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1 **Marine diatom *Navicula jeffreyi*: from biochemical composition and**
2 **physico-chemical surface properties to understanding the first step of**
3 **benthic biofilm formation**

4

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27 **Abstract**

28 To understand the first step of marine benthic microbial mat formation and biofouling
29 phenomena, caused by diatoms in the marine environment, the surface properties of the
30 epipellic diatom *Navicula jeffreyi* were studied and the composition of its bound Extracellular
31 Polymeric Substances (EPS) was determined. These parameters are determining factors for
32 the initial adhesion step of diatoms to other constituents that start marine fouling. Surface
33 energy of a diatom cell layer was determined using the sessile drop technique and highlights
34 that diatoms show a moderate hydrophobic character (contact angle with water $> 68^\circ$), no
35 Lewis acid character ($\gamma^+ < 1 \text{ mJ/m}^2$) and a low Lewis basic character ($\bar{\gamma} = 16,1 \text{ mJ/m}^2$). An
36 extraction procedure using a cationic resin subtracted only the bound EPS. Biochemical
37 assays showed that there were 2.5 times more proteins than sugars. The propensity of
38 *Navicula jeffreyi* diatom to adhere to five different solid surfaces, showing a gradient in their
39 hydrophobic and hydrophilic character, was measured. The attachment densities were high on
40 hydrophobic surfaces such as polytetrafluoroethylene and very low on substrata with surface
41 free energy over 40-50 mJ/m². Using a thermodynamic approach, the free energy of adhesion
42 of the diatom to the five substrata was determined, and led to a very strong correlation with
43 attachment densities for polytetrafluoroethylene, polyamide, polyethylene and stainless steel.

44

45 **Keywords:** Bioadhesion, Contact angle measurements, Diatom, Extracellular Polymeric
46 Substances, Microalgae, Physico-chemical surface properties.

47

48 **Introduction**

49 Diatoms are the most common early autotrophic colonizers of surfaces in seawater and are an
50 important constituent of the biofouling community in the marine environment, together with
51 bacteria and other algae [1,2]. Diatoms form an ubiquitous group of unicellular microalgae
52 characterized by their highly ornate, siliceous cell walls, associated with organic extracellular

53 polymers [3]. Marine biofouling phenomena cause serious and costly problems for surfaces
54 immersed in seawater, such as energy loss for boats, increased risk of mechanical failure for
55 static marine structures as well as safety problems [4-7]. During their assemblage diatoms
56 produce copious amounts of Extracellular Polymeric Substances (EPS), rich in proteins and
57 carbohydrates [8,9], which form an adhesive mucilage and allow them to build and, at a later
58 stage, to hold the biofilm together [10]. EPS also permit the attachment of other fouling
59 organisms, such as bacteria, to the sediment or to immersed solid surfaces [11]. These EPS are
60 secreted from a slit in the cell wall called the raphe. A common feature of adhesion in raphid
61 diatoms is that adhesive mucilages appear to be processed in several complex steps over time,
62 and these depend on the stage of adhesion as well as the nature of both the organism and the
63 substratum [12]. Most fouling diatoms have an initial adhesive mucilage that they use for
64 traction and movement (initial mucilage) and then a more permanent adhesive mucilage when
65 they eventually settle down to divide and form a biofilm (biofilm mucilage). There are also
66 several distinct types of EPS; motility, outer capsule, and matrix EPS, that can all participate
67 in the adhesion process [12]. The goal of the present paper is to study the transitory physico-
68 chemical interactions that occur between diatoms with initial mucilage and different substrata.
69 In general, adhesion of microorganisms is influenced by environmental (temperature, pH and
70 ionic strength), interfacial (surface charge and hydrophobic or hydrophilic character of the
71 microbial cells, chemical composition) and physiological factors (type of microorganisms)
72 [13-15]. The roughness, or the mechanical properties, of the substratum [16], as well as the
73 roughness of the cell surface, can also influence the adhesion of cells to surfaces. Among the
74 parameters outlined, interfacial properties can be most readily altered by using solid surfaces
75 with coatings that prevent adhesion of microorganisms.

