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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 -NH₂ terminal groups. At pH 7, electrostatics prevents interactions between bacteria and SiNH₂ NPs, regardless of the NPs concentration considered (0-10⁻² g L⁻¹ 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 -NH₂ 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 λ red recombination (Chaverocche 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 μ m to 100 μ 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⁻¹ KH₂PO₄ (Sigma-Aldrich[®]), 2 g L⁻¹ (NH₄)₂SO₄ (Emsure[®]), 0.1 g L⁻¹ MgSO₄·7H₂O
168 (Emsure[®]), 5·10⁻⁴ g L⁻¹ FeSO₄·7H₂O (Sigma-Aldrich[®]), 1·10⁻³ g L⁻¹ thiamine (Roth[®]) and 4 g L⁻¹ 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 OD₆₀₀
171 of 0.05, and finally cultured at 37°C, 160 rpm until an OD₆₀₀ 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 KNO₃ (Sigma-Aldrich[®], purity > 99%) so as to reach an OD₆₀₀
175 of 0.2. Then, batches of cells suspended in 1 mM to 250 mM KNO₃ final electrolyte concentration
176 were prepared in such a way that a final OD₆₀₀ of 0.02 was reached. For that purpose, appropriate
177 volumes of 2M KNO₃ electrolyte, of ultrapure water (milli-Q[®] water) and of the aforementioned cells
178 suspensions (OD₆₀₀=0.2, 10 mM KNO₃) 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 HNO₃
180 and KOH (10⁻²-10⁻¹ 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 KNO₃ (Sigma-Aldrich®, purity > 99%) so as to reach an OD₆₀₀
186 of 0.4. Appropriate volumes of (i) separately prepared 1 mM KNO₃ NPs suspension at 10⁻¹ g L⁻¹ (pH 3
187 or 7) obtained after 20 min sonication, of (ii) bacteria suspension (OD₆₀₀=0.4, 1 mM KNO₃) and of (iii)
188 1 mM KNO₃ 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⁻¹-
190 10⁻² g L⁻¹, and an OD₆₀₀ 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 SiNH₂ NPs. Experiments were carried out twice on freshly prepared samples. Under the conditions
195 applicable in **Figure 4**, the OD_{600nm} is 0.02 and the maximum NPs concentration tested is 10⁻² 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 NH₂ surface groups per nm², 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 SiNH₂ NPs were dispersed in 0.1 mM or 1
208 mM KNO₃ electrolyte to obtain a final 0.1 g L⁻¹ NPs concentration. For that purpose, the appropriate
209 weight of NPs was added to half of the desired volume of KNO₃ solution under 1000 rpm agitation
210 conditions for 1 min, and the volume was filled with 0.1 mM or 1 mM KNO₃ 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 (μ) 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 μ m (size measurements) and 5 nm to 10 μ m (electrophoretic mobility
219 measurements), and the latter for larger systems with size in the range 0.1 μ m to 50 μ 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 SiNH₂ NPs was measured at solution pH 3 and 7 as a function of KNO₃
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 KNO₃ salt and pH adjustment using HNO₃ (10⁻¹-10⁻²M Fluka®) or
232 KOH (10⁻¹-10⁻²M, Fluka®).

233 **Electrophoretic mobility measurements**

234 The electrophoretic mobility of SiNH₂ NPs was measured as a function of pH (3 to 9) and KNO₃ 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⁻¹) 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⁻¹), 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 KNO₃ 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⁻¹, 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⁻¹ 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⁻¹ PEI solution (Sigma-Aldrich®, 7.5 10⁶ Mw, 50 wt. % in H₂O) for 30 min and finally rinsed with
263 milli-Q® water. 1.4 mL of bacterial suspension (of pH 3 or 7, and OD₆₀₀ = 0.4) was deposited onto the
264 PEI-coated slides. After 30 min, the sample was extensively rinsed with 1 mM KNO₃ 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 KNO₃ solution at the sample pH (3 or 7). For AFM imaging of binary systems, bacteria
267 suspensions (OD₆₀₀ = 0.4) were first mixed with 1 mM KNO₃ NPs solution of final concentration 10⁻³
268 g L⁻¹ 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 KNO₃ solution, pH 3 or 7. For each tested condition
271 (pH 3 or 7, presence/absence of SiNH₂ 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.

