



**HAL**  
open science

## Phenotypic Heterogeneity in Attachment of Marine Bacteria toward Antifouling Copolymers Unraveled by AFM

Sofiane El-Kirat-Chatel, Aurore Puymège, The Duong, Perrine van Overtvelt, Christine Bressy, Lénaïk Bélec, Yves Dufrêne, Maëlle Molmeret

► **To cite this version:**

Sofiane El-Kirat-Chatel, Aurore Puymège, The Duong, Perrine van Overtvelt, Christine Bressy, et al.. Phenotypic Heterogeneity in Attachment of Marine Bacteria toward Antifouling Copolymers Unraveled by AFM. *Frontiers in Microbiology*, 2017, 8, 10.3389/fmicb.2017.01399 . hal-01898961

**HAL Id: hal-01898961**

**<https://hal.univ-lorraine.fr/hal-01898961>**

Submitted on 31 Jan 2022

**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           **Phenotypic heterogeneity in attachment of marine bacteria**

## 2           **towards antifouling copolymers unraveled by AFM**

3 Sofiane El-Kirat-Chatel<sup>1,4</sup>, Aurore Puymege<sup>2</sup>, The Hy Duong<sup>2,3</sup>, Perrine Van Overtvelt<sup>2</sup>,  
4 Christine Bressy<sup>2</sup>, Lénaïk Belec<sup>2</sup>, Yves F. Dufrêne<sup>4</sup>, Maëlle Molmeret<sup>\*2</sup>

5 <sup>1</sup>CNRS and Université de Lorraine, Laboratoire de Chimie Physique et Microbiologie pour  
6 l'Environnement (LCPME), UMR 7564, Nancy, France

7 <sup>2</sup>Université de Toulon, Laboratoire Mapiem, EA4323, La Garde, France

8 <sup>3</sup>The University of Danang, University of Science and Technology, 54 Nguyen Luong Bang, Danang,  
9 Vietnam

10 <sup>4</sup>Institute of Life Sciences, Université catholique de Louvain, Croix du Sud 4-5, B-1348 Louvain-la-  
11 Neuve, Belgium

### 12   **Correspondence:**

13 Maëlle Molmeret, Laboratoire MAPIEM EA 4323, Université de Toulon

14 Avenue de l'université - BP 20132, 83957 La Garde Cedex

15 E-mail: molmeret@univ-tln.fr or maellemolmeret@hotmail.com

### 16   **Co-authors e-mails:**

17 El-Kirat-Chatel S. : elkirat1@univ-lorraine.fr

18 Puymege A: aurore1821@wanadoo.fr

19 Duong T.H.: dthy@dut.udn.vn

20 Van Overtvelt P.: [perrine.vanovertvelt@gmail.com](mailto:perrine.vanovertvelt@gmail.com)

21 Bressy C.: christine.bressy@univ-tln.fr

22 Belec L.: belec@univ-tln.fr

23 Dufrêne Y.F. : Yves.Dufrene@uclouvain.be

24 Molmeret M.: molmeret@univ-tln.fr

25   **Keywords:** Marine bacteria, adhesion, heterogeneity, AFM, copolymers surfaces

26   **Running title:** Phenotypic heterogeneity in bacterial adhesion

27

28 **Summary**

29 Up to recent years, bacterial adhesion have mostly been evaluated at the population level. Single  
30 cell level have improved in the past few years allowing a better comprehension of the  
31 implication of individual behaviors as compared to the one of a whole community. A new  
32 approach using atomic force microscopy to measure adhesion forces between a live bacterium  
33 attached via a silica microbead to the AFM tipless cantilever and the surface have been recently  
34 developed. The objectives of this study is to examine the bacterial adhesion to a surface  
35 dedicated to ship hulls at the population and the cellular level to understand to what extent these  
36 2 levels could be correlated. Adhesion of marine bacteria on inert surfaces are poorly studied  
37 in particular when substrata are dedicated to ship hulls. Studying these interactions in this  
38 context are worthwhile as they may involve different adhesion behaviors, taking place in salty  
39 conditions, using different surfaces than the ones usually utilized in the literacy. FRC (fouling  
40 release coatings)–SPC (self-polishing coatings) hybrids antifouling coatings have been used as  
41 substrata and are of particular interest for designing environmentally-friendly surfaces,  
42 combining progressive surface erosion and low adhesion properties. In this study, a hybrid  
43 coating has been synthetized and used to study the adhesion of three marine bacteria, displaying  
44 different surface characteristics, using microplate assays associated with confocal scanning  
45 laser microscopy and, AFM. This study shows that the bacterial strain that appeared to have the  
46 weakest adhesion and biofilm formation abilities when evaluated at the population level using  
47 microplates assays and CSLM, displayed stronger adhesion forces on the same surfaces at the  
48 single cell level using AFM. In addition, one of the strains tested which presented a strong  
49 ability to adhere and to form biofilm at the population level, displayed a heterogeneous  
50 phenotypic behaviour at the single cell level. Therefore, these results suggest that the evaluation  
51 of adhesion at the population level cannot always be correlated with adhesion forces measured

52 individually by AFM and that some bacteria are prone to phenotypic heterogeneity among their  
53 population.

54

55 (339 words)

## 56 **Introduction**

57

58 Little is known on adhesion of marine bacteria on surfaces in particular when they are dedicated  
59 to ship hulls. The comprehension of bacterial adhesion on these surfaces should help finding  
60 potential environmentally less toxic anti-adhesion or anti-fouling strategies. The specific  
61 intrinsic nature of marine bacteria, that are poorly studied and characterized, may modify the  
62 type of interactions that can be observed between a cell and its surface, particularly when the  
63 interactions take place in marine conditions, making them worthwhile studying. Overall,  
64 molecular or cellular mechanisms of bacterial adhesion have been in fact mostly evaluated at  
65 the population level but very rarely at the single cell level. Indeed, very few information are  
66 factually available on individual behaviors of bacteria regarding adhesion. Due to the  
67 development of new single cell level approaches, individual cells can be studied with the  
68 purpose of understanding how a single cell behaves as compared to its population of origin and  
69 if bacterial cells behave all similarly within a supposedly clonal population, after  
70 synchronization in growth culture, or if important behavioral differences exist between them.  
71 Recently, the idea that bacterial population could be composed of heterogeneous individuals  
72 has emerged, even when coming from a single cell or a group of clonal or genetically identical  
73 individuals (Grote et al., 2015; Martins and Locke, 2015). Differential gene expression could  
74 explain these phenotypic fluctuations. Some bacterial strains are also more prone to allelic  
75 variations than others (Davis and Isberg, 2016). In adhesion studies, AFM approaches have  
76 been used to study adhesion forces at the single cell level between a cell and a surface. They  
77 have been improved during the past few years, making it possible to study these interactions  
78 with alive bacteria (Kang and Elimelech, 2009; Loskill et al., 2012; Beaussart et al., 2013;  
79 Beaussart et al., 2014; El-Kirat-Chatel et al., 2014b). Indeed, a new approach using a silica  
80 microbead fixed on the tipless cantilever allow the attachment of a single cell that can stay alive

81 during the time of the measurement. These approaches have been proven very useful to decipher  
82 adhesion of bacteria such as *Staphylococcus aureus*, *Escherichia coli* toward glass and  
83 functionalized surfaces.

84  
85 In the marine context, all artificial surfaces immersed in seawater are subjected to the  
86 accumulation of marine organisms such as microorganisms and macrofoulers, known as marine  
87 biofouling. Current antifouling strategies rely on the widely use of self-polishing coatings  
88 (SPC), which releases toxic biocides with a constant rate controlled by the coating erosion  
89 process (Yebra et al., 2004). The erosion of the coating is achieved through the hydrolysis of  
90 the polymeric binder in seawater making the polymer water-soluble. Fouling Release Coatings  
91 (FRC) represent a second type of antifouling coatings, which are able to release organisms  
92 settled on the surface while boats are navigating (Lejars et al., 2012). Their efficacy relies on  
93 hydrophobicity, low surface energy and low elastic modulus of its poly(dimethylsiloxane)  
94 (PDMS) cross-linked matrix, which decreases the adhesion strength of marine organisms and  
95 enhance their removal. Despite, the clear environmentally friendly advantage of this antifouling  
96 solution, FRCs are inefficient when vessels are docked. During navigation, the coating is able  
97 to release the macrofouling but retains a microfouling film (composed mainly of bacteria and  
98 diatoms), which is still responsible for 10% of drag resistance (Schultz, 2007). An attractive  
99 option in developing such coatings is the synthesis of new polymers which are both  
100 hydrolyzable and hydrophobic/low-surface energy materials. Poly(dimethylsiloxane) blocks  
101 could be inserted in silylated-based polymers to provide access to a wide variety of materials  
102 with tunable hydrophobicity, water resistance and mechanical properties. Bressy and coworkers  
103 have synthesized tri-alkylsilylester-based statistical copolymers by conventional radical  
104 polymerization and several diblock copolymers using the reversible addition-fragmentation  
105 chain transfer (RAFT) polymerization for developing erodible binders for marine antifouling

106 coatings (Bressy and Margailan, 2009; Bressy et al., 2010; Bressy et al., 2014; Lejars et al.,  
107 2014). Hybrid copolymers with PDMS blocks or side-chains and silylated side groups have  
108 been reported to exhibit surface erosion and hydrophobic surfaces depending on the relative  
109 content of the two components (Lejars et al., 2013). These hybrid surfaces displaying SPC and  
110 FRC properties have been characterized including for their antifouling efficacy (Duong et al.,  
111 2014; Duong et al., 2015).