76 Interfacial properties are linked to the surface energy of both the microorganism cell surface
77 and the solid substrate surfaces, that is a measure of the capacity of a surface to interact
78 spontaneously with other materials by forming new bonds. While the effect of the surface

79 energy of solid substrata on the adhesion strength of diatoms has often been studied by
80 employing widely different materials, ranging from urethanes and epoxies (high surface
81 energy), to silicones and fluorinated materials (low surface energy) [6,10,17-19], the
82 measurement of the surface energy of diatom cells themselves has rarely been performed [20].
83 We feel that such measurements could help in understanding the first step of benthic biofilm
84 formation, and thus help to prevent adhesion of microalgae to surfaces submerged by the sea.
85 In this report, the first part is devoted to a study of the surface properties of the epipellic
86 diatom *Navicula jeffreyi*, a diatom largely involved in the formation of microphytobenthic
87 biofilms. The cell length, width, and volume of *Navicula jeffreyi* are respectively equal to
88 $11.18 \pm 0.82 \mu\text{m}$, $7.56 \pm 0.60 \mu\text{m}$ and $366.71 \pm 85.50 \mu\text{m}^3/\text{cell}$ (mean \pm SE) (n = 6) [21].
89 Diatom cells were grown on an orbital shaker, so that they do not form a biofilm, and then
90 freeze-dried, in order to have diatoms with their initial mucilage only. The surface energy of
91 the diatom was determined by applying the sessile drop technique to a cohesive layer of
92 diatom cells resuspended in water and was calculated using the Lifshitz van der Waals acid-
93 base (LW-AB) method. The second part of the report evaluates the propensity of these diatom
94 cells to adhere to five different solid surfaces showing a gradient in their hydrophobic and
95 hydrophilic character. The role of the initial EPS in adhesion was also investigated, in
96 particular, by extracting the bound polymers and characterizing their biochemical
97 composition. Finally the experimentally observed adhesion of *N. jeffreyi* diatom to the five
98 different substrata was compared with that predicted by a thermodynamic approach that uses
99 the calculation of the free energy of adhesion.

100

101 **1. Materials and Methods**

102 **1.1 Microalgal strain and growth conditions**

103 The *N. jeffreyi* strain (CS-46/8) was from CSIRO Marine and Atmospheric Research
104 (Australia). 20 mL of F/2 liquid medium (Sigma-Aldrich) were suspended in 1L of Artificial

105 Sea Water (ASW, Tropic Marin Sea Salts, Wartenberg, Germany, 30 Practical Salinity Units).
106 100 mg/L of sodium metasilicate were added to the medium then the pH was adjusted to 8.2.
107 This solution was used as growth medium for *N. jeffreyi*. Cells were grown on an orbital
108 shaker (50 rpm) and kept for 20 days at 18°C, in conditions of natural alternating day/night.
109 After 20 days, corresponding to the end of the exponential growth phase, microalgal cells
110 were harvested by centrifugation for 10 min at 6000 g and 4°C then washed twice with, and
111 resuspended in NaCl 0,9 %. Cells were then collected and resuspended in 10 mL of ASW
112 diluted 1: 1000. Finally, the suspensions were freeze-dried and stored at 4°C until they were
113 used for the following experiments.

114

115 **1.2 Extraction of the Extracellular Polymeric Substances (EPS) bound to *N. jeffreyi* cells**

116 The extraction of EPS bound to *N. jeffreyi* cells was performed with Dowex resin, as this
117 method was developed for culture pellets of *N. jeffreyi* cells and was shown to provoke the
118 minimum release of internal compounds (protein, ATP) and the lowest proportions of glucose
119 compared with the water-extracted EPS [22], from which the high glucose content must be
120 inferred as contamination by the chrysolaminaran found in the vacuoles of the diatoms [23].
121 20 mL of ASW were added to 30 mg freeze-dried cells. 5 g of activated Dowex (Marathon C,
122 previously activated in Phosphate Buffer Saline for 1h in the dark) were gently mixed with the
123 sample at 4°C for 1h in the dark. The solution was then centrifuged at 3500 g and 4°C for 10
124 min and the supernatant was collected, freeze-dried and stored at -80°C prior to biochemical
125 analysis.

126

127 **1.3 EPS composition**

128 Total sugar and protein content of EPS and also sugar composition of polysaccharidic fraction
129 of EPS were determined by previously used methods for EPS from the *Navicula* genus of
130 diatoms [24-26]. Total sugar content was determined using the phenol-sulfuric acid assay,

131 using glucose as standard [27]. Protein content was determined using the bicinchoninic acid
132 assay, using bovine serum albumin as standard [28]. The sulfate content was measured by the
133 Azure A assay [29], using dextran sulfate as standard.

