

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

Elise Vouriot, Isabelle Bihannic, Audrey Beaussart, Yves Waldvogel, Angelina Razafitianamaharavo, Tânia Ribeiro, José Paulo S. Farinha, Christophe Beloin, Jérôme F.L Duval

# ▶ To cite this version:

Elise Vouriot, Isabelle Bihannic, Audrey Beaussart, Yves Waldvogel, Angelina Razafitianamaharavo, et al.. Electrophoresis as a simple method to detect deleterious actions of engineered nanoparticles on living cells. Environmental Chemistry, 2019, 17, pp.39-53. 10.1071/EN19190 . hal-02351027

# HAL Id: hal-02351027 https://hal.univ-lorraine.fr/hal-02351027

Submitted on 6 May 2020  $\,$ 

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1	Electrophoresis as a simple method to detect
2	deleterious actions of engineered nanoparticles on living cells
3	
4	Elise Vouriot, <sup>a</sup> Isabelle Bihannic, <sup>a,<math>\perp</math></sup> Audrey Beaussart, <sup>a,<math>\perp</math></sup> Yves Waldvogel, <sup>a</sup>
5	Angelina Razafitianamaharavo, <sup>a</sup> Tania Ribeiro, <sup>b</sup> José Paulo S. Farinha, <sup>b</sup> Christophe Beloin, <sup>c</sup>
6	Jérôme F.L. Duval <sup>a,*</sup>
7	
8 9 10 11 12 13	<ul> <li><sup>a</sup> Université de Lorraine, CNRS, LIEC (Laboratoire Interdisciplinaire des Environnements Continentaux), UMR 7360, Vandoeuvre-lès-Nancy F-54501, France.</li> <li><sup>b</sup> Centro de Química-Física Molecular and IN - Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, University of Lisbon, 1049-001, Lisboa, Portugal.</li> <li><sup>c</sup> Institut Pasteur, Unité Génétique des Biofilms, F-75724 Paris 15, France.</li> </ul>
14	$^{\perp}$ These authors equally contributed to this work.
15 16	* Corresponding author: jerome.duval@univ-lorraine.fr

17 Abstract:

18 The release of engineered nanoparticles (NPs) in the environment may have profound implications 19 for the health of aquatic biota. In this study, we evidence that the initial stage of NPs action on 20 bacteria can be detected upon measurement of the electrophoretic fingerprints of mixed NPs-cells 21 dispersions. Such electrokinetic signatures reflect a modification of the physicochemical cells and NPs 22 surface properties following changes in cell envelope organization, subsequent intracellular material 23 release and/or biomolecules cell excretion. The demonstration is based on a thorough investigation 24 of the electrohydrodynamic features of genetically engineered Escherichia coli bacteria exhibiting 25 distinct surface phenotypes (presence of adhesive YeeJ large proteins or F-pili proteinaceous 26 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 27 28 concentration considered ( $0-10^{-2}$  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 29 30 determinants of cells and NPs are generated due to cells envelope alteration and bio-corona 31 acquisition by NPs. Differentiated roles of the cell surface appendages in the mediation of NPs 32 impacts are evidenced by the measured dependence of electropherograms on cell surface 33 phenotype and NPs concentration. Cell morphology and surface roughness, evaluated by Atomic 34 Force Microscopy (AFM) in liquid, confirm the pH and NPs concentration conditions where NPs-cells 35 interactions are operational. Combination of electrokinetics and AFM further pinpoints 36 heterogeneities in cells response at the single cell and population scales. Altogether, results show 37 that electrokinetics is suitable to detect the preliminary stage of events leading to NPs toxicity 38 toward microorganisms.

#### 39 Environmental context:

40 Attractive interactions and subsequent contacts between NPs and microorganisms are the first steps 41 of a chain of events leading to adverse NPs effects toward cells. Herein we show that the 42 electrophoretic response of complex mixtures of engineered NPs (silica NPs) and bacteria (*E. coli*) reflects initial NPs-mediated cell surface damages. We thus provide a promising option for a rapid
 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 (Yang et al. 2013), or to affect cells growth (El Badawy et al. 2011), cells viability (Choi et al. 2010; 53 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

potentialities of electrophoresis for assessing the first stage of NPs action toward the surface ofbiological 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 µm to 100 µm 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).