112

113 In this study, three strains isolated from the Mediterranean sea, presenting different  
114 phenotypical traits, have been used to evaluate their ability to adhere on a new antifouling  
115 coating dedicated to ship hulls at the population and the cellular level (Brian-Jaisson et al.,  
116 2014). TC5 belonging to the *Polaribacter* genus, a non-motile marine bacteria, is the most  
117 hydrophobic of the 3 strains according to Microbial Adhesion to Solvents (MATS) assays and  
118 has a poor ability to form biofilm on polystyrene when studied in microplates (Brian-Jaisson et  
119 al., 2014). TC10 and TC11 are 2 different strains of *Shewanella*, which are overall more  
120 hydrophilic and are motile. TC11 is able of a stronger adhesion and a faster capacity to form a  
121 biofilm on polystyrene while for TC10, it takes more time to form its biofilm. In this context,  
122 adhesion have been tested on an hybrid block copolymer SPC-FRC coating called MC3MB6  
123 (PDMS-*b*-p(SiMA-*stat*-BMA)). The results have been compared to its SPC block alone called  
124 MB6 (SiMA-*stat*-BMA). In contrast with conventional SPC, MB6 has no biocide but retaining  
125 the ability of self-hydrolysis. Both surfaces have been synthesized and their properties  
126 characterized similarly as previously done (Duong et al., 2014; Duong et al., 2015). The  
127 adhesion of these 3 marine strains on the hybrid coatings have been performed through a  
128 microplate assay associated with CLSM and AFM, in order to verify if the adhesion forces  
129 measured at the single cell level could be correlated with the evaluation of the population  
130 adhesion. Bacterial adhesion has been very rarely evaluated at the same time, at the population

131 and the cellular level to understand to what extent these 2 levels could be correlated for each of  
132 the strain.

133

## 134 **Material and Methods**

### 135 Substrates

136 Two copolymers based on *Tert*-butyldimethylsilyl methacrylate (SiMA) were synthesized as  
137 previously reported (Duong et al., 2014) . Butyl methacrylate was used as co-monomer of SiMA  
138 to prepare films without cracking (Table 1). Each copolymer was dissolved in toluene, at a 40–  
139 50 wt% solid content, and applied on abraded poly(vinyl chloride) (PVC) substrates with a bar-  
140 coater resulting in about 100  $\mu\text{m}$  dried thickness coatings. The surfaces of the samples for the  
141 contact angle measurement and for AFM measurements were 25mm x 45 mm and 10 mm x 10  
142 mm, respectively. The coated plates were left to dry in the open air for 15 days.

143

### 144 Characterization methods

145 The number-average molar mass ( $M_n$ ) and dispersity ( $\mathcal{D}$ ) of polymers were determined by triple  
146 detection size exclusion chromatography (TD-SEC). Analyses were performed on a Viscotek  
147 apparatus, composed of a GPC Max (comprising a degasser, a pump and an autosampler) with  
148 a TDA-302 (RI refractive index detector, right and low angle light scattering detector at 670  
149 nm and viscometer) and an UV detector ( $\lambda = 298 \text{ nm}$ ). The following columns were used: a  
150 Viscotek HHR-H precolumn and two Viscotek ViscoGel GMHHR-H columns. THF was used  
151 as the eluent with a flow rate of  $1.0 \text{ mL min}^{-1}$  at  $30 \text{ }^\circ\text{C}$ . For each precipitated polymer, the  
152 refractive index increment ( $dn/dc$ ) was determined using the OmniSec software, from a solution  
153 of known concentration (ca.  $10 \text{ mg mL}^{-1}$ ) filtered through a 0.2  $\mu\text{m}$  PTFE filter.



154 Differential scanning calorimetric (DSC) measurements were performed on a DSC Q10  
155 apparatus from TA Instruments calibrated with indium. Polymer samples weighing 15-20 mg  
156 were run at equal heating and cooling rates,  $10\text{ }^{\circ}\text{C min}^{-1}$ , under a constant stream of nitrogen.  
157 The MC3MB6 sample was first scanned from room temperature to  $100\text{ }^{\circ}\text{C}$  (PDMS-*block*-  
158 P(SiMA-*stat*-BMA)). The sample was then cooled to  $-165\text{ }^{\circ}\text{C}$ . This temperature was held for 5  
159 min to allow the system to attain thermal equilibrium before the second heating scan. The first  
160 heating ramp of each sample was discarded for this work. The glass transition temperature ( $T_g$ )  
161 values were determined as the midpoint between the onset and the end of a step transition using  
162 the TA Instruments Universal Analysis 2000 software.

163 Static contact angle measurements were carried out at room temperature using a sessile drop  
164 method with a DIGIDROP contact angle meter from GBX Instruments. Two test liquids:  
165 deionized water and diiodomethane (Sigma-Aldrich) were used. The liquid drop volume was 1  
166  $\mu\text{L}$  and  $0.5\text{ }\mu\text{L}$  for water and diiodomethane, respectively. A picture of the liquid drop on the  
167 surface was taken 4s after its formation for contact angle measurement. The reported contact  
168 angles were an average of five individual measurements in different regions of the same coating  
169 ( $\pm\sigma$ ). Surface free energies of the coatings ( $\gamma_s$ ) and their dispersive ( $\gamma_s^D$ ) and polar components  
170 ( $\gamma_s^P$ ) were calculated using the Owens-Wendt method. Dynamic contact angle measurements  
171 were carried out under ambient conditions by using the dynamic sessile drop technique. A water  
172 drop with a volume of around  $1\text{ }\mu\text{L}$  is growing on a syringe tip and picked up by the surface.  
173 The syringe tip never leaves the liquid drop. The water was inflated and sucked up from the  
174 surface and the advancing and receding angles were obtained.

175

176 AFM characterization of the surface

177 AFM measurements were performed on a Nanoscope V controller equipped with a Multimode  
178 V Atomic Force Microscope, with a 8610 JVLR type scanner. Tapping mode cantilever probes

179 (RTESP model from BRUKER) were used to show the topography of the supported polymer  
180 films and to evaluate their Young modulus values. The system sensitivity and cantilever spring  
181 constant  $k_c$  are successively determined from force measurements on a rigid sample and from  
182 the thermal tune method (Butt et al., 2005), implemented in Bruker Nanoscope (V7.3) software.  
183 The topography was initially scanned in tapping mode with a cantilever spring constant around  
184 48 N/m and a resonance frequency of  $\sim 380$  kHz. AFM force curves were performed with  
185 maximum forces lower than  $1.5 \mu\text{N}$  (The slope of the force–displacement approach curve in the  
186 linear elastic range gives an apparent stiffness  $k_{\text{eff}}$  which is directly linked to the sample  
187 stiffness  $k_s$  knowing the cantilever stiffness (Butt et al., 2005). In the case of a perfectly elastic  
188 tip with a spherical end and a homogeneous sample, with no adhesive effects, the Hertz model  
189 can give an estimation of Young's modulus from  $k_s$  measurement (Butt et al., 2005; Belec et  
190 al., 2015). The slope was calculated on the approach curves (between 400 and 450 nm of  
191 deflection). The standard deviation is calculated on 7 measurements.

192

193 Microorganisms and growth conditions.

194 Bacterial strains used in this study (TC for Toulon Collection) are listed in Table 4. They were  
195 isolated from biofilms formed on inert surfaces immersed in the Mediterranean Sea (bay of  
196 Toulon, France,  $43^{\circ}06'23''\text{N}$ - $5^{\circ}57'17''\text{E}$ ) (Camps et al., 2011; Briand et al., 2012). TC strains  
197 were grown in Vaatanen nine salt solution (VNSS) at  $20^{\circ}\text{C}$  in a rotary shaker (120rpm)  
198 (Mardén, 1985) up to post-exponential phase prior to analysis.

199

200 Adhesion assay on polystyrene

201 Post exponential phase grown cells were centrifuged and resuspended in artificial sea water  
202 (ASW). Then  $200 \mu\text{L}$  of cells were inoculated at  $\text{OD}_{600\text{nm}} 0.3$  in triplicate in black microplates  
203 (sterile black PS; Nunc, Fisher Scientific, Illkirch, france). After 24h of incubation at  $20^{\circ}\text{C}$ , the

204 non-adhered bacteria were eliminated by three successive washes (36g.L<sup>-1</sup> sterile NaCl  
205 solution). The adhered bacteria were stained by both Syto 61 Red and Syto 9 Green fluorescent  
206 markers targeting bacterial DNA (Life technology). After 10 min, the excess stain was  
207 eliminated by one wash. Fluorescence intensity (FI) was measured using an Infinit 200  
208 microplate fluorescence reader (Tecan, Lyon, France). A fluorescent intensity was calculated  
209 per well: Fluorescent intensity (FI) = FI average assay / FI average negative control. Three  
210 independent assays were done for each strain tested. Same results were found with both stains  
211 (data not shown).

212

### 213 Adhesion assay on copolymers

214 Adhesion assay on copolymers was performed as described for adhesion assay on polystyrene  
215 excepted for the following points. PVC coverslips of 13 mm of diameter were coated with the  
216 MB6 and MC3MB6 polymers. Each copolymer previously dissolved in toluene, at a 40–50  
217 wt% solid content, were applied on PVC coverslips with a bar-coater resulting in about 100 µm  
218 dried thickness coatings. Coverslips were inserted in 24 well microplates (sterile transparent  
219 PS; VWR) and sterilized 15 minutes with UV. Post-exponential bacterial strains were  
220 resuspended in ASW and inoculated at OD<sub>600nm</sub> 1 in the microplates. After 24h of incubation  
221 at 20°C strains were labelled with 5µM of Syto 9 Green fluorescent nucleic acid stain (Life  
222 technology). After 10 min, the excess stain was eliminated by 3 washes. Fluorescence intensity  
223 (FI) was measured using an Infinit 200 microplate fluorescence reader (Tecan, Lyon, France).  
224 A fluorescent intensity was calculated per well: Fluorescent intensity (FI) = FI average assay /  
225 FI average negative control. Three independent assays were done for each strain tested.

226

227 CSLM observation

228 The same coverlips were used for the observation of the bacteria on the surfaces using CSLM  
229 Zeiss LSM 510. Briefly, the coated coverlips were glued onto a glass slide and covered with  
230 prolong antifade (Life technology) and a new glass coverslips. After 48 h drying, the samples  
231 were stored at 4°C until use for CSLM observation.

232

233 Atomic force microscopy imaging

234 AFM contact mode images were obtained in air, at room temperature, using a Nanoscope VIII  
235 Multimode AFM (Nano Surfaces Business, Bruker Corporation, Santa Barbara, CA), MSCT  
236 cantilevers with a nominal spring constant of ~0.01 N/m (calculated with the thermal noise  
237 method), and a scanning rate of 2 Hz. One hundred  $\mu$ l of cell suspension from post-exponential  
238 growth phase was put in contact with freshly cleaved mica supports mounted on steel pucks.  
239 The samples were incubated for 2 h at 30 °C, gently rinsed in three successive baths of ultrapure  
240 water (Elga, purelab), and allowed to dry at 30°C overnight.

241

242 Cell probes

243 For single-bacterial cell force spectroscopy, cell probes were prepared using a recently  
244 developed protocol that combines colloidal probe cantilevers and bioinspired polydopamine  
245 wet adhesives (Beaussart et al., 2013). Briefly, silica microspheres (6.1  $\mu$ m diameter, Bangs  
246 laboratories) were attached on triangular shaped tipless cantilevers (NP-O10, Microlevers,  
247 Bruker Corporation) using UV-curable glue (NOA 63, Norland Edmund Optics). The  
248 cantilevers were then immersed for 1 h in a 10 mM Tris Buffer solution (pH 8.5) containing 4  
249 mg ml<sup>-1</sup> dopamine hydrochloride (99%, Sigma), and dried with N<sub>2</sub> flow. Single bacteria were  
250 then attached onto polydopamine-coated colloidal probes using a Bioscope Catalyst AFM

251 (Bruker corporation, Santa Barbara, CA). To this end, 2  $\mu$ l of a cell suspension were added to  
252 4 ml of ASW solution (pH 8, Sea salts, Sigma) in a glass Petri dish containing MB6 and  
253 MC3MB6 substrates. A single probe was brought into contact with an isolated cell for 3 min,  
254 and the obtained cell probe was then transferred over a solid substrate for further force  
255 measurements. Viability of the attached bacteria was checked using a Live-dead *BacLight*  
256 viability kit (Invitrogen, kit L7012) following the manufacturer instructions.

257

258 **Single-cell force spectroscopy measurements**

259 SCFS measurements were performed at room temperature (20 °C) in in ASW solution pH 8  
260 and using a Bioscope Catalyst AFM (Bruker AXS Corporation, Santa Barbara, CA). The  
261 nominal spring constant of the colloidal probe cantilever was  $\sim 0.06 \text{ N m}^{-1}$ , as determined by  
262 the thermal noise method. Multiple force-distance curves were recorded on various spots of  
263 MB6 and MC3MB6 substrates using a maximum applied force of 250 pN, a contact time of 100  
264 ms or 1s, and constant approach and retraction speeds of  $1000 \text{ nm s}^{-1}$ . For each condition, the  
265 interaction forces of 3 bacterial cells from independent cultures were measured and  $n > 400$   
266 force curves were recorded for each bacteria.

267

268 **Results and discussion**

269

270 **Polymers synthesis and characterization of copolymer surfaces**

271 Well-defined diblock copolymers combining a tert-butyldimethylsilyl methacrylate (SiMA)-  
272 based block, as hydrolyzable “SPC-type” monomer, with a poly(dimethylsiloxane) (PDMS)  
273 block, as hydrophobic, “FRC-type” monomer have been investigated. The synthesis of the  
274 PDMS-*b*-p(SiMA-*stat*-BMA) block copolymer called MC3MB6 was achieved from  
275 copolymerizations of *tert*-butyldimethylsilyl methacrylate (SiMA) and butyl methacrylate on

276 PDMS macro-RAFT agents. The methodology relies on the synthesis of PDMS monofunctional  
277 chain transfer agents easily available in one synthetic step from commercially available  
278 hydroxylated PDMSs (Duong et al., 2014). A statistical copolymer P(SiMA-*stat*-BMA) called  
279 MB6, with a composition similar to the second block of MC3MB6, has also been prepared  
280 (Table 1). As these copolymers might be used in marine environment as coatings, their ability  
281 to form films without cracking is required. Good film properties have been displayed for MB6  
282 and MC3MB6 due to their low glass transition temperature of 45-46°C corresponding to  
283 P(SiMA-*stat*-BMA) block. In the case of MC3MB6, the  $T_g$  of the PDMS block (from -127°C  
284 to -124°C) was not visible because of a low amount of DMS monomer units within the  
285 copolymer (Duong et al., 2014). Surface properties including wetting properties and  
286 smoothness have been investigated. Table 2 shows that the water contact angle values increased  
287 and the polar component of the surface free energy ( $\gamma_s^P$ ) decreased when the PDMS block was  
288 added within the copolymer. Taken together these results show that MC3MB6 is more  
289 hydrophobic than its MB6 counterpart which could suggest according to the literature a close  
290 packing of the pendant methyl groups of the flexible siloxane chain at the film/air interface  
291 (Lejars et al., 2012). Tapping-mode AFM analysis shows the topography of the PDMS-based  
292 films to be smoother than the MB6 PDMS-free coating (Figure 1). In addition, the flexibility  
293 of the PDMS block coming from its low  $T_g$  value and the flexibility of the methacrylic block  
294 coming from the presence of BMA monomer units provided soft samples. A lower Young's  
295 modulus value and a higher indentation were found for the PDMS-based sample (Table 3).  
296 Taken together these results show that MC3MB6 surface is softer than MB6 one.  
297 When immersing these silylester-based polymers in artificial seawater, the hydrophilic  
298 character of the two coating surfaces increased with time as the water contact angle  $\theta_{H_2O}$   
299 decreases with immersion time (Figure 2). This result is in agreement with the well-known  
300 hydrolysis reaction of the hydrophobic silyl ester groups of SiMA units into hydrophilic sodium

301 carboxylate groups in artificial seawater (Bressy and Margailan, 2009). Nevertheless, the  
302 surface of the PDMS-based coating MC3MB6 remains more hydrophobic than the MB6 one.

303

#### 304 **Adhesion tests of marine bacteria on polystyrene**

305 Biofilm formation has been previously evaluated in different rich marine media for a number  
306 of marine bacteria isolated from the Mediterranean sea (Camps et al., 2011; Brian-Jaisson et  
307 al., 2014). Five of these marine bacterial strains (Table S1), which were all isolated from  
308 biofilms formed on immersed supports in the bay of Toulon (France), were analyzed for their  
309 adhesion ability in artificial sea water (ASW) on polystyrene (Figure 3). Three of them (TC9-  
310 TC10 and TC11) belong to *Shewanella* genus. TC5 and TC8 belong respectively to  
311 *Polaribacter* and *Pseudoalteromonas* genus (Table S1). All strains except TC5, were able to  
312 form a biofilm in laboratory conditions (Brian-Jaisson et al., 2014). In this study, strains  
313 exhibited different adhesion patterns on polystyrene after 24h (Figure 3) in a poor medium,  
314 artificial sea water (ASW). Bacterial adhesion of TC11 was the strongest. TC8 adhered to  
315 polystyrene but fluorescent intensity was 1.6 times less than TC11. Adhesion on polystyrene  
316 was weak for TC5 and very weak for the TC10 and TC9 strains. Three profiles based on  
317 adhesion on polystyrene and biofilm formation pattern can be identified : i) a weak adhesion  
318 profile in ASW with an incapacity to form biofilm in rich media (Brian-Jaisson et al., 2014) for  
319 TC5; ii) a strong adhesion on polystyrene with a strong ability to form biofilm in rich media for  
320 TC8 and TC11; iii) a weaker ability to adhere on polystyrene in ASW and a slower capacity to  
321 form a biofilm in rich media for TC9 and TC10, corresponding thus to an intermediary  
322 phenotypic between the 2 first groups. For the following approaches, we therefore chose to  
323 work with one strain of each group. TC5 belonging to the *Polaribacter* genus, a non-motile  
324 marine bacteria, is the most hydrophobic of the 3 strains according to Microbial Adhesion to  
325 Solvents (MATS) assays, has a weak adhesion profile and has a poor ability to form biofilm

326 (Brian-Jaisson et al., 2014). TC10 and TC11 are 2 different strains of *Shewanella*, which are  
327 overall more hydrophilic and are motile. TC11 is able of a stronger adhesion and a faster  
328 capacity to form a biofilm on polystyrene while for TC10, it takes more time to form its biofilm  
329 (Brian-Jaisson et al., 2014).

330

### 331 **AFM imaging to unravel morphological features of bacteria species.**

332 We used AFM contact mode imaging in air to visualize the general cell topography of TC5,  
333 TC10 and TC11. For all strains, bacteria were small rod-shaped, which agree well with  
334 observations performed previously (Brian-Jaisson et al., 2014). TC5 and TC10 were about 3.4  
335 micrometer long. TC11 seemed to be smaller and was about 2.6 micrometers long (Figure 4).  
336 Pili and flagella are major contributors to mobility, adhesion and biofilm formation (Mattick,  
337 2002; Telford et al., 2006; Pelicic, 2008; Belas, 2014; Lavery et al., 2014). As suspected  
338 flagella were clearly present on the surface of TC10 and TC11, while they were not seen for  
339 TC5, previously described as non-motile (Figure 4) (Brian-Jaisson et al., 2014). Furthermore,  
340 in few images of TC10, we observed a smaller and thinner structure, which could be pili, on  
341 the surface of this strain (Figure 4D, white thin arrow). Overall, pili were more difficult to  
342 observe than flagella. It is possible that pili were broken during the preparation of the cells for  
343 the AFM observation. Despite the presence of small residues particles most likely coming from  
344 the culture medium, spherical particles, which surround TC5 strain seem to be of different  
345 nature (Figure 4B, black triangle). We hypothesized that this strain produces outer membrane  
346 vesicles (OMV). A wide variety of Gram negative bacteria secrete OMV including marine  
347 bacteria such as *Prochlorococcus* or *Shewanella vesiculosa* (Beveridge, 1999; Perez-Cruz et  
348 al., 2013; Biller et al., 2014). OMV are implicated in many functions such as bacterial survival,  
349 pathogenicity, enzyme delivery and biofilm formation (Beveridge, 1999; Schooling and



350 Beveridge, 2006; Lee et al., 2008; Yonezawa et al., 2009; Baumgarten et al., 2012; van Hoek,  
351 2013; Altindis et al., 2014; Murphy et al., 2014; Orench-Rivera and Kuehn, 2016). Taken  
352 together, these observations show that the *Polaribacter* TC5 strain presents different features  
353 from the 2 *Shewanella* strains as it has no flagella and seems to present OMV at its surface.