134 The sugar composition of the bound EPS fraction was determined as follows. *N. jeffreyi*
135 bound EPS fraction was dissolved in 2M HCl at 50 mg/mL and heated at 100°C for 20 h.
136 Polysaccharides were completely hydrolyzed in monomers, then the preparation was freeze-
137 dried and stored at -20°C. Analysis of the carbohydrate fraction was carried out by GC/MS
138 using a Varian CP-3800 GC/Varian Saturn 2000. Operating conditions were based on the
139 methodology of Pierre [26]: 200µL of pyridine and 200µL of BSTFA(N,O-
140 bis(trimethylsilyl)trifluoroacetamide):TMCS(trimethylchlorosilane) (99:1) per mg of
141 hydrolyzed EPS were added. The solution was mixed for 2 h at room temperature and injected
142 into a DB-1701 J&W Scientific column (30 m, 0.32 mm, 1 µm). The helium pressure was 8.8
143 psi and the flow rate was 1 mL/min. The temperature of the injector was set at 250°C. The
144 rise in temperature in the oven was programmed for a first step at 150°C, then an increment of
145 10 °C/min up to 200°C with a final step at 200°C for 35 min. The ionization was performed
146 by Electronic Impact (70 eV), the trap temperature was set at 150°C, the transfer line
147 temperature was defined at 180°C and the target ion was fixed at 40-650 m/z.

148 In order to check that the freeze-drying step for the diatom cells did not have any influence on
149 this composition, the bound EPS extraction procedure was applied to diatoms cell with and
150 without freeze-drying and the monosaccharide composition of polysaccharides was
151 determined for both samples of bound EPS.

152

153 **1.4 Determination of surface energy of substrata and diatom cells and the free energy of** 154 **adhesion ΔG_{adh}^{Total} between substrata and diatoms**

155 There are many thermodynamic approaches in the literature to evaluate the cells or solid
156 substrata surface energy. All of them are based on contact angle measurements with different

157 liquids with known surface tension, which were shown to give reproducible and accurate
158 results for both inorganic material surfaces and microbial cell surfaces [30-32]. However the
159 values of calculated surface energy from contact angles measurements depends on the
160 followed approach: Fowkes, Equation of state, Geometric mean and Lifshitz van der Waals
161 acid-base (LW-AB) approaches. In 2002, a remarkable study was published about the surface
162 energy of 140 bacterial and 7 yeast cell surfaces, determined by the four different approaches
163 mentioned above [30]. LW- AB was found to give the most consistent results. That is why we
164 chose this last method to evaluate surface energy of the five different substrata and the
165 epipellic diatom *N. jeffreyi*.

166 Five solid materials at monolithic and film state were used. The roughness and porosity of all
167 these materials were considered as insignificant. Four materials were provided by
168 Goodfellow: Stainless Steel AISI 316L (0.5 mm thick, noted SS316), Polytetrafluoroethylene
169 (0.5 mm thick, noted PTFE), Polyamide-nylon 6 (0.5 mm thick, noted PA) and Polyethylene
170 (0.5 thick, noted PE), and Glass (1 mm thick microscope slides) was provided by Thermo.
171 The films were cut into 4 cm² pieces and washed in 2 % PCC-54 (v/v, phosphate-free
172 surfactant, Thermo Scientific) for 10 min then rinsed 5 times with sterile milliQ water.

173 The surface energy of *N. jeffreyi* was measured by producing a uniform and cohesive layer of
174 cells deposited on membrane filters [31]. The layer of cells was prepared by depositing 40 mL
175 of microalga suspended in milliQ water (approximately 10¹⁰ cells/mL) on a cellulose
176 triacetate membrane filter (with a pore diameter of 0.45 µm, Sartorius) by filtration of the
177 suspension using low depression. The filters with the diatoms were then placed in a petri dish
178 on the surface of a layer of 1 % agar (w/v) in water containing 10 % (v/v) glycerol to preserve
179 constant moisture content [32]. The filters were stored for 30 min, at room temperature. Three
180 separate filters from three different cultures were used and the results were averaged. The
181 filters were then placed on empty Petri dish and allowed to air dry for 30 to 45 min [31,32].
182 After this drying time, same contact angles were measured for water droplets deposited on the

183 diatom layer several minutes apart, indicating that water evaporation from the layer was
 184 achieved. The contact angle was consistent for 30 min up to 120 min of drying time.
 185 The contact angle (CA) measurements were performed with a goniometer G40 (Krüss,
 186 Germany) at room temperature (23°C) with an accuracy of $\pm 2^\circ\text{C}$, employing the sessile drop
 187 technique and using three pure solvents whose surface tension components were known
 188 (Table 1): distilled water (Infilco), diiodomethane 99% (Sigma-Aldrich) and formