneethynylene) constructs.  
916 *Angew. Chemie - Int. Ed.* **47**, 2590–2594. <https://doi.org/10.1002/anie.200703369>
- 917 Planchon, M., Ferrari, R., Guyot, F., Gélabert, A., Menguy, N., Chanéac, C., Thill, A., Benedetti, M.F.,  
918 Spalla, O., 2013. Interaction between *Escherichia coli* and TiO<sub>2</sub> nanoparticles in natural and  
919 artificial waters. *Colloids Surfaces B Biointerfaces* **102**, 158–164.  
920 <https://doi.org/10.1016/j.colsurfb.2012.08.034>
- 921 Reisner, A., Haagensen, J.A.J., Schembri, M.A., Zechner, E.L., Molin, S., 2003. Development and  
922 maturation of *Escherichia coli* K-12 biofilms. *Mol. Microbiol.* **48**, 933–946. <https://doi.org/10.1046/j.1365-3113.2003.02900.x>  
923 [pii]
- 924 Ribeiro, T., Baleizão, C., Farinha, J.P.S., 2017. Artefact-free evaluation of metal enhanced  
925 fluorescence in silica coated gold nanoparticles. *Sci. Rep.* **7**, 1–12.  
926 <https://doi.org/10.1038/s41598-017-02678-0>
- 927 Ribeiro, T., Raja, S., Rodrigues, A.S., Fernandes, F., Baleizão, C., Farinha, J.P.S., 2014. NIR and visible  
928 perylene diimide-silica nanoparticles for laser scanning bioimaging. *Dye. Pigment.* **110**, 227–234.