354

### 355 **Evaluation of adhesion on copolymers at the population level**

356 In order to evaluate bacterial adhesion on the hybrid MC3MB6 and its control MB6, these  
357 polymers were coated onto round PVC coverslips (as they did not stick well on glass) and  
358 inserted in 24 well plates. Glass coverslips, widely used in fluorescence or CLSM microscopy  
359 experiments, served as a reference. Bacteria were then left to seed onto the surface for 24 h,  
360 washed off to remove non adherent bacteria and then stained using the fluorescent marker  
361 Syto9. Because some polymers can present an autofluorescence, a direct observation of the  
362 same samples was performed using CLSM. This double approach is rarely undergone when  
363 such coatings are used. Figure 5A and B show that for each coating tested, TC11 was the strain  
364 displaying stronger adhesive properties, in particular with glass alone. TC11 adhered 2 and 1.5  
365 times more than TC5 or TC10 on MB6 or MC3MB6 respectively (Figure 5B). All 3 strains  
366 adhered better on glass coverslips than on the polymers (Figure 5A and C), with no significant  
367 difference in adhesion between MB6 and MC3MB6 (Figure 5A). These results have been  
368 corroborated with the CSLM observation as very few bacteria can be seen on either MC3MB6  
369 or MB6 surfaces in sharp contrast with the glass surface (Figure 5C). Taken together, these  
370 results suggest that copolymers MB6 and MC3MB6 prevent bacterial adhesion. While the  
371 adhesion was overall very weak, TC11 appears to adhere slightly better on these 2 surfaces.  
372 MB6 alone, composed of SiMA-*stat*-BMA with self-hydrolysis properties (with no biocide) is  
373 self-sufficient for the inhibition of bacterial adhesion in these conditions. The hydrophobic  
374 PDMS blocks, do not add in efficacy whichever bacteria studied despite their different

375 hydrophilic surface properties (Brian-Jaisson et al., 2014). As previously described,  
376 hydrophobic/hydrophilic interactions can be easily overcome by the presence of extracellular  
377 appendages and covalent bindings, in particular in the stage of the “irreversible adhesion”  
378 (Garrett et al., 2008). Bacteria can adapt to their environment i.e. the presence of a surface, very  
379 rapidly by, temporarily and in coordinated manner, specifically expressing numbers of proteins  
380 anchored in the membrane or being part of the extracellular appendages that can modify and  
381 overcome such interactions.

382

### 383 **Single-cell adhesion force analysis.**

384 The results of the previous experiments reflect the behavior of the bacteria at the population  
385 level. In order to understand how each bacterium behaves on these surfaces at the single cell  
386 level, AFM was used in single-cell force spectroscopy (SCFS) mode (Helenius et al., 2008;  
387 Muller et al., 2009) to quantify the adhesive properties of individual TC5, TC10 and TC11  
388 bacterial cells towards surfaces MB6 and MC3MB6 (Beaussart et al., 2013). Briefly, a colloidal  
389 probe cantilever coated with polydopamine bioadhesive was used to pick up single cells without  
390 altering their viability (assessed using the Live-dead *BacLight* viability kit) and to measure  
391 force-distance curves between the bacterium and the surfaces MB6 and MC3MB6 (Figure 6).  
392 The 3 bacteria tested stayed alive during the course of the experiment.

393 We first used SCFS to investigate the adhesion force of single cells towards MB6 surfaces and  
394 the effect of contact time between cells and substrates. Figure 7 shows the adhesion force and  
395 rupture length histograms, together with representative force curves, obtained between TC5  
396 (Figure 7A and B), TC10 (Figure 7C and D) and TC11 (Figure 7E and F) cells and MB6  
397 surfaces at short (Figure 7A, C, and E) and prolonged (Figure 7B, D, and F) contact times.  
398 Consecutive force curves were recorded on different spots of the substrate and no changes were  
399 observed regarding the general features of the curves, indicating the cells were not damaged

400 and cell surface properties were not altered by force measurements. Cell from independent  
401 cultures were analysed and generally yielded similar behaviour although sometimes one cell  
402 showed differences (Figure 7C, D, and F) that we attribute to heterogeneity of the bacterial  
403 population. This phenotypic heterogeneity was less pronounced for TC5, whereas it was more  
404 obvious for TC11 whether at a short or long contact time. At short contact time, the adhesion  
405 frequency of TC5 cells on MB6 surfaces was ~30-35 % with adhesive force curves presenting  
406 force of 50-400 pN and rupture distances of 100-900 nm (Figure 7A). Prolonged contact time  
407 (1 s) led to increased adhesion frequencies (75-90 %), increased adhesion forces (from 300 to  
408 2400 pN) and rupture lengths in the same range as at short contact time, yet with higher  
409 frequencies of short rupture distances (Figure 7B). At short contact time, most force distance  
410 curves presented multiple well-defined individual peaks of 50-100 pN (Figure 7A, right  
411 histogram inset). According to previous observations, we attribute those multiple peaks  
412 signatures with flat regions preceding each peak to type IV pili interaction with MB6 surfaces  
413 (Touhami et al., 2006; Biais et al., 2010). The absence of such structures on bacterial images  
414 (Figure 4A and B) suggest that TC5 pili are fragile, short or could be retracted during sample  
415 drying. On 1 s contact time force curves, similar peaks were sometimes observed but the short  
416 distances interaction at higher forces (>300 pN) seemed to govern the adhesion of cell on MB6  
417 surfaces. Such first large force and short distance adhesive events phenomenon could be  
418 attributed to the outer membrane surface property itself that needs longer contact time for  
419 interaction rather than appendages or adhesives molecules that would lead to longer rupture  
420 lengths. Analysis of TC10 cells led to similar results, still with few differences. Increasing the  
421 contact time did not significantly increases the adhesion frequency or the range of forces. As  
422 for TC5, force curves signatures obtained for TC10 suggested type IV pili interaction and this  
423 conclusion was confirmed by AFM images (Figure 4D). For TC11 cells, the adhesion frequency  
424 did not increase with contact time. However, adhesion forces of some cells significantly

425 increased up to 10 nN (Figure 7F). These high forces corresponded to short rupture distances  
426 peak suggesting strong hydrophobic interaction between the cell surface and the MB6 substrate.  
427 At short contact time, force curves frequently showed a first adhesive event with sometimes a  
428 sawtooth pattern (Figure 7E, inset in right histogram, upper curve). This first peak may  
429 correspond to cell surface proteins interacting with MB6 surface and strengthened in force and  
430 number under prolonged contact time. On short contact time curves, although no pili were  
431 detected on bacterial images (Figure 4E and F), peaks following the initial adhesive event  
432 presented signatures that could be attributed to pili and as for TC5, we hypothesize that those  
433 pili were fragile, short or could be retracted on image samples. Force curves obtained after  
434 prolonged contact time revealed sawtooth pattern with regular peaks and long rupture distances.  
435 Based on previous observations, these signatures could correspond to proteins interacting with  
436 the surface and containing multiple repeats that are unfolded upon bacterial pulling from the  
437 substrate (Alsteens et al., 2009; Beaussart et al., 2014; El-Kirat-Chatel et al., 2014a).

438

439 The MC3MB6 surface was used similarly to evaluate the impact of the surface chemistry  
440 change on bacterial adhesion of TC5, TC10 and TC11 cells. Figure 8 shows the adhesion force  
441 and rupture length histograms, together with representative force curves, obtained between TC5  
442 (Figure 8A and B), TC10 (Figure 8C and D) and TC11 (Figure 8 E and F) cells and surfaces  
443 MC3MB6 at short (Figure 8A, C, and E) and prolonged (Figure 8B, D, and F) contact times.  
444 TC5 cells presented high frequency adhesion towards surface MC3MB6 (more than 70 % at  
445 short contact times and about 100 % at prolonged contact times). Increasing contact time  
446 resulted in higher adhesion force (from 300-2000 pN to 1200-4800 pN). Force curves recorded  
447 for TC5 on surface MC3MB6 presented large initial force peaks followed by smaller forces that  
448 may correspond to stretching of cell surface molecules. Compared to results obtained on  
449 surfaces MB6, force curves recorded on surface MC3MB6 rarely presented pili signature,

450 suggesting that TC5 pili are mostly involved in interaction with surface MB6 and that the  
451 interaction with surface MC3MB6 is governed by the cell wall itself together with surface  
452 adhesive molecules. TC10 cells presented slightly similar adhesive profile on surface MC3MB6  
453 and surface MB6. Its adhesion to both surfaces is lower in term of frequency and force than the  
454 adhesion of TC5. Based on force curves shape, this adhesion seems to be mainly controlled by  
455 pili at short contact time (small peaks at long distance and visualisation of pili on image Figure  
456 4) and at longer contact time, the cell adhesion through pili seems to be reinforced by cell wall  
457 and surface molecule (large initial peak and sawtooth pattern of molecules unfolding). TC11  
458 cells were slightly more adhesive to surface MC3MB6 than what was observed for surfaces  
459 MB6. Still, the interaction looks similar with almost no pili signature but rather protein  
460 unfolding and large initial peaks suggesting that TC11 adhere mainly through cell wall  
461 hydrophobicity and adhesive macromolecules containing repeated domains unfolded upon  
462 pulling. Phenotypic heterogeneity in adhesion was also more obvious on MC3MB6 for TC11  
463 than for the 2 other strains, whether on short or long contact time.