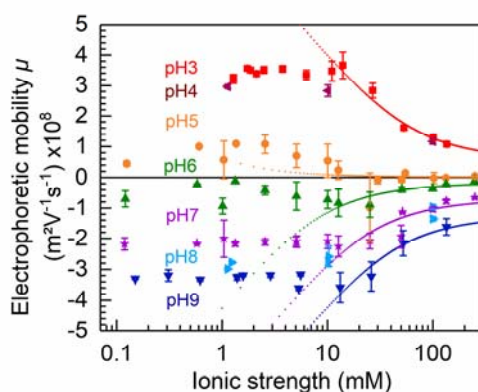
275

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 SiNH₂ NPs, we discuss below the electrophoretic mobility properties of SiNH₂
279 NPs and of the adopted *E. coli* strains, taken separately.

280 3.1 Electrokinetics of SiNH₂ NPs and of bacteria

281 The dependence of the electrophoretic mobility μ of SiNH₂ NPs on KNO₃ electrolyte concentration
282 and pH is reported in **Figure 1**.



283

284 **Figure 1: Electrophoretic mobility of SiNH₂ NPs as a function of pH (indicated) and solution ionic**
285 **strength** (changed upon modification of KNO₃ 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 SiNH₂ 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 μ of SiNH₂ NPs is negative over the whole range of tested KNO₃ concentrations at pH>6
291 and it is positive for pH values lower than 5-6. The underlying reversal of SiNH₂ 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 NH₂ surface groups per
295 nm²), 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 -NH₂ 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 -NH₂
301 groups under such pH conditions. The comparison between $|\mu|$ reached at pH 9 and pH 3 in *e.g.*
302 10 mM KNO₃ suggests that the NPs surface concentration of -NH₂ 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 μ at such low KNO_3 concentrations (here, below 10 mM): (i) double layer polarization
 308 effects, that tend to reduce the dependence of μ 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 μ 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 SiNH_2 NPs to 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 μ 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 ρ_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. η and ε are the dynamic viscosity
 327 and dielectric permittivity of the medium, respectively, κ_m the reciprocal Debye layer thickness in the
 328 soft surface layer, ψ^0 the surface potential located at the edge of the soft layer, and ψ^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 ψ^D , ψ^0 and κ_m all depend on charge density ρ_0 and
 336 electrolyte concentration c^∞ (1:1 symmetrical electrolyte is considered here) according to (Ohshima
 337 1995)

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

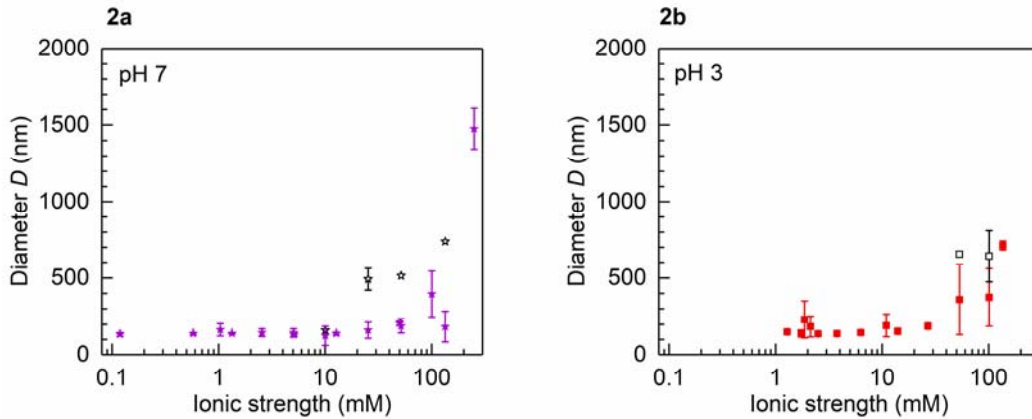
$$339 \quad \psi^0 = \psi^D - \frac{RT}{F} \tanh \left(\frac{F\psi^D}{2RT} \right) \quad (3)$$

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

341 , where R is the gas constant, T the absolute temperature, F the Faraday number and κ the salinity-
 342 dependent reciprocal Debye layer thickness. **Equations 1-4** successfully fitted the experimental data
 343 above 10 mM KNO_3 concentration in **Figure 1** upon adjustment of ρ_0 and $1/\lambda_0$ *via* Levenberg-
 344 Marquardt procedure. Results are given in **Table 2** for the various tested pH conditions with ρ_0
 345 expressed in molar concentration of equivalent monovalent charges. In agreement with conclusions
 346 from previous work (Škvarla 2007), 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 -NH₂ surface groups. In detail, the hydrodynamic softness of SiNH₂ 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 SiNH₂ NPs behave as soft particles. Ohshima's model overestimates the magnitude
359 of μ for all pH conditions at KNO₃ 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 SiNH₂ 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 SiNH₂ NPs are probably less significant than for latex NPs, which qualitatively
368 agrees with the ρ_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⁻ or -NH₃⁺ ionogenic
372 surface groups, a feature evidenced for nanoparticulate dendrimers (Moussa et al. 2015). Following
373 these conclusions, and exploiting the unexpected reversal in SiNH₂ NPs mobility at pH 5, the pH 7 and
374 pH 3 conditions were selected for deciphering below the impacts of SiNH₂ NPs on cell electrokinetic
375 properties (§3.2).

376 In order to ascertain that suspensions of SiNH₂ 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**), SiNH₂ 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 SiNH₂ 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 SiNH₂ 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).



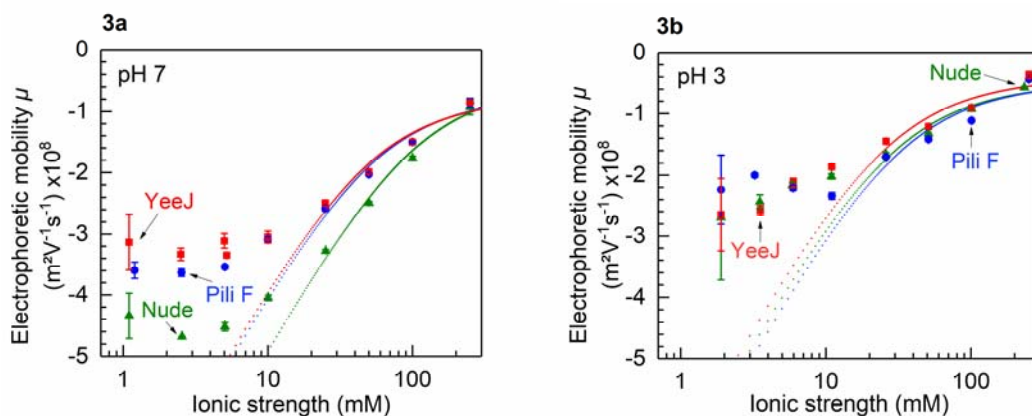
394

395 **Figure 2: SiNH₂ 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 KNO₃ electrolyte.