Using the relevant antibiotics listed in Table 1, bacteria were pre-grown in Lysogeny Broth (LB) 163 164 medium (BD Difco<sup>®</sup>) 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 appropriate antibiotics (hereafter called M63B1glu medium). In details, this M63B1glu medium 166 contained 13.6 g  $L^{-1}$  KH<sub>2</sub>PO<sub>4</sub> (Sigma-Aldrich<sup>®</sup>), 2 g  $L^{-1}$  (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (Emsure<sup>®</sup>), 0.1 g  $L^{-1}$  MgSO<sub>4</sub>,7H<sub>2</sub>O 167 (Emsure<sup>®</sup>),  $5.10^{-4}$  g L<sup>-1</sup> FeSO<sub>4</sub>,7H<sub>2</sub>O (Sigma-Aldrich<sup>®</sup>),  $1.10^{-3}$  g L<sup>-1</sup> thiamine (Roth<sup>®</sup>) and 4 g L<sup>-1</sup> glucose 168 169 (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  $5000 \times g$  and then re-suspended in 10 mM KNO<sub>3</sub> (Sigma-Aldrich<sup>®</sup>, 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  $OD_{600}$  of 0.02 was reached. For that purpose, appropriate 177 volumes of 2M KNO<sub>3</sub> electrolyte, of ultrapure water (milli-Q<sup>®</sup> 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<sup>®</sup>) 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  $5000 \times g$  and then re-suspended in 1 mM KNO<sub>3</sub> (Sigma-Aldrich<sup>®</sup>, purity > 99%) so as to reach an OD<sub>600</sub> of 0.4. Appropriate volumes of (i) separately prepared 1 mM KNO<sub>3</sub> NPs suspension at  $10^{-1}$  g L<sup>-1</sup> (pH 3 186 187 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  $10^{-2}$  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 applicable in Figure 4, the  $OD_{600nm}$  is 0.02 and the maximum NPs concentration tested is  $10^{-2}$  g/L, 195 196 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 synthetized 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  $\pm$  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  $L^{-1}$  NPs concentration. For that purpose, the appropriate weight of NPs was added to half of the desired volume of KNO<sub>3</sub> solution under 1000 rpm agitation 209 210 conditions for 1 min, and the volume was filled with 0.1 mM or 1 mM KNO<sub>3</sub> to reach the final targeted volume. The so-obtained NPs suspension (pH 7) was finally stirred for 24 h at 1000 rpm. 211 212 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 µm to 50 µm (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  $SiNH_2 NPs$  was measured at solution pH 3 and 7 as a function of  $KNO_3$ 

salt concentration (0.1-250 mM) using Dynamic Light Scattering (DLS) (Zetasizer NanoZS, Malvern®)

with a 633 nm laser. The particle diffusion coefficient was obtained from the correlation of the measured scattered intensity and converted to hydrodynamic diameter using Stokes-Einstein equation. Results are reported here in the form of particle number-size distributions. The NPs suspensions used for size measurements were prepared from the NPs suspensions obtained in §2.2 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<sup>®</sup>) or KOH (10<sup>-1</sup>-10<sup>-2</sup>M, Fluka<sup>®</sup>).

#### 233 Electrophoretic mobility measurements

The electrophoretic mobility of  $SiNH_2$  NPs was measured as a function of pH (3 to 9) and KNO<sub>3</sub> salt concentration in the range 0.1 mM to 250 mM at 25°C using Zetasizer NanoZS. A constant direct current electric field (25 V cm<sup>-1</sup>) applied between two electrodes immersed in the NPs-containing solution imposes a displacement of the particles. Electrophoretic mobility was then determined using Phase Analysis Light Scattering (PALS) technology (Malvern Instruments). NPs suspensions were prepared in a way similar to that adopted for size measurements. Experiments were carried out 3 to 5 times on freshly prepared samples.

241 242 2.3.2 Cells electrophoretic mobility measurements and electrokinetics of mixed NPsbacteria 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 spring constant of 0.12 N m<sup>-1</sup>, Bruker, AXS<sup>®</sup>, Palaiseau, France). Prior to experiments, glass slides 258 259 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<sup>®</sup>, 7.5  $10^6$  Mw, 50 wt. % in H<sub>2</sub>O) for 30 min and finally rinsed with 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 263 264 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  $10^{-3}$ 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

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

275

#### 276 **3. Results and Discussions**

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

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

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



283

Figure 1: Electrophoretic mobility of SiNH<sub>2</sub> NPs as a function of pH (indicated) and solution ionic strength (changed upon modification of KNO<sub>3</sub> electrolyte concentration in solution). Colored symbols: experimental data. Lines: data reconstruction by means of Ohshima's theory (eqs 1-4). Measurements, performed with Zetasizer NanoZS equipment (Malvern Instruments<sup>®</sup>, UK), were carried out on 2 to 5 freshly prepared SiNH<sub>2</sub> NPs suspensions, and each of these measurements was 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  $SiNH_2$  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  $-NH_2$  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  $-NH_2$  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 KNO<sub>3</sub> 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 SiNH<sub>2</sub> NPs to KNO<sub>3</sub> 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 
$$\mu = \frac{\rho_0}{\eta \lambda_0^2} + \frac{\varepsilon}{\eta} \frac{\psi^{\circ} / \kappa_{\rm m} + \psi^{\rm D} / \lambda_{\rm o}}{1 / \kappa_{\rm m} + 1 / \lambda_{\rm o}} \quad (1)$$