464

465 To validate the specificity of the measured adhesion forces and rule out the possibility of  
466 artefact associated with the cell probe preparation, a control experiment was performed using a  
467 1s contact time (Figure S2). Use of polydopamine-coated probes instead of bacterial probes led  
468 to a major reduction of adhesion frequency and no signatures similar to what we observed for  
469 cells were present. This control indicates that the adhesive events measured above reflect the  
470 interactions between bacteria and coatings.

471

472 Taken together, these results show first that TC5 was the most adhesive of the 3 strains on both  
473 surfaces in terms of frequency and presented large adhesion forces, in particular on the  
474 hydrophobic surface, MC3MB6, while TC10 showed a weaker adhesion on both surfaces with

475 adhesion forces averaging 1000 or 1200 pN. The influence of surface chemistry is mostly  
476 observed for TC5. This comes in contrast with the results found at the population level, as TC11  
477 was the bacteria that adhered the most efficiently on polystyrene, glass as well as on MB6 and  
478 MC3MB6, even though adhesion on the antifouling surfaces was overall very low (Figure 4).  
479 Second, different extracellular components seem to be involved in the 3 strains adhesion on the  
480 surfaces. Short distance interactions at higher forces govern adhesion of TC5 and TC10 on the  
481 surfaces (Dufrene, 2015). Adhesion seems to be controlled by pili, cell wall on MB6 (and  
482 MC3MB6 for TC10) and by cell wall and stretching of surface molecules on MC3MB6. TC11  
483 adheres mainly through cell wall hydrophobicity and adhesive macromolecules containing  
484 repeated domains unfolded upon pulling. Third, some TC11 cells were slightly more adhesive  
485 to both surfaces (with long rupture distances) than others. While TC5 and in a lesser extent  
486 TC10 showed a more homogenous response towards the surfaces, TC11 presented  
487 heterogeneous adhesion profiles toward both surfaces, with some bacterial cells presenting  
488 weak adhesion forces and some of them presenting very strong ones. Phenotypic heterogeneity  
489 within a population, which corresponds to the expression of substantial phenotypical  
490 differences by individuals when they are in a similar context, is thought to allow better chance  
491 of survival for the population as a whole entity. A subpopulation may be then better equipped  
492 to face stressful situations and settle in new environmental niches. This heterogeneity can come  
493 from variations of gene expression at the single cell level but also from allelic variations (Davis  
494 and Isberg, 2016). Some bacteria are more susceptible to genetic variations than others (for  
495 instance the ones undergoing phase variation). These phenomena have been described for  
496 instance in biofilm with the apparition of persisters as well as in relation with QS dependent  
497 mechanisms due to highly heterogeneous gene expression at a single cell level (Grote et al.,  
498 2015). This is most likely a widespread phenomenon, which just started to be highlighted in the  
499 literature with the development of single cell approaches, even though this variability may

500 differs from a bacterium to another. This emerging evidence of phenotypical variability need to  
501 be studied more precisely at the molecular and cellular level in order to understand how these  
502 variations can make a subpopulation adapt and survive in an environmental niche. It is possible  
503 that TC11 fits to this description and can present at the population level a better fitness when  
504 facing a new environment than TC5 or TC10. Phenotypic heterogeneity, and differential  
505 bacterial-bacterial collaborative interactions involved in biofilm formation could explain the  
506 reason why, despite a weaker adhesion at the single cell level, TC11 is able to form more biofilm  
507 than TC5 including in ASW (data not shown). Further studies would be required using, for  
508 instance, other single cell techniques, such as the newly developed single cell RNA-seq,  
509 allowing a broader vision of the variability to confirm these results (Davis and Isberg, 2016).

510

511

## 512 **Supporting Information**

513 Additional supporting information may be found in the online version of this article at the  
514 publisher's web-site:

515 Detailed experimental procedures

516 Table S1

517 Figure S1

518 Figure S2

519

## 520 **Conflict of interests**

521 The authors declare that they have no conflict of interests.

522

## 523 **Funding**

524 This work was supported by MAPIEM laboratory of the Université de Toulon, France and by  
525 the institute of Life Sciences of the Université catholique de Louvain, Belgium. PVO is the recipient of  
526 a French PACA Region doctoral fellowship.

527

## 528 **Acknowledgments**

529 none

## 530 **References**

- 531 Alsteens, D., Dupres, V., Klotz, S.A., Gaur, N.K., Lipke, P.N., and Dufrene, Y.F. (2009)  
532 Unfolding individual als5p adhesion proteins on live cells. *ACS Nano* **3**: 1677-1682.
- 533 Altindis, E., Fu, Y., and Mekalanos, J.J. (2014) Proteomic analysis of *Vibrio cholerae* outer  
534 membrane vesicles. *Proc Natl Acad Sci U S A* **111**: E1548-1556.
- 535 Baumgarten, T., Sperling, S., Seifert, J., von Bergen, M., Steiniger, F., Wick, L.Y., and  
536 Heipieper, H.J. (2012) Membrane vesicle formation as a multiple-stress response mechanism  
537 enhances *Pseudomonas putida* DOT-T1E cell surface hydrophobicity and biofilm formation.  
538 *Appl Environ Microbiol* **78**: 6217-6224.
- 539 Beaussart, A., El-Kirat-Chatel, S., Herman, P., Alsteens, D., Mahillon, J., Hols, P., and Dufrene,  
540 Y.F. (2013) Single-cell force spectroscopy of probiotic bacteria. *Biophys J* **104**: 1886-1892.
- 541 Beaussart, A., El-Kirat-Chatel, S., Sullan, R.M.A., Alsteens, D., Herman, P., Derclaye, S., and  
542 Dufrene, Y.F. (2014) Quantifying the forces guiding microbial cell adhesion using single-cell  
543 force spectroscopy. *Nat Protocols* **9**: 1049-1055.
- 544 Belas, R. (2014) Biofilms, flagella, and mechanosensing of surfaces by bacteria. *Trends*  
545 *Microbiol* **22**: 517-527.
- 546 Belec, L., Nguyen, T., Nguyen, D., and Chailan, J. (2015) Comparative effects of humid  
547 tropical weathering and artificial ageing on a model composite properties from nano-to macro-  
548 scale. *Composites Part A: Applied Science and Manufacturing* **68**: 235-241.
- 549 Beveridge, T.J. (1999) Structures of gram-negative cell walls and their derived membrane  
550 vesicles. *J Bacteriol* **181**: 4725-4733.
- 551 Biais, N., Higashi, D.L., Brujic, J., So, M., and Sheetz, M.P. (2010) Force-dependent  
552 polymorphism in type IV pili reveals hidden epitopes. *Proc Natl Acad Sci U S A* **107**: 11358-  
553 11363.
- 554 Biller, S.J., Schubotz, F., Roggensack, S.E., Thompson, A.W., Summons, R.E., and Chisholm,  
555 S.W. (2014) Bacterial vesicles in marine ecosystems. *Science* **343**: 183-186.
- 556 Bressy, C., and Margaillan, A. (2009) Erosion study of poly (trialkylsilyl methacrylate)-based  
557 antifouling coatings. *Progress in Organic Coatings* **66**: 400-405.
- 558 Bressy, C., NGuyen, M.N., Tanguy, B., Ngo, V.G., and Margaillan, A. (2010) Poly (trialkylsilyl  
559 methacrylate) s: a family of hydrolysable polymers with tuneable erosion profiles. *Polymer*  
560 *Degradation and Stability* **95**: 1260-1268.



561 Bressy, C., Hellio, C., Nguyen, M.N., Tanguy, B., Maréchal, J.-P., and Margaillan, A. (2014)  
562 Optimized silyl ester diblock methacrylic copolymers: A new class of binders for chemically  
563 active antifouling coatings. *Progress in Organic Coatings* **77**: 665-673.

564 Brian-Jaisson, F., Ortalo-Magne, A., Guentas-Dombrowsky, L., Armougom, F., Blache, Y.,  
565 and Molmeret, M. (2014) Identification of bacterial strains isolated from the Mediterranean Sea  
566 exhibiting different abilities of biofilm formation. *Microb Ecol* **68**: 94-110.

567 Briand, J.F., Djeridi, I., Jamet, D., Coupe, S., Bressy, C., Molmeret, M. et al. (2012) Pioneer  
568 marine biofilms on artificial surfaces including antifouling coatings immersed in two  
569 contrasting French Mediterranean coast sites. *Biofouling* **28**: 453-463.

570 Butt, H.-J., Cappella, B., and Kappl, M. (2005) Force measurements with the atomic force  
571 microscope: Technique, interpretation and applications. *Surface science reports* **59**: 1-152.

572 Camps, M., Briand, J.F., Guentas-Dombrowsky, L., Culioli, G., Bazire, A., and Blache, Y.  
573 (2011) Antifouling activity of commercial biocides vs. natural and natural-derived products  
574 assessed by marine bacteria adhesion bioassay. *Mar Pollut Bull* **62**: 1032-1040.

575 Davis, K.M., and Isberg, R.R. (2016) Defining heterogeneity within bacterial populations via  
576 single cell approaches. *Bioessays* **38**: 782-790.

577 Dufrene, Y.F. (2015) Sticky microbes: forces in microbial cell adhesion. *Trends Microbiol* **23**:  
578 376-382.

579 Duong, T.H., Bressy, C., and Margaillan, A. (2014) Well-defined diblock copolymers of poly  
580 (tert-butyltrimethylsilyl methacrylate) and poly (dimethylsiloxane) synthesized by RAFT  
581 polymerization. *Polymer* **55**: 39-47.

582 Duong, T.H., Briand, J.-F., Margaillan, A., and Bressy, C. (2015) Polysiloxane-Based Block  
583 Copolymers with Marine Bacterial Anti-Adhesion Properties. *ACS Appl Mater Interfaces* **7**:  
584 15578-15586.

585 El-Kirat-Chatel, S., Boyd, C.D., O'Toole, G.A., and Dufrene, Y.F. (2014a) Single-molecule  
586 analysis of *Pseudomonas fluorescens* footprints. *ACS Nano* **8**: 1690-1698.

587 El-Kirat-Chatel, S., Beaussart, A., Boyd, C.D., O'Toole, G.A., and Dufrêne, Y.F. (2014b)  
588 Single-Cell and Single-Molecule Analysis Deciphers the Localization, Adhesion, and  
589 Mechanics of the Biofilm Adhesin LapA. *ACS Chem Biol* **9**: 485-494.

590 Garrett, T.R., Bhakoo, M., and Zhang, Z. (2008) Bacterial adhesion and biofilms on surfaces.  
591 *Progress in Natural Science* **18**: 1049-1056.

592 Grote, J., Krysciak, D., and Streit, W.R. (2015) Phenotypic Heterogeneity, a Phenomenon That  
593 May Explain Why Quorum Sensing Does Not Always Result in Truly Homogenous Cell  
594 Behavior. *Appl Environ Microbiol* **81**: 5280-5289.

595 Helenius, J., Heisenberg, C.P., Gaub, H.E., and Muller, D.J. (2008) Single-cell force  
596 spectroscopy. *J Cell Sci* **121**: 1785-1791.

597 Kang, S., and Elimelech, M. (2009) Bioinspired Single Bacterial Cell Force Spectroscopy.  
598 *Langmuir* **25**: 9656-9659.

599 Lavery, G., Gorman, S.P., and Gilmore, B.F. (2014) Biomolecular Mechanisms of  
600 *Pseudomonas aeruginosa* and *Escherichia coli* Biofilm Formation. *Pathogens* **3**: 596-632.

601 Lee, E.Y., Choi, D.S., Kim, K.P., and Gho, Y.S. (2008) Proteomics in gram-negative bacterial  
602 outer membrane vesicles. *Mass Spectrom Rev* **27**: 535-555.

603 Lejars, M., Margaillan, A., and Bressy, C. (2012) Fouling release coatings: a nontoxic  
604 alternative to biocidal antifouling coatings. *Chem Rev* **112**: 4347-4390.

605 Lejars, M., Margaillan, A., and Bressy, C. (2013) Well-defined graft copolymers of tert-  
606 butyltrimethylsilyl methacrylate and poly (dimethylsiloxane) macromonomers synthesized by  
607 RAFT polymerization. *Polymer Chemistry* **4**: 3282-3292.

608 Lejars, M., Margaillan, A., and Bressy, C. (2014) Synthesis and characterization of diblock and  
609 statistical copolymers based on hydrolyzable siloxy silylester methacrylate monomers. *Polymer*  
610 *Chemistry* **5**: 2109-2117.

611 Loskill, P., Hahl, H., Thewes, N., Kreis, C.T., Bischoff, M., Herrmann, M., and Jacobs, K.  
612 (2012) Influence of the subsurface composition of a material on the adhesion of staphylococci.  
613 *Langmuir* **28**: 7242-7248.

614 Mardén, P.T., A.; Malmcrona-Friberg, K.; Odham, G.; and Kjelleberg, S. (1985) Physiological  
615 and morphological changes during short term starvation of marine bacterial isolates. In *Archives*  
616 *of Microbiology*, p. 7.

617 Martins, B.M., and Locke, J.C. (2015) Microbial individuality: how single-cell heterogeneity  
618 enables population level strategies. *Curr Opin Microbiol* **24**: 104-112.

619 Mattick, J.S. (2002) Type IV pili and twitching motility. *Annu Rev Microbiol* **56**: 289-314.

620 Muller, D.J., Helenius, J., Alsteens, D., and Dufrene, Y.F. (2009) Force probing surfaces of  
621 living cells to molecular resolution. *Nat Chem Biol* **5**: 383-390.

622 Murphy, K., Park, A.J., Hao, Y., Brewer, D., Lam, J.S., and Khursigara, C.M. (2014) Influence  
623 of O polysaccharides on biofilm development and outer membrane vesicle biogenesis in  
624 *Pseudomonas aeruginosa* PAO1. *J Bacteriol* **196**: 1306-1317.

625 Orench-Rivera, N., and Kuehn, M.J. (2016) Environmentally controlled bacterial vesicle-  
626 mediated export. *Cell Microbiol* **18**: 1525-1536.

627 Owen, D., and Wendt, R. (1969) Estimation of the surface free energy of polymer. *J Appl Polym*  
628 *Sci* **13**: 1741-1747.

629 Pelicic, V. (2008) Type IV pili: e pluribus unum? *Mol Microbiol* **68**: 827-837.

630 Perez-Cruz, C., Carrion, O., Delgado, L., Martinez, G., Lopez-Iglesias, C., and Mercade, E.  
631 (2013) New type of outer membrane vesicle produced by the Gram-negative bacterium  
632 *Shewanella vesiculosa* M7T: implications for DNA content. *Appl Environ Microbiol* **79**: 1874-  
633 1881.

634 Schooling, S.R., and Beveridge, T.J. (2006) Membrane vesicles: an overlooked component of  
635 the matrices of biofilms. *J Bacteriol* **188**: 5945-5957.

636 Schultz, M.P. (2007) Effects of coating roughness and biofouling on ship resistance and  
637 powering. *Biofouling* **23**: 331-341.

638 Telford, J.L., Barocchi, M.A., Margarit, I., Rappuoli, R., and Grandi, G. (2006) Pili in gram-  
639 positive pathogens. *Nat Rev Microbiol* **4**: 509-519.

640 Touhami, A., Jericho, M.H., Boyd, J.M., and Beveridge, T.J. (2006) Nanoscale characterization  
641 and determination of adhesion forces of *Pseudomonas aeruginosa* pili by using atomic force  
642 microscopy. *J Bacteriol* **188**: 370-377.

643 van Hoek, M.L. (2013) Biofilms: an advancement in our understanding of *Francisella* species.  
644 *Virulence* **4**: 833-846.

645 Yebra, D.M., Kiil, S., and Dam-Johansen, K. (2004) Antifouling technology—past, present and  
646 future steps towards efficient and environmentally friendly antifouling coatings. *Progress in*  
647 *organic coatings* **50**: 75-104.

648 Yonezawa, H., Osaki, T., Kurata, S., Fukuda, M., Kawakami, H., Ochiai, K. et al. (2009) Outer  
649 membrane vesicles of *Helicobacter pylori* TK1402 are involved in biofilm formation. *BMC*  
650 *Microbiol* **9**: 197.

651

## 652 **Illustrations and figures**

653

654 **Tables and figures legends**

655 Table 1. Characteristics of diblock and statistical copolymers prepared by RAFT  
 656 polymerization of SiMA and BMA from PDMS-macro RAFT agent at 70 °C in toluene.

Polymer	% mol	% mass	%	$M_n$ (g.mol <sup>-1</sup> )*	$D^*$
	(DMS/SiMA/BMA)	(DMS/SiMA/BMA)	$vol_{PDMS}$		
MC3MB6	31/10/59	18/16/66	19	59,700	1.1
MB6	0/14/86	0/18/82	0	49,500	1.1

657 \* assessed by triple detection size exclusion chromatography (TD-SEC)

658

659 Table 2. Wetting properties of the coating surfaces.

Polymer	Contact angle (°)				Surface energies (mJ.m <sup>-2</sup> )*		
	$\theta_{H_2O}$	$\sigma$	$\theta_{CH_2I_2}$	$\sigma$	$\gamma_s$	$\gamma_s^D$	$\gamma_s^P$
MC3MB6	101.9	1	71.5	0.6	22	20.9	1.1
MB6	91.3	0.5	64.3	4.6	26.7	23.3	3.3

660 \*using Owens-Wendt's method (Owen and Wendt, 1969)

661

662 Table 3. Young's modulus at the coating surfaces measured by AFM.

Polymer	$E$ (MPa)	Indentation (nm)
MC3MB6	73 ± 4	207 ± 4
MB6	88 ± 7	145 ± 5

663

664

665 **Figure 1. Height AFM images of A) MB6 and B)MC3MB6**

666 Rms is  $2.3 \pm 0.10$  and  $5.4 \pm 1$  for MC3MB6 and MB6 respectively with Ra of  $8.6$   
667  $\pm 0.6$  and  $14 \pm 7$  respectively.

668

669 **Figure 2. Evolution of the static water contact angle of MC3MB6 and MB6 with ASW**  
670 **immersion time.**

671

672 **Figure 3. Evaluation of the adhesion of five marine bacterial strains on polystyrene**

673 After 24h of incubation at  $20^{\circ}\text{C}$ , bacteria were stained by Syto 9 Green and fluorescent intensity  
674 was measured as a representation of bacterial adhesion. Bars represent the standard deviation  
675 obtained from three independent measures.

676

677 **Figure 4. Imaging bacterial surface in air.** AFM deflection and height (insets) images  
678 (contact mode) of post-exponentially growing cells that were directly deposited on mica and  
679 dried prior analysis. Vertical cross sections taken in the height images (dashed lines) are also  
680 shown to emphasize sizes of cellular structures.

681

682 **Figure 5. Adhesion of TC5, TC10 and TC11 on MB6 and MC3MB6 surfaces.** Adhesion  
683 was first measured at the population level in 24 well plates. A and B are different statistical  
684 analysis displays of the same measurement. After 24h of incubation at  $20^{\circ}\text{C}$ , bacteria were  
685 stained by Syto 9 green fluorescent nucleic and fluorescent intensity was measured using a  
686 TECAN microplate reader as a representation of bacterial adhesion. Bars represent the standard

687 deviation obtained from three independent measures. Statistical significance was accepted at  
688  $p < 0.05$ . \* for  $p < 0.05$ , \*\* for  $p < 0.01$  and \*\*\* for  $p < 0.001$ . C show the CSLM visualization of  
689 adhered bacteria on the surfaces. The same coverslips were used in the microplate assay and in  
690 CSLM. Glass coverslips were used as a control.