- 929 <https://doi.org/10.1016/j.dyepig.2014.03.026>
- 930 Rowenczyk, L., Duclairoir-poc, C., Barreau, M., Picard, C., Hucher, N., Orange, N., Grisel, M.,  
931 Feuilloley, M., 2017. Impact of coated TiO<sub>2</sub>-nanoparticles used in sunscreens on two  
932 representative strains of the human microbiota : Effect of the particle surface nature and aging.  
933 *Colloids Surfaces B Biointerfaces* **158**, 339–348. <https://doi.org/10.1016/j.colsurfb.2017.07.013>
- 934 Shang, L., Nienhaus, K., Nienhaus, G.U., 2014. Engineered nanoparticles interacting with cells: Size  
935 matters. *J. Nanobiotechnology* **12**, 1–11. <https://doi.org/10.1186/1477-3155-12-5>
- 936 Sheng, G.P., Yu, H.Q., Yue, Z.B., 2005. Production of extracellular polymeric substances from  
937 *Rhodospseudomonas acidophila* in the presence of toxic substances. *Appl. Microbiol. Biotechnol.*  
938 **69**, 216–222. <https://doi.org/10.1007/s00253-005-1990-6>
- 939 Shrivastava, S., Bera, T., Roy, A., 2007. Characterization of enhanced antibacterial effects of novel  
940 silver nanoparticles. *Nanotechnology* **18**. <https://doi.org/10.1088/0957-4484/18/22/225103>
- 941 Silva, T., Pokhrel, L.R., Dubey, B., Tolaymat, T.M., Maier, K.J., Liu, X., 2014. Particle size, surface  
942 charge and concentration dependent ecotoxicity of three organo-coated silver nanoparticles:  
943 Comparison between general linear model-predicted and observed toxicity. *Sci. Total Environ.*  
944 **468–469**, 968–976. <https://doi.org/10.1016/j.scitotenv.2013.09.006>
- 945 Silverman, P.M., 1997. Towards a structural biology of bacterial conjugation. *Mol. Microbiol.* **23**, 423–  
946 429. <https://doi.org/10.1046/j.1365-2958.1997.2411604.x>
- 947 Škvarla, J., 2007. Hard versus soft particle electrokinetics of silica colloids. *Langmuir* **23**, 5305–5314.  
948 <https://doi.org/10.1021/la0635451>
- 949 Stöber, W., Fink, A., Bohn, E., 1968. Controlled growth of monodisperse silica spheres in the micron  
950 size range. *J. Colloid Interface Sci.* **26**, 62–69. [https://doi.org/10.1016/0021-9797\(68\)90272-5](https://doi.org/10.1016/0021-9797(68)90272-5)
- 951 Stoimenov, P.K., Klinger, R.L., Marchin, G.L., Klabunde, K.J., 2002. Metal oxide nanoparticles as  
952 bactericidal agents. *Langmuir* **18**, 6679–6686. <https://doi.org/10.1021/la0202374>
- 953 Svenson, S., 2009. Dendrimers as versatile platform in drug delivery applications. *Eur. J. Pharm.*  
954 *Biopharm.* **71**, 445–462. <https://doi.org/10.1016/j.ejpb.2008.09.023>
- 955 Tajarobi, F., El-Sayed, M., Rege, B.D., Polli, J.E., Ghandehari, H., 2001. Transport of poly amidoamine  
956 dendrimers across Madin-Darby canine kidney cells. *Int. J. Pharm.* **215**, 263–267.  
957 [https://doi.org/10.1016/S0378-5173\(00\)00679-7](https://doi.org/10.1016/S0378-5173(00)00679-7)
- 958 Thill, A., Zeyons, O., Spalla, O., Chauvat, F., Rose, J., Auffan, M., Flank, A.M., 2006. Cytotoxicity of  
959 CeO<sub>2</sub> nanoparticles for *Escherichia coli*. Physico-chemical insight of the cytotoxicity mechanism.  
960 *Environ. Sci. Technol.* **40**, 6151–6156. <https://doi.org/10.1021/es060999b>
- 961 Velzeboer, I., Hendriks, A.J., Ragas, M.J., De Meent, D., 2008. Nanomaterials in the environment  
962 aquatic ecotoxicity tests of some nanomaterials. *Environ. Toxicol. Chem.* **27**, 1942–1947.  
963 <https://doi.org/10.1897/07-509.1>
- 964 Van Der Wal, A., Norde, W., Zehnder, A.J.B., Lyklema, J., 1997. Determination of the total charge in  
965 the cell walls of Gram-positive bacteria. *Colloids Surfaces B Biointerfaces* **9**, 81–100.  
966 [https://doi.org/10.1016/S0927-7765\(96\)01340-9](https://doi.org/10.1016/S0927-7765(96)01340-9)
- 967 Wang, Y.A., Yu, X., Silverman, P.M., Harris, R.L., Egelman, E.H., 2009. The structure of F-pili. *J. Mol.*  
968 *Biol.* **385**, 22–29. <https://doi.org/10.1016/j.jmb.2008.10.054>
- 969 Yang, Y., Quensen, J., Mathieu, J., Wang, Q., Wang, J., Li, M., Tiedje, J.M., Alvarez, P.J.J., 2013.  
970 Pyrosequencing reveals higher impact of silver nanoparticles than Ag<sup>+</sup> on the microbial

971 community structure of activated sludge. *Water Res.* **48**, 317–325.  
972 <https://doi.org/10.1016/j.watres.2013.09.046>

973 Zeyons, O., Thill, A., Chauvat, F., Menguy, N., Cassier-Chauvat, C., Oréar, C., Daraspe, J., Auffan, M.,  
974 Rose, J., Spalla, O., 2009. Direct and indirect CeO<sub>2</sub> nanoparticles toxicity for *Escherichia coli* and  
975 *Synechocystis*. *Nanotoxicology* **3**, 284–295. <https://doi.org/10.3109/17435390903305260>

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978 **Tables**979 **Table 1: Characteristics of the bacteria adopted in this work.**

Strains	Expressed surface appendages	Genotype	Antibiotic resistance	Antibiotics for culture	Reference
E2152 (nude)	no flagella, no YeeJ protein, no Ag43, no type 1 fimbriae, no F factor	MG1655_gfp_Δfli_Δflu::km_ΔfimA-H::zeo	Amp <sup>R</sup> , Cm <sup>R</sup> , Km <sup>R</sup> , Zeo <sup>R</sup>	Ampicillin (Amp): 100 μg mL <sup>-1</sup> Kanamycin (Km): 50 μg mL <sup>-1</sup>	(Francius et al. 2011)
E2551 (YeeJ)	no flagella, no Ag43, no type 1 fimbriae, no F factor, constitutive expression of YeeJ	MG1655_gfp_Δfli_Δflu_ΔfimA-H::zeo_kmPcLYeeJ	Amp <sup>R</sup> , Cm <sup>R</sup> , Km <sup>R</sup> , Zeo <sup>R</sup>	Ampicillin: 100 μg mL <sup>-1</sup> Kanamycin: 50 μg mL <sup>-1</sup>	(Martinez-Gil et al. 2017)
E2302 (F+)	no flagella, no Ag43, no type 1 fimbriae, no YeeJ protein, constitutive expression of F-pili	MG1655_gfp_Δfli_Δflu::km_ΔfimA-H::zeo_F'tet	Amp <sup>R</sup> , Cm <sup>R</sup> , Km <sup>R</sup> , Zeo <sup>R</sup> , Tet <sup>R</sup>	Ampicillin: 100 μg mL <sup>-1</sup> Tetracyclin (Tet): 50 μg mL <sup>-1</sup>	(Francius et al. 2011)

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981 **Table 2: Values of charge density  $\rho_0$  (in mM) and hydrodynamic penetration length  $1/\lambda_0$  (in nm)**  
 982 obtained from eqs 1-4 adopted to recover the electrokinetic data given in Figure 1.  $\rho_0$  is expressed in  
 983 molar concentration of equivalent monovalent cationic or anionic charges (depending on pH), *i.e.* the  
 984 ratio between charge density (in C m<sup>-3</sup>) and Faraday number.

SiNH <sub>2</sub> NPs	pH 3 and 4	pH 5	pH 6	pH 7	pH 9
$\rho_0$ (mM)	105	1.3	-17.7	-50.5	-73.7
$1/\lambda_0$ (nm)	0.74	1.07	1.00	1.11	1.30

985

986 **Table 3: Values of charge density  $\rho_0$  and hydrodynamic penetration length  $1/\lambda_0$  at different pH**  
 987 values for the nude (E2152), YeeJ (E2551) and F-pili (E2302) strains obtained from Ohshima's model  
 988 (eqs 1-4).  $\rho_0$  is expressed in molar concentration of equivalent monovalent anionic charges, *i.e.* from  
 989 the ratio between charge density (in C m<sup>-3</sup>) and Faraday number.

	Nude (E2152)		YeeJ (E2551)		F-pili (E2302)	
	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7
$\rho_0$ (mM)	-57.5	-195	-53.7	-92.5	-64.6	-101

$1/\lambda_0$ (nm)	0.91	0.51	0.85	0.88	0.85	0.83
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991 **Table 4: Values of the slopes  $\omega$  corresponding to the linear plots  $\sigma_2$  vs.  $\sigma_1$**  for nude (E2152), YeeJ  
 992 (E2551) and F-pili (E2302) cells at pH 7 and pH 3 in the absence or presence of SiNH<sub>2</sub> NPs (indicated).  
 993 Data correspond to mean values  $\pm$  standard deviation as obtained from analysis of 3 to 7 cells per  
 994 examined condition. Data given at pH 3, without specification of standard deviation, correspond to  
 995 the analysis of 1 F-pili cell as we encountered significant difficulties in imaging more of these cells  
 996 under such pH condition, which is possibly due to AFM tip contamination by NPs and/or detached F-  
 997 pili structures during sample scanning (**Figure S2**).

	pH 7	SiNH <sub>2</sub> NPs pH 7	pH 3	SiNH <sub>2</sub> NPs pH 3
Nude (E2152)	0.64 $\pm$ 0.13	0.79 $\pm$ 0.07	0.61 $\pm$ 0.07	1.26 $\pm$ 0.50
YeeJ (E2551)	1.23 $\pm$ 0.17	1.01 $\pm$ 0.14	1.96 $\pm$ 0.30	1.29 $\pm$ 0.80
F-pili (E2302)	1.43 $\pm$ 0.15	0.85 $\pm$ 0.18	1.15	1.73

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