400 As our intention is to assess the electrokinetic cell features (in mixed cell-SiNH₂ NPs suspensions)
 401 as a function of NPs concentrations in solution, we further identified the range of SiNH₂ NPs
 402 concentration in line with constant SiNH₂ 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 SiNH₂ NPs at pH 3 (pH 7, respectively) in 1 mM solution
 405 ionic strength is $\mu=2.1\pm0.6\times10^{-8}$ m²V⁻¹s⁻¹ at 10⁻³ g L⁻¹ ($\mu=-1.8\pm0.1\times10^{-8}$ m²V⁻¹s⁻¹, respectively) and
 406 $\mu=2.5\pm0.5\times10^{-8}$ m²V⁻¹s⁻¹ at 10⁻² g L⁻¹ ($\mu=-2.0\pm0.4\times10^{-8}$ m²V⁻¹s⁻¹, respectively), as obtained with
 407 Zetaphoremeter IV equipment (CAD Instrumentations®). Measurements with Zetasizer NanoZS
 408 (Malvern) lead at pH 3 (pH 7, respectively) to $\mu=2.4\pm0.6\times10^{-8}$ m²V⁻¹s⁻¹ at 10⁻² g L⁻¹ ($\mu=-1.9\pm0.7\times10^{-8}$
 409 m²V⁻¹s⁻¹, respectively) and $\mu=3.0\pm0.7\times10^{-8}$ m²V⁻¹s⁻¹ at 10⁻¹ g L⁻¹ ($\mu=-1.7\pm0.9\times10^{-8}$ m²V⁻¹s⁻¹, respectively).
 410 At fixed pH (3 or 7), within experimental error μ values therefore remain constant in the 10⁻³ g L⁻¹ (or
 411 lower) to 10⁻² g L⁻¹ 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, μ 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 ρ_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 ρ_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 KNO_3 electrolyte.

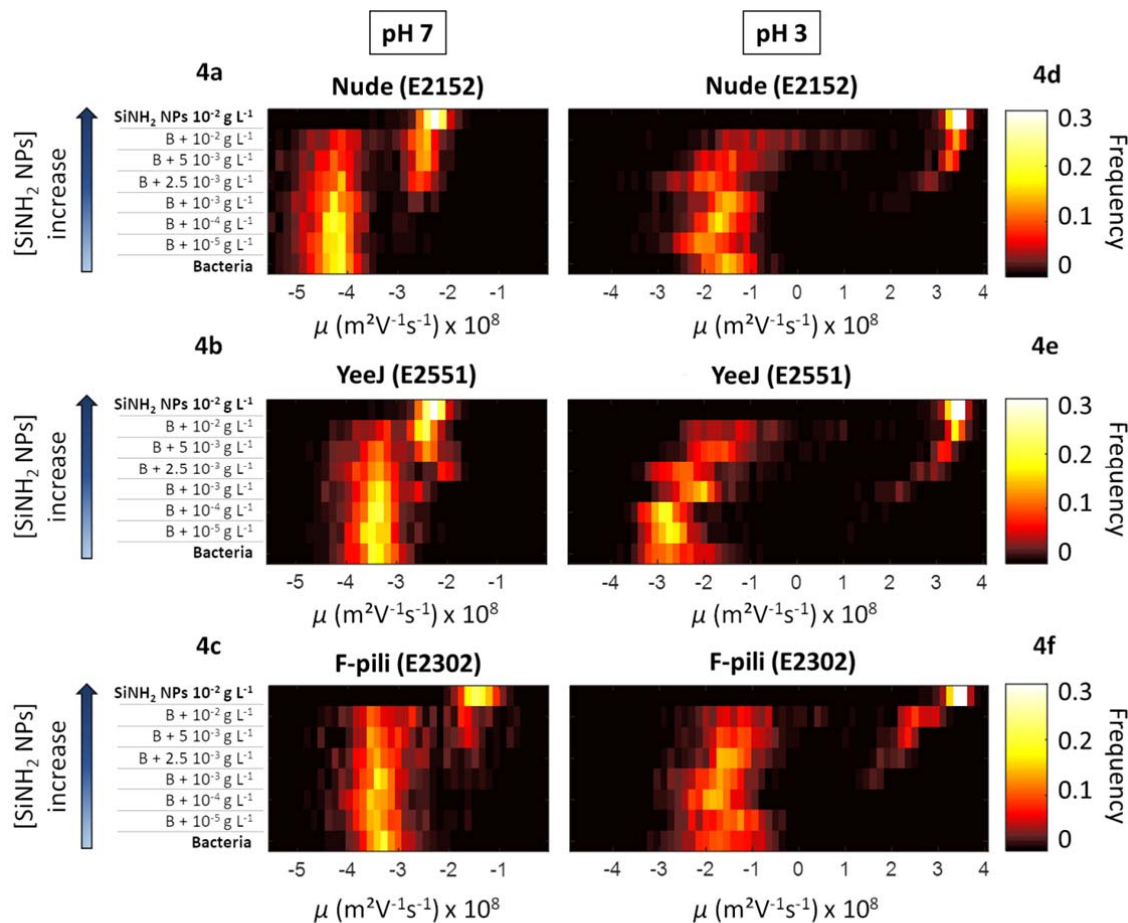
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453 3.2 Electrokinetics of mixed bacteria-NPs suspensions

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 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 SiNH_2 NPs and bacteria, respectively. The electrophoretic fingerprints of the cells and
 461 those of the 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 μ (the width of each class is set here to 0.15×10^{-8}
464 $\text{m}^2\text{V}^{-1}\text{s}^{-1}$ increment in μ) corresponds to the ratio between the number of particle trajectories
465 detected at these selected μ 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* μ (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 -4×10^{-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 -3×10^{-8} $\text{m}^2\text{V}^{-1}\text{s}^{-1}$ (E2551, YeeJ) and from -4 to -2.5×10^{-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 SiNH_2 NPs concentration up to 10^{-3} 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} g L^{-1} to 10^{-2} g L^{-1} . The most important conclusion at this stage
484 is the absence of deformation of the cells-electrokinetic plume with increasing SiNH_2 NPs, which
485 suggests that the electrohydrodynamic properties of E2152 and E2551 cells remain basically
486 unaffected in the presence of 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 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 SiNH_2 NPs from 0 to 10^{-2} 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-SiNH₂ NPs suspensions at pH 7 and pH 3**
 501 **(indicated) as a function of SiNH₂ 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 SiNH₂ 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 SiNH₂ 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 SiNH₂ 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

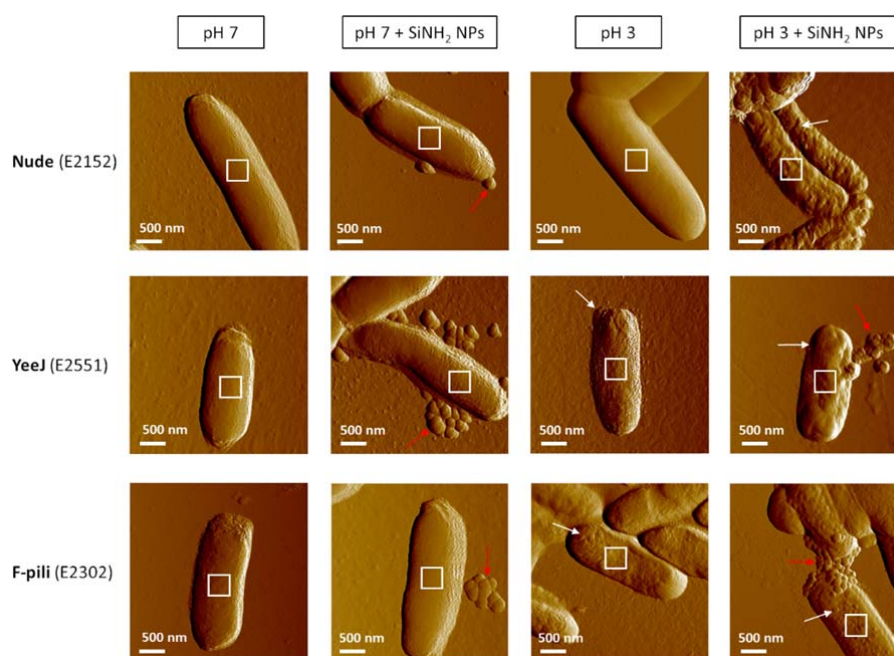
520 removal of charged bio-residues originally anchored at the cell membrane (Shrivastava et al. 2007).
521 As a result, the effective charge density of the so-modified cell envelope probed by electrokinetics
522 becomes less negative, in line with the cells-plume features given in **Figures 4e**. In 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 SiNH₂ 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 SiNH₂
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 SiNH₂ NPs electrokinetic plume. Obviously, the higher the SiNH₂
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 SiNH₂ 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/SiNH₂ NPs electrokinetic diagram
541 at pH 3 are also observed for the mixed E2152/SiNH₂ NPs at this pH (**Figure 4d**). However, the
542 distortions of the SiNH₂ NPs- and E2152-plumes take place at larger NPs concentrations, *i.e.* 2.5×10^{-3}
543 g L^{-1} and 10^{-2} g L^{-1} , respectively, instead of 10^{-3} 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 SiNH₂ NPs. In both scenarios, YeeJ proteins promote contacts between cells
549 and SiNH₂ NPs and, therewith, NPs-mediated modifications of the overall cell electro-surface
550 characteristics. Finally, it is found that the situation for E2302/SiNH₂ NPs (**Figure 4f**) is intermediate
551 between that for E2551/SiNH₂ NPs and E2152/SiNH₂ 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 SiNH₂ NPs signal in the presence of E2302 cells is
554 qualitatively more similar to that of SiNH₂ NPs in presence of E2551 cells (**Figure 4e**). Further
555 comparison between **Figure 4e** and **Figure 4f** reveals that modifications of the SiNH₂ NPs surface
556 properties with increasing SiNH₂ 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 SiNH₂ 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 SiNH₂ NPs at
578 pH 7 and pH 3.



579

580 **Figure 5: AFM peak force error images (3.5 μm × 3.5 μm, 256 pixels × 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 × 400 nm spatial regions where cell surface roughness
584 features were evaluated.

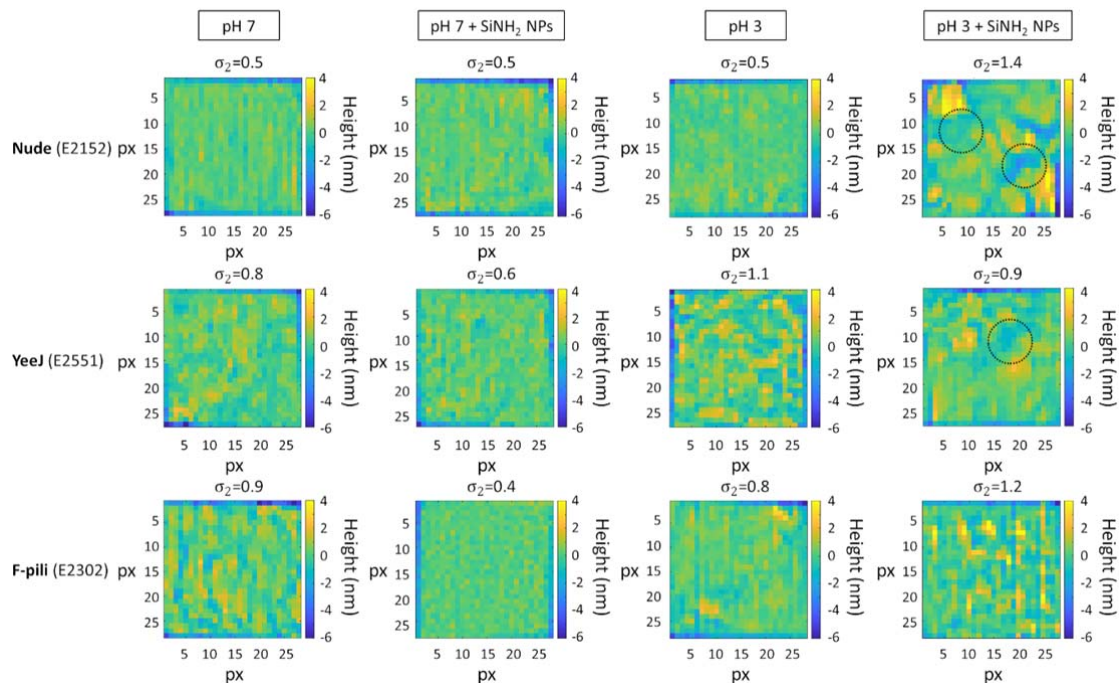
585 The AFM cell pictures obtained in the absence of SiNH₂ NPs at pH 7 (left column, **Figure 5**) display
586 normal bacterial morphologies with aspects that agree well with those detailed in previous reports
587 (Beloin et al. 2010; Francius et al. 2011). In particular, identification of YeeJ and pili 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 SiNH₂ NPs (2nd
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 SiNH₂ 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 SiNH₂ 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 SiNH₂ 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 SiNH₂ NPs (third column from left in **Figure 5**). Images of certain YeeJ-
601 decorated cells collected at pH 3 without SiNH₂ 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 SiNH₂ NPs-induced changes in cell surface integrity recall those
608 identified on *E. coli* bacteria (MRE 162 strain) exposed to negatively-charged modified SiO₂ NPs
609 (diameter < 80 nm) or to positively-charged functionalized SiO₂ 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 SiNH₂ 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 σ_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 σ_2 , that
631 varies linearly with σ_1 . The (dimensionless) slope (denoted as ω) of the obtained σ_2 versus σ_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 ω 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} g L^{-1} SiNH₂ NPs (indicated). σ_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 σ_2 .

649 For the nude (E2152) strain imaged at pH 7 in the absence of NPs, σ_2 evaluated at $\sigma_1=1$ is 0.5 (**Figure**
 650 **6**). For the YeeJ (E2551) and F-pili (E2302) strains, σ_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 SiNH₂ 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 SiNH₂ 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, σ_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 ω -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 σ_1
 666 values (0.4 to 1).

667 Concerning nude (E2152) bacteria, ω is of the same order of magnitude at pH 7 and at pH 3 in
668 the absence of SiNH₂ NPs, which is in line with qualitative observations of **Figure 5**. For the YeeJ
669 (E2551) strain, ω 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 SiNH₂ 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, ω pertaining to
675 E2152 and E2551 cells is not significantly impacted by the presence of SiNH₂ NPs. At pH 3, ω for
676 E2152 cells is *ca.* two times higher in the presence of SiNH₂ 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 SiNH₂ NPs as their diameter (*ca.* 150 nm) qualitatively compares
679 with that of SiNH₂ 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, ω 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 β -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 β -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 SiNH₂ 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 SiNH₂ 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 SiNH₂ 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 SiNH₂ NPs (10⁻³ g L⁻¹) and for F-pili (E2302) strain at pH 3 in the presence of SiNH₂ NPs (10⁻³ g L⁻¹)
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**

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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 ^R , Cm ^R , Km ^R , Zeo ^R	Ampicillin (Amp): 100 μg mL ⁻¹ Kanamycin (Km): 50 μg mL ⁻¹	(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 ^R , Cm ^R , Km ^R , Zeo ^R	Ampicillin: 100 μg mL ⁻¹ Kanamycin: 50 μg mL ⁻¹	(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 ^t tet	Amp ^R , Cm ^R , Km ^R , Zeo ^R , Tet ^R	Ampicillin: 100 μg mL ⁻¹ Tetracyclin (Tet): 50 μg mL ⁻¹	(Francius et al. 2011)

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981 **Table 2: Values of charge density ρ_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. ρ_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⁻³) and Faraday number.

SiNH ₂ NPs	pH 3 and 4	pH 5	pH 6	pH 7	pH 9
ρ_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 ρ_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). ρ_0 is expressed in molar concentration of equivalent monovalent anionic charges, *i.e.* from
 989 the ratio between charge density (in C m⁻³) and Faraday number.

	Nude (E2152)		YeeJ (E2551)		F-pili (E2302)	
	pH 3	pH 7	pH 3	pH 7	pH 3	pH 7
ρ_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 ω corresponding to the linear plots σ_2 vs. σ_1** for nude (E2152), YeeJ
 992 (E2551) and F-pili (E2302) cells at pH 7 and pH 3 in the absence or presence of SiNH₂ 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 ₂ NPs pH 7	pH 3	SiNH ₂ 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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