691

692 **Figure 6. The use of a microbead for single-cell force spectroscopy analysis.** A. Principle  
693 of single cell force spectroscopy with tip less cantilevers modified with colloidal beads and  
694 coated with polydopamine to attach a single bacteria (green) and probe it towards surfaces. B.  
695 Optical microscope image of a single bacterium attached to the colloidal cantilever probes  
696 documenting that the cell is properly located and alive (green fluorescence).

697

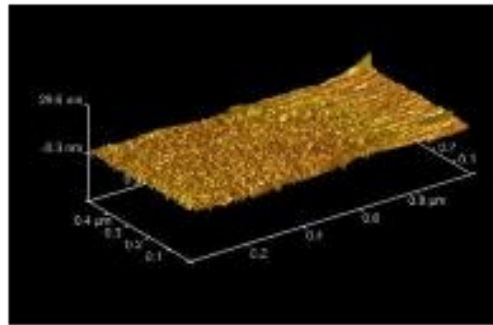
698 **Figure 7. Single-cell force spectroscopy analysis on surface MB6.** Adhesion force (left) and  
699 rupture length histograms with representative retraction force profiles (right) obtained by  
700 recording multiple force–distance curves between single TC5 (A and B), TC10 (C and D) or  
701 TC11 (E and F) bacteria and surface MB6 at short (100 ms, A, C and E) or prolonged (1s, B,  
702 D, F) contact times. Black, red, and blue colors represent results from three cells from  
703 independent cultures ( $n > 400$  force–distance curves for each cell).

704

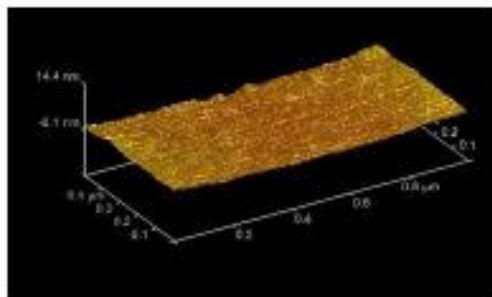
705 **Figure 8. Single-cell force spectroscopy analysis on surface MC3MB6.** Adhesion force (left)  
706 and rupture length histograms with representative retraction force profiles (right) obtained by  
707 recording multiple force–distance curves between single TC5 (A and B), TC10 (C and D) or  
708 TC11 (E and F) bacteria and surface MC3MB6 at short (100 ms, A, C and E) or prolonged (1s,  
709 B, D, F) contact times. Black, red, and blue colors represent results from three cells from  
710 independent cultures ( $n > 400$  force–distance curves for each cell).

711

A.



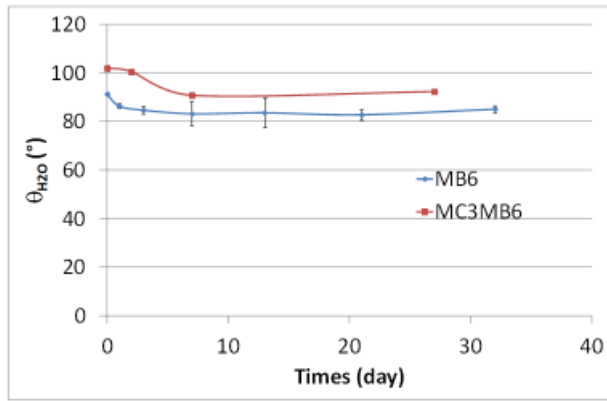
B.

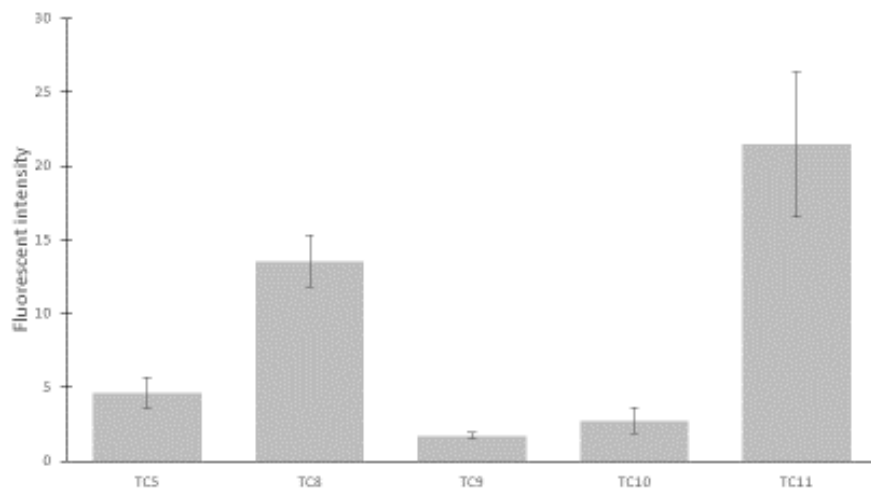


712

713

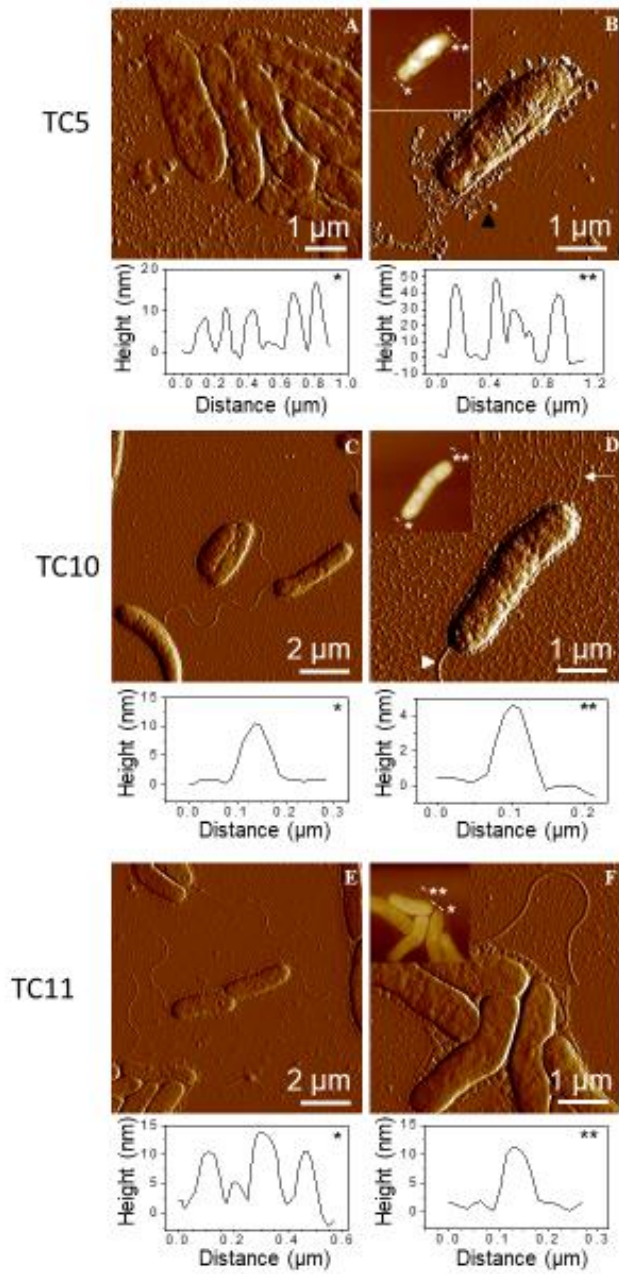
El-Kirat-Chatel et al. 2017 Figure 1.



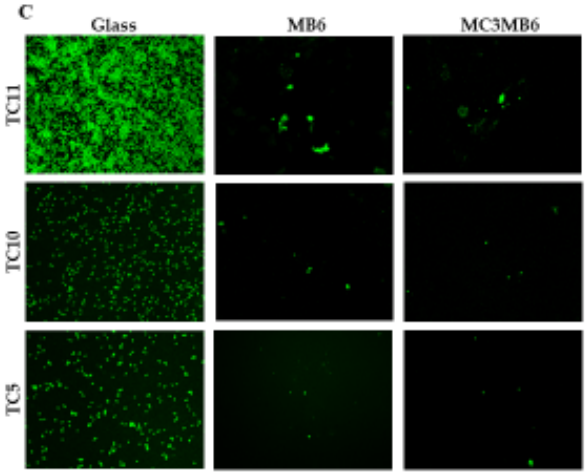
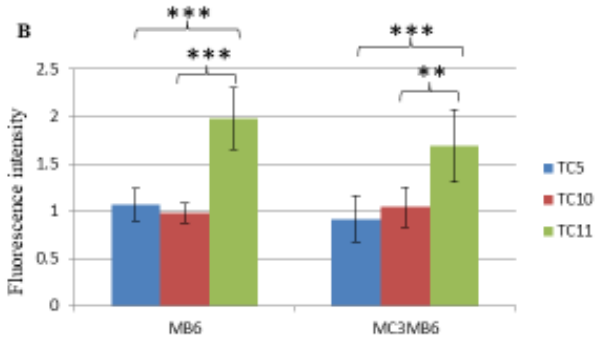
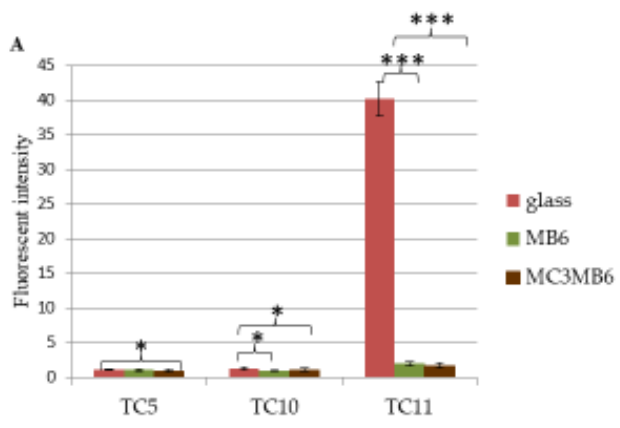


El-Kirat-Chatelet *et al.* 2017 Figure 3.