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  $\psi^0$  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.*  $\kappa a >> 1$ ,  $\kappa d >> 1$ ,  $\lambda_d >> 1$ . 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 and Ohshima 2006; Ohshima 1995). The quantities  $\psi^{D}$ ,  $\psi^{0}$  and  $\kappa_{m}$  all depend on charge density  $\rho_{0}$  and 335 electrolyte concentration  $c^{\infty}$  (1:1 symmetrical electrolyte is considered here) according to (Ohshima 336 337 1995)

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

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

340 
$$\kappa_{\rm m} = \kappa \left\{ \cosh\left(\frac{F\psi^{\rm D}}{RT}\right) \right\}^{1/2}$$
 (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 KNO<sub>3</sub> 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), SiNH<sub>2</sub> 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  $pH\sim5$ . The nanometric length scale 364  $1/\lambda_0$  (= 1 ± 0.3 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  $-0^{\circ}$  or  $-NH_{3}^{+}$  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  $140 \pm 10$  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

Figure 2: SiNH<sub>2</sub> NPs hydrodynamic diameter D at pH 7 (2a) and pH 3 (2b) as a function of solution ionic strength. Filled symbols correspond to the first mode detected in the number-size distributions obtained by DLS. Empty bullet points correspond to the second mode if applicable. Due to the contribution of protons to the overall solution ionic strength, there are no data points in panel b at 1 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 ionic strength is  $\mu = 2.1 \pm 0.6 \times 10^{-8} \text{ m}^2 \text{V}^{-1} \text{s}^{-1}$  at  $10^{-3} \text{ g L}^{-1}$  ( $\mu = -1.8 \pm 0.1 \times 10^{-8} \text{ m}^2 \text{V}^{-1} \text{s}^{-1}$ , respectively) and 405  $\mu$ =2.5±0.5×10<sup>-8</sup> 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±0.4×10<sup>-8</sup> m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup>, respectively), as obtained with 406 407 Zetaphoremeter IV equipment (CAD Instrumentations®). Measurements with Zetasizer NanoZS (Malvern) lead at pH 3 (pH 7, respectively) to  $\mu$ =2.4±0.6×10<sup>-8</sup> 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±0.7×10<sup>-8</sup> 408  $m^{2}V^{-1}s^{-1}$ , respectively) and  $\mu = 3.0\pm 0.7 \times 10^{-8} m^{2}V^{-1}s^{-1}$  at  $10^{-1} g L^{-1} (\mu = -1.7 \pm 0.9 \times 10^{-8} m^{2}V^{-1}s^{-1}$ , respectively). 409 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 410 lower) to  $10^{-2}$  g L<sup>-1</sup> NPs concentration range: this NP concentration range is therefore adopted in §3.2 411 412 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  $\rho_0$  (~97±4 mM) and similar Brinkman lengths (1/ $\lambda_0$ ~0.85 nm), their 434 permeability to electroosmotic flow appears to be somewhat larger than that for nude cells  $(1/\lambda_0 \sim 0.5)$ 435 nm). Surface appendages thus contribute here to slightly increase flow permeability (*i.e.* increase of 436  $1/\lambda_{0}$ ) 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 error the electrokinetic properties of the three strains of interest are similar at pH 3 over the whole 439 440 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  $1/\lambda_0$  (= 0.7±0.2 nm, with 443 no marked dependence on pH) and  $\rho_0$  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

Figure 3: Electrophoretic mobility of the adopted bacterial strains (nude (E2152), F-pili (E2302) and YeeJ (E2551) cells, indicated) at pH 7 (3a) and pH 3 (3b) as a function of solution ionic strength. Symbols: experimental data. Dotted lines: theory (eqs 1-4). Due to the contribution of protons to the overall solution ionic strength, there are no data points in panel b at 1 mM ionic strength after addition of KNO<sub>3</sub> 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 SiNH<sub>2</sub> 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<sub>2</sub> NPs and bacteria, respectively. The electrophoretic fingerprints of the cells and 461 those of the SiNH<sub>2</sub> 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

for a given class of electrophoretic mobility values  $\mu$  (the width of each class is set here to 0.15×10<sup>8</sup> 463 464  $m^2 V^{-1} s^{-1}$  increment in  $\mu$ ) corresponds to the ratio between the number of particle trajectories detected at these selected  $\mu$  and the total number of trajectories measured over the whole range of 465 466 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.

At pH 7, in line with data in **Figure 3**, cells electrophoretic mobility ranges from -5 to  $-4 \times 10^{-8}$  m<sup>2</sup>V<sup>-</sup> 474  $^{1}s^{-1}$  (E2152, nude cells), from -4 to -3×10<sup>-8</sup> m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup> (E2551, YeeJ) and from -4 to -2.5 x 10<sup>-8</sup> m<sup>2</sup>V<sup>-1</sup>s<sup>-1</sup> 475 (E2302, F-pili decorated). The width and frequencies defining the mobility distributions of E2152 and 476 477 E2551 cells remain basically unchanged upon increase of SiNH<sub>2</sub> NPs concentration up to  $10^{-3}$  g L<sup>-1</sup> (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 increasing NP concentrations from  $10^{-3}$  g L<sup>-1</sup> to  $10^{-2}$  g L<sup>-1</sup>. The most important conclusion at this stage 483 484 is the absence of deformation of the cells-electrokinetic plume with increasing SiNH<sub>2</sub> NPs, which 485 suggests that the electrohydrodynamic properties of E2152 and E2551 cells remain basically 486 unaffected in the presence of SiNH<sub>2</sub> 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<sub>2</sub> NPs as pictured in Figure 3a and Figure 1, respectively. Similar conclusions may be formulated 489 for E2302 cells even though, gualitatively, the width of their mobility distributions tends to slightly increase with increasing SiNH<sub>2</sub> NPs from 0 to 10<sup>-2</sup> g L<sup>-1</sup>, resulting in an overlap of the cells- and NPs-490 plumes that is significantly more pronounced than for E2152 cells. This finding underpins that a 491 492 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

Figure 4: Electrophoretic fingerprints of mixed bacteria-SiNH<sub>2</sub> NPs suspensions at pH 7 and pH 3
 (indicated) as a function of SiNH<sub>2</sub> NPs concentration in solution for the three cell surface
 phenotypes of interest (indicated). Solution ionic strength: 1 mM at pH 7 and 2 mM at pH 3. See text
 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 SiNH<sub>2</sub> NPs (Figure 1) and negatively charged E2551 cells (Figure 3b) at pH 3 induce significant changes 515 516 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 $_2$ 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 distortions of the SiNH<sub>2</sub> NPs- and E2152-plumes take place at larger NPs concentrations, *i.e.* 2.5×10<sup>-3</sup> 542 543 g L<sup>-1</sup> and  $10^{-2}$  g L<sup>-1</sup>, respectively, instead of  $10^{-3}$  g L<sup>-1</sup> and  $5 \times 10^{-3}$  g L<sup>-1</sup> for the E2551/NPs system. This suggests that the resistance of the nude E2152 bacteria against NPs action is more efficient than that 544 545 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 electrosurface 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

roles played by cells surface bio-compounds in promoting or not NPs action. In the next section, we provide AFM images of individual cells exposed to  $SiNH_2$  NPs as an additional support for the trends given in **Figure 4**, recalling that electrokinetic measurements refer to averages of 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

Figure 5: AFM peak force error images (3.5 µm × 3.5 µm, 256 pixels × 256 pixels) taken in liquid for
 E2152, E2551 and E2302 bacteria (indicated) at pH 7 and 3 (indicated) prior to and after incubation
 with NPs (see details in §2). Red arrows correspond to NPs, white arrows to asperities or
 depressions, white squares define 400 nm × 400 nm spatial regions where cell surface roughness
 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 ( $2^{nd}$ 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 synthetized 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.q. 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



639 Figure 6: Illustrative spatial mapping (400 nm  $\times$  400 nm, 28 pixels  $\times$  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<sup>-1</sup> 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  $\sigma_2$ .

638

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 region of the protein (Martinez-Gil et al. 2017). The only passenger domain can be cleaved from the 692 693 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).

# **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 gualitatively 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

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

- 733
- 734 **6. Conflicts of interest**
- The authors declare no conflicts of interest.

#### 736 **7. Acknowledgements**

- 737 This research did not receive any specific funding.
- 738
- 739 References
- El Badawy, A.M., Silva, R.G., Morris, B., Scheckel, K.G., Suidan, M.T., Tolaymat, T.M., 2011. Surface
  charge-dependent toxicity of silver nanoparticles. *Environ. Sci. Technol.* 45, 283–287.
  https://doi.org/10.1021/es1034188
- Beaussart, A., Beloin, C., Ghigo, J.-M., Chapot-Chartier, M.-P., Kulakauskas, S., Duval, J.F.L., 2018a.
  Probing the influence of cell surface polysaccharides on nanodendrimer binding to Gramnegative and Gram-positive bacteria using single-nanoparticle force spectroscopy. *Nanoscale*10, 12743–12753. https://doi.org/10.1039/c8nr01766b
- Beaussart, A., Caillet, C., Bihannic, I., Zimmermann, R., Duval, J.F.L., 2018b. Remarkable reversal of
  electrostatic interaction forces on zwitterionic soft nanointerfaces in a monovalent aqueous
  electrolyte: An AFM study at the single nanoparticle level. *Nanoscale* 10, 3181–3190.
  https://doi.org/10.1039/c7nr07976a
- Beddoes, C.M., Case, C.P., Briscoe, W.H., 2015. Understanding nanoparticle cellular entry: A
  physicochemical perspective. *Adv. Colloid Interface Sci.* 218, 48–68.
  https://doi.org/10.1016/j.cis.2015.01.007
- Beloin, C., Roux, A., Ghigo, J., 2010. Escherichia coli biofilms. *Curr Top Microbiol Immunol* 322, 249–
   389. 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.

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

- Duval, J.F.L., Gaboriaud, F., 2010. Progress in electrohydrodynamics of soft microbial particle
   interphases. *Curr. Opin. Colloid Interface Sci.* 15, 184–195.
   https://doi.org/10.1016/j.cocis.2009.12.002
- Buval, J.F.L., Leermakers, F.A.M., Van Leeuwen, H.P., 2004. Electrostatic interactions between double
   layers: Influence of surface roughness, regulation, and chemical heterogeneities. *Langmuir* 20, 5052–5065. https://doi.org/10.1021/la030404f
- Buval, J.F.L., Ohshima, H., 2006. Electrophoresis of diffuse soft particles. *Langmuir* 22, 3533–3546.
   https://doi.org/10.1021/la0528293
- Flemming, H.C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., Kjelleberg, S., 2016. Biofilms: An
  emergent form of bacterial life. *Nat. Rev. Microbiol.* 14, 563–575.
  https://doi.org/10.1038/nrmicro.2016.94
- Francius, G., Polyakov, P., Merlin, J., Abe, Y., Ghigo, J.M., Merlin, C., Beloin, C., Duval, J.F.L., 2011.
  Bacterial surface appendages strongly impact nanomechanical and electrokinetic properties of
  escherichia coli cells subjected to osmotic stress. *PLoS One* 6: e20066.
  https://doi.org/10.1371/journal.pone.0020066
- Geusebroek, J., Smeulders, A.W.M., van de Weijer, J., 2003. Fast anisotropic gauss filtering 12, 938–
  943.
- Hardij, J., Cecchet, F., Berquand, A., Gheldof, D., Chatelain, C., Mullier, F., Chatelain, B., Hardij, J.,
  Cecchet, F., Berquand, A., Gheldof, D., Chatelain, C., 2013. Characterisation of tissue factorbearing extracellular vesicles with AFM: Comparison of air-tapping-mode AFM and liquid Peak
  Force AFM. J. Extracell. Vesicles 3078. https://doi.org/10.3402/jev.v2i0.21045
- Hartlen, K.D., Athanasopoulos, A.P.T., Kitaev, V., 2008. Facile preparation of highly monodisperse
   small silica spheres (15 to >200 nm) suitable for colloidal templating and formation of ordered
   arrays. *Langmuir* 24, 1714–1720. https://doi.org/10.1021/la7025285
- Hayden, S.C., Zhao, G., Saha, K., Phillips, R.L., Li, X., Miranda, O.R., Rotello, V.M., El-Sayed, M.A.,
  Schmidt-Krey, I., Bunz, U.H.F., 2012. Aggregation and interaction of cationic nanoparticles on
  bacterial surfaces. J. Am. Chem. Soc. 134, 6920–6923. https://doi.org/10.1021/ja301167y
- Hill, R.J., Saville, D.A., Russel, W.B., 2003. Electrophoresis of spherical polymer-coated colloidal
   particles. J. Colloid Interface Sci. 258, 56–74. https://doi.org/10.1016/S0021-9797(02)00043-7
- Huang, Z., Zheng, X., Yan, D., Yin, G., Liao, X., Kang, Y., Yao, Y., Huang, D., Hao, B., 2008. Toxicological
  effect of ZnO nanoparticles based on bacteria. *Langmuir* 24, 4140–4144.
  https://doi.org/10.1021/la7035949
- Ikuma, K., Decho, A.W., Lau, B.L.T., 2015. When nanoparticles meet biofilms-interactions guiding the
   environmental fate and accumulation of nanoparticles. *Front. Microbiol.* 6, 1–6.
   https://doi.org/10.3389/fmicb.2015.00591
- Ingale, A.G., Chaudhari, A.N., 2013. Biogenic synthesis of nanoparticles and potential applications : an
  eco-friendly approach. *Nanomedecine Nanotechnol.* 4. https://doi.org/10.4172/21577439.1000165
- Jacobson, K.H., Gunsolus, I.L., Kuech, T.R., Troiano, J.M., Melby, E.S., Lohse, S.E., Hu, D., Chrisler,
  W.B., Murphy, C.J., Orr, G., Geiger, F.M., Haynes, C.L., Pedersen, J.A., 2015. Lipopolysaccharide
  density and structure govern the extent and distance of nanoparticle interaction with actual
  and model bacterial outer membranes. *Environ. Sci. Technol.* 49, 10642–10650.
  https://doi.org/10.1021/acs.est.5b01841

- Jandt, K.D., 2001. Atomic force microscopy of biomaterials surfaces and interfaces. *Surf. Sci.* 491, 303–332. https://doi.org/10.1016/S0039-6028(01)01296-1
- Jiang, W., Yang, K., Vachet, R.W., Xing, B., 2010. Interaction between oxide nanoparticles and
  biomolecules of the bacterial cell envelope as examined by infrared spectroscopy. *Langmuir* 26,
  18071–18077. https://doi.org/10.1021/la103738e
- Ju-Nam, Y., Lead, J.R., 2008. Manufactured nanoparticles: An overview of their chemistry,
  interactions and potential environmental implications. *Sci. Total Environ.* 400, 396–414.
  https://doi.org/10.1016/j.scitotenv.2008.06.042
- Kimkes, T.E.P., Heinemann, M., 2018. Reassessing the role of the Escherichia coli CpxAR system in
   sensing surface contact. *PLoS One* 13, 1–11. https://doi.org/10.1371/journal.pone.0207181
- Kleinstreuer, N.C., Sullivan, K., Allen, D., Edwards, S., Mendrick, D.L., Embry, M., Matheson, J.,
  Rowlands, J.C., Munn, S., Maull, E., Casey, W., 2016. Adverse outcome pathways: From research
  to regulation scientific workshop report. *Regul. Toxicol. Pharmacol.* 76, 39–50.
  https://doi.org/10.1016/j.yrtph.2016.01.007
- Li, N., Zeng, S., He, L., Zhong, W., 2010. Probing nanoparticle-protein interaction by capillary electrophoresis. *Anal. Chem.* **82**, 7460–7466. https://doi.org/10.1021/ac101627p
- van Loosdrecht, M.C.M., Lyklema, J., Norde, W., Zehnder, A.J.B., 1990. Influence of interfaces on
   microbial activity. *Microbiol. Rev.* 54, 75–87.
- López-León, T., Carvalho, E.L.S., Seijo, B., Ortega-Vinuesa, J.L., Bastos-González, D., 2005.
  Physicochemical characterization of chitosan nanoparticles: Electrokinetic and stability
  behavior. J. Colloid Interface Sci. 283, 344–351. https://doi.org/10.1016/j.jcis.2004.08.186
- López-Viota, J., Mandal, S., Delgado, A. V., Toca-Herrera, J.L., Möller, M., Zanuttin, F., Balestrino, M.,
  Krol, S., 2009. Electrophoretic characterization of gold nanoparticles functionalized with human
  serum albumin (HSA) and creatine. J. Colloid Interface Sci. 332, 215–223.
  https://doi.org/10.1016/j.jcis.2008.11.077
- Majdalani, N., Heck, M., Stout, V., Gottesman, S., 2005. Role of RcsF in signaling to the Rcs
  phosphorelay pathway in Escherichia coli. J. Bacteriol. 187, 6770–6778.
  https://doi.org/10.1128/JB.187.19.6770-6778.2005
- Malvern Instruments Limited, 2012. A basic guide to particle characterization. White Pap. 1–26.
   https://doi.org/10.1021/ac00110a016
- Martinez-Gil, M., Goh, K.G.K., Rackaityte, E., Sakamoto, C., Audrain, B., Moriel, D.G., Totsika, M.,
  Ghigo, J.M., Schembri, M.A., Beloin, C., 2017. YeeJ is an inverse autotransporter from
  Escherichia coli that binds to peptidoglycan and promotes biofilm formation. *Sci. Rep.* 7, 1–16.
  https://doi.org/10.1038/s41598-017-10902-0
- 877 Mathelié-guinlet, M., 2017. Etude de l'intéraction nanoparticules-bactéries : application à
   878 l'élaboration d'un biocapteur. Université de Bordeaux.
- Mathelié-Guinlet, M., Béven, L., Moroté, F., Moynet, D., Grauby-Heywang, C., Gammoudi, I., Delville,
  M.H., Cohen-Bouhacina, T., 2017. Probing the threshold of membrane damage and cytotoxicity
  effects induced by silica nanoparticles in Escherichia coli bacteria. *Adv. Colloid Interface Sci.* 245,
  81–91. https://doi.org/10.1016/j.cis.2017.04.012
- Mathelié-Guinlet, M., Grauby-Heywang, C., Martin, A., Février, H., Moroté, F., Vilquin, A., Béven, L.,
   Delville, M.H., Cohen-Bouhacina, T., 2018. Detrimental impact of silica nanoparticles on the
   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
- Maurya, S.K., Gopmandal, P.P., Ohshima, H., 2018. Electrophoresis of concentrated suspension of
   soft particles with volumetrically charged inner core. *Colloid Polym. Sci.* 296, 721–732.
   https://doi.org/10.1007/s00396-018-4292-0
- McBroom, A.J., Kuehn, M.J., 2007. Release of outer membrane vesicles by Gram-negative bacteria is
  a novel envelope stress response. *Mol. Microbiol.* 63, 545–558. https://doi.org/10.1111/j.13652958.2006.05522.x
- Mecke, A., Majoros, I.J., Patri, A.K., Baker, J.R., Banaszak Holl, M.M., Orr, B.G., 2005. Lipid bilayer
   disruption by polycationic polymers: The roles of size and chemical functional group. *Langmuir* 21, 10348–10354. https://doi.org/10.1021/la050629l
- Monopoli, M.P., Åberg, C., Salvati, A., Dawson, K.A., 2012. Biomolecular coronas provide the
  biological identity of nanosized materials. *Nat. Nanotechnol.* 7, 779–786.
  https://doi.org/10.1038/nnano.2012.207
- Moore, M.N., 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic
   environment? *Environ. Int.* 32, 967–976. https://doi.org/10.1016/j.envint.2006.06.014
- Moussa, M., Caillet, C., Town, R.M., Duval, J.F.L., 2015. Remarkable electrokinetic features of charge stratified soft nanoparticles: Mobility reversal in monovalent aqueous electrolyte. *Langmuir* 31, 5656–5666. https://doi.org/10.1021/acs.langmuir.5b01241
- Neal, A.L., 2008. What can be inferred from bacterium-nanoparticle interactions about the potential
   consequences of environmental exposure to nanoparticles? *Ecotoxicology* 17, 362–371.
   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
- Pagnout, C., Jomini, S., Dadhwal, M., Caillet, C., Thomas, F., Bauda, P., 2012. Role of electrostatic
   interactions in the toxicity of titanium dioxide nanoparticles toward Escherichia coli. *Colloids Surfaces B Biointerfaces* 92, 315–321. https://doi.org/10.1016/j.colsurfb.2011.12.012
- Peulen, T.O., Wilkinson, K.J., 2011. Diffusion of nanoparticles in a biofilm. *Environ. Sci. Technol.* 45, 3367–3373. https://doi.org/10.1021/es103450g
- Phillips, R.L., Miranda, O.R., You, C.C., Rotello, V.M., Bunz, U.H.F., 2008. Rapid and efficient identification of bacteria using gold-nanoparticle-poly(para-phenyleneethynylene) constructs.
  Angew. Chemie Int. Ed. 47, 2590–2594. https://doi.org/10.1002/anie.200703369
- Planchon, M., Ferrari, R., Guyot, F., Gélabert, A., Menguy, N., Chanéac, C., Thill, A., Benedetti, M.F.,
  Spalla, O., 2013. Interaction between Escherichia coli and TiO2 nanoparticles in natural and
  artificial waters. *Colloids Surfaces B Biointerfaces* 102, 158–164.
  https://doi.org/10.1016/j.colsurfb.2012.08.034
- Reisner, A., Haagensen, J.A.J., Schembri, M.A., Zechner, E.L., Molin, S., 2003. Development and
  maturation of Escherichia coli K-12 biofilms. *Mol. Microbiol.* 48, 933–946. https://doi.org/3490
  [pii]
- Ribeiro, T., Baleizão, C., Farinha, J.P.S., 2017. Artefact-free evaluation of metal enhanced
  fluorescence in silica coated gold nanoparticles. *Sci. Rep.* 7, 1–12.
  https://doi.org/10.1038/s41598-017-02678-0
- Ribeiro, T., Raja, S., Rodrigues, A.S., Fernandes, F., Baleizão, C., Farinha, J.P.S., 2014. NIR and visible
   perylenediimide-silica nanoparticles for laser scanning bioimaging. *Dye. Pigment.* 110, 227–234.

- 929 https://doi.org/10.1016/j.dyepig.2014.03.026
- Rowenczyk, L., Duclairoir-poc, C., Barreau, M., Picard, C., Hucher, N., Orange, N., Grisel, M.,
   Feuilloley, M., 2017. Impact of coated TiO2-nanoparticles used in sunscreens on two
   representative strains of the human microbiota : Effect of the particle surface nature and aging.
   *Colloids Surfaces B Biointerfaces* 158, 339–348. https://doi.org/10.1016/j.colsurfb.2017.07.013
- Shang, L., Nienhaus, K., Nienhaus, G.U., 2014. Engineered nanoparticles interacting with cells: Size
   matters. J. Nanobiotechnology 12, 1–11. https://doi.org/10.1186/1477-3155-12-5
- Sheng, G.P., Yu, H.Q., Yue, Z.B., 2005. Production of extracellular polymeric substances from
   Rhodopseudomonas acidophila in the presence of toxic substances. *Appl. Microbiol. Biotechnol.* 69, 216–222. https://doi.org/10.1007/s00253-005-1990-6
- Shrivastava, S., Bera, T., Roy, A., 2007. Characterization of enhanced antibacterial effects of novel
   silver nanoparticles. *Nanotechnology* 18. https://doi.org/10.1088/0957-4484/18/22/225103
- Silva, T., Pokhrel, L.R., Dubey, B., Tolaymat, T.M., Maier, K.J., Liu, X., 2014. Particle size, surface
  charge and concentration dependent ecotoxicity of three organo-coated silver nanoparticles:
  Comparison between general linear model-predicted and observed toxicity. *Sci. Total Environ.*468–469, 968–976. https://doi.org/10.1016/j.scitotenv.2013.09.006
- Silverman, P.M., 1997. Towards a structural biology of bacterial conjugation. *Mol. Microbiol.* 23, 423–
  429. https://doi.org/10.1046/j.1365-2958.1997.2411604.x
- Škvarla, J., 2007. Hard versus soft particle electrokinetics of silica colloids. *Langmuir* 23, 5305–5314.
   https://doi.org/10.1021/la0635451
- 949Stöber, W., Fink, A., Bohn, E., 1968. Controlled growth of monodisperse silica spheres in the micron950size range. J. Colloid Interface Sci. 26, 62–69. https://doi.org/10.1016/0021-9797(68)90272-5
- Stoimenov, P.K., Klinger, R.L., Marchin, G.L., Klabunde, K.J., 2002. Metal oxide nanoparticles as
   bactericidal agents. *Langmuir* 18, 6679–6686. https://doi.org/10.1021/la0202374
- Svenson, S., 2009. Dendrimers as versatile platform in drug delivery applications. *Eur. J. Pharm. Biopharm.* 71, 445–462. https://doi.org/10.1016/j.ejpb.2008.09.023
- Tajarobi, F., El-Sayed, M., Rege, B.D., Polli, J.E., Ghandehari, H., 2001. Transport of poly amidoamine
  dendrimers across Madin-Darby canine kidney cells. *Int. J. Pharm.* 215, 263–267.
  https://doi.org/10.1016/S0378-5173(00)00679-7
- Thill, A., Zeyons, O., Spalla, O., Chauvat, F., Rose, J., Auffan, M., Flank, A.M., 2006. Cytotoxicity of
   CeO2 nanoparticles for Escherichia coli. Physico-chemical insight of the cytotoxicity mechanism.
   *Environ. Sci. Technol.* 40, 6151–6156. https://doi.org/10.1021/es060999b
- Velzeboer, I., Hendriks, A.J., Ragas, M.J., De Meent, D., 2008. Nanomaterials in the environment
  aquatic ecotoxicity tests of some nanomaterials. *Environ. Toxicol. Chem.* 27, 1942–1947.
  https://doi.org/10.1897/07-509.1
- Van Der Wal, A., Norde, W., Zehnder, A.J.B., Lyklema, J., 1997. Determination of the total charge in
   the cell walls of Gram-positive bacteria. *Colloids Surfaces B Biointerfaces* 9, 81–100.
   https://doi.org/10.1016/S0927-7765(96)01340-9
- Wang, Y.A., Yu, X., Silverman, P.M., Harris, R.L., Egelman, E.H., 2009. The structure of F-pili. *J. Mol. Biol.* 385, 22–29. https://doi.org/10.1016/j.jmb.2008.10.054
- Yang, Y., Quensen, J., Mathieu, J., Wang, Q., Wang, J., Li, M., Tiedje, J.M., Alvarez, P.J.J., 2013.
   Pyrosequencing reveals higher impact of silver nanoparticles than Ag+ 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
- Zeyons, O., Thill, A., Chauvat, F., Menguy, N., Cassier-Chauvat, C., Oréar, C., Daraspe, J., Auffan, M.,
   Rose, J., Spalla, O., 2009. Direct and indirect CeO2 nanoparticles toxicity for Escherichia coli and
- 975 Synechocystis. *Nanotoxicology* **3**, 284–295. https://doi.org/10.3109/17435390903305260
- 976

977

#### 978 **Tables**

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_∆fli _∆flu::km_∆fimA- 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_∆fli _ 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_∆fli_∆ flu::km_∆fimA- 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)

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

980

Table 2: Values of charge density  $\rho_0$  (in mM) and hydrodynamic penetration length  $1/\lambda_0$  (in nm) obtained from eqs 1-4 adopted to recover the electrokinetic data given in Figure 1.  $\rho_0$  is expressed in 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	рН 6	pH 7	рН 9
$ ho_{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

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

	Nude (E2152)		YeeJ (E2551)		F-pili (E2302)	
	рН 3	pH 7	рН 3	pH 7	рН 3	рН 7
$ ho_{ m o}$ (mM)	-57.5	-195	-53.7	-92.5	-64.6	-101

|--|

990

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

	pH 7	SiNH <sub>2</sub> NPs	рН 3	SiNH <sub>2</sub> NPs
		рН 7		рН 3
Nude (E2152)	$0.64 \pm 0.13$	0.79 ± 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

998