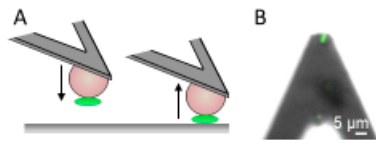




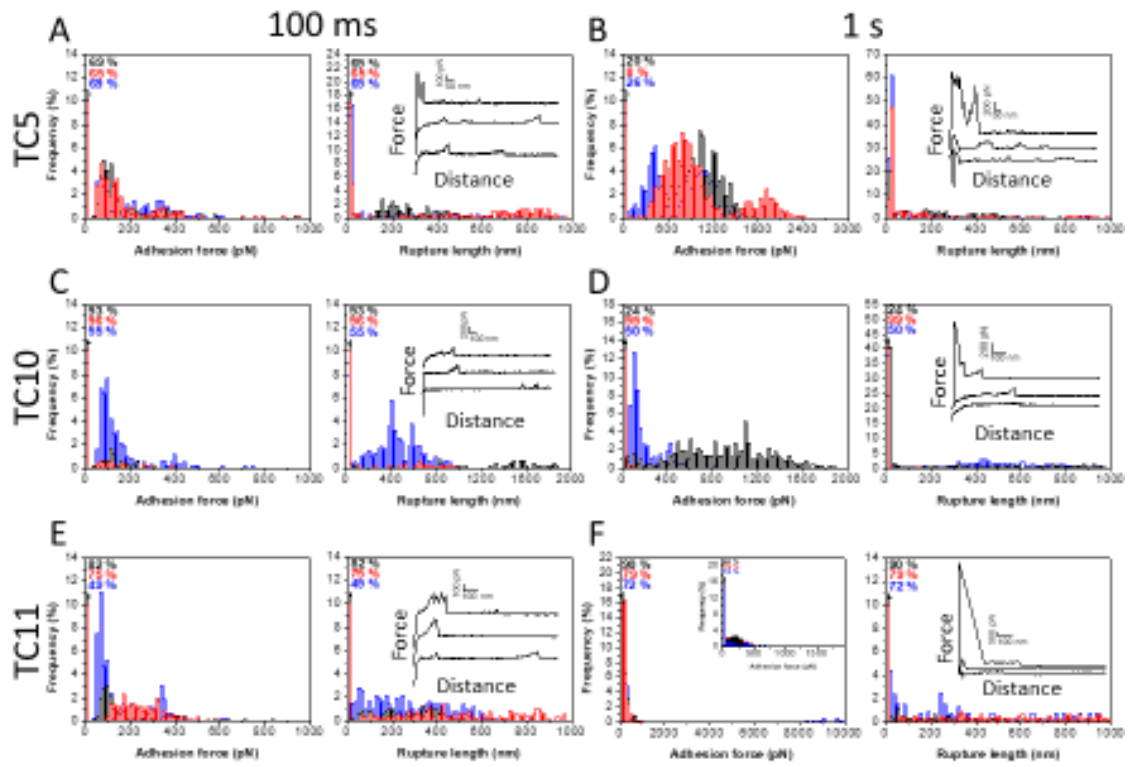
El-Kirat-Chatel *et al.*  
2017 Figure 4.



El-Kirat-Chatelet *et al.*  
2017  
Figure 5.

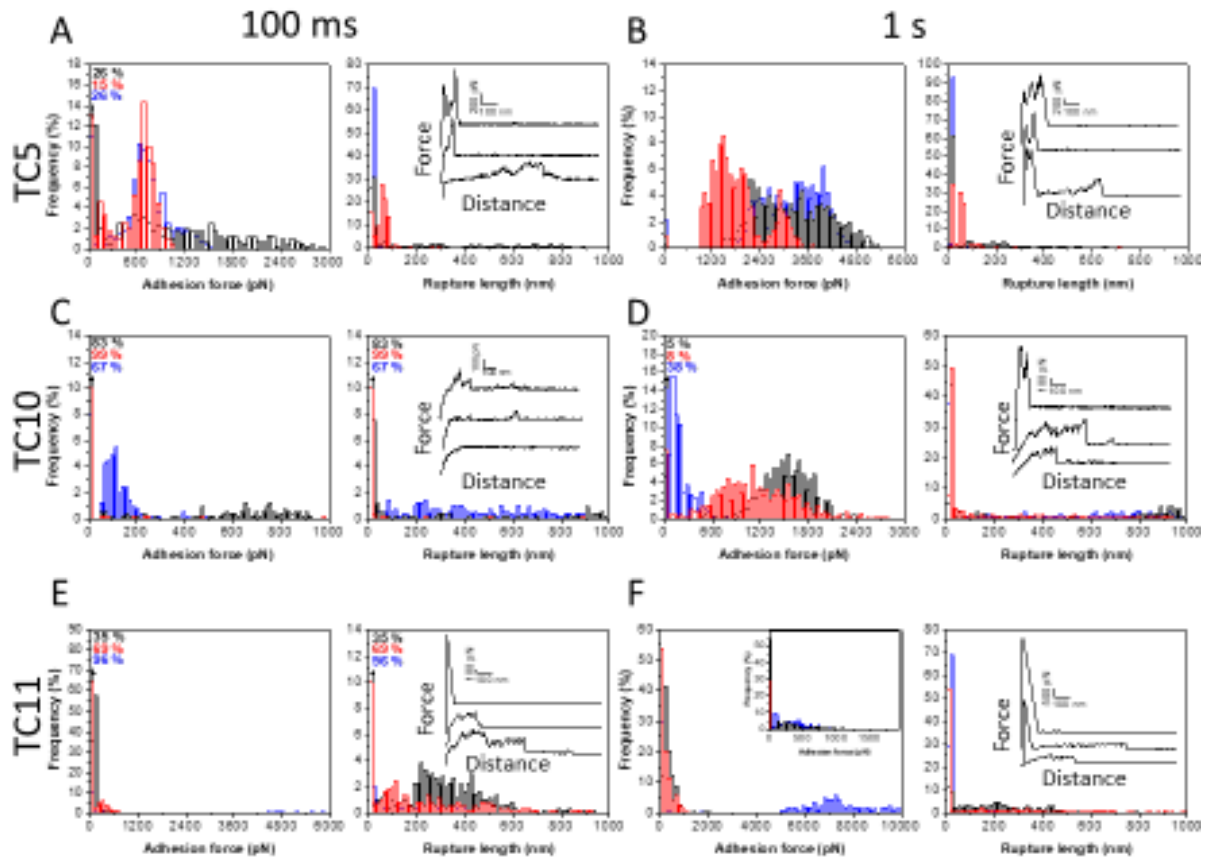


Surface MB6 (p(MASI-Co-BMA))

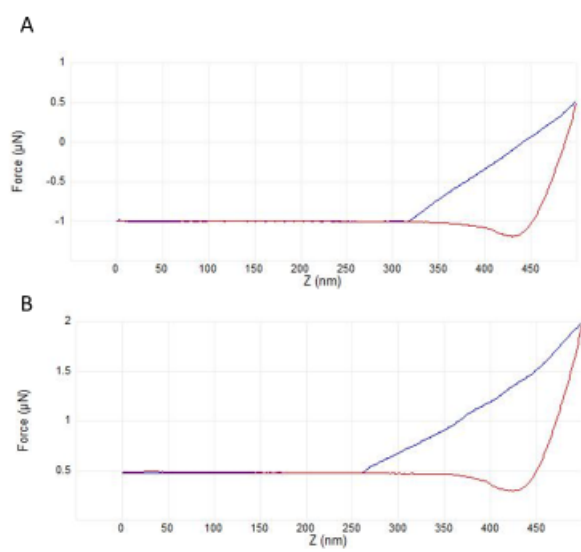


El-Kirat-Chatel *et al.* 2017 Figure 7.

Surface MC3MB6 (PDMS-b-P(MASI-Co-BMA))

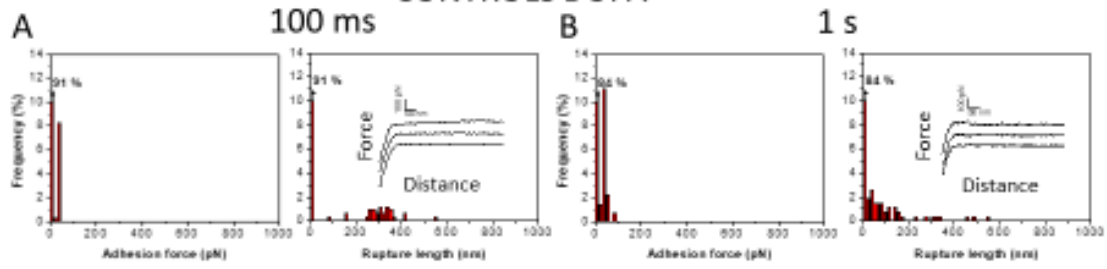


El-Kirat-Chatel *et al.* 2017 Figure 8.



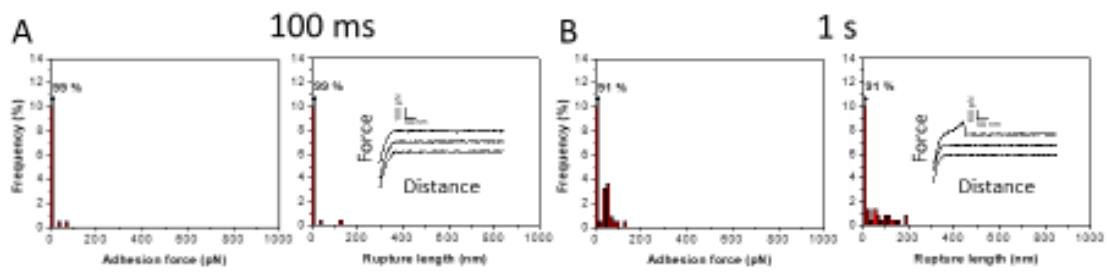
MB6 surface (P(SIMA-*stat*-BMA))

CONTROLS DOPA



MC3MB6 surface (PDMS-*b*-p(SIMA-*stat*-BMA))

CONTROLS DOPA



El-Kirat-Chatel *et al.* 2017 Figure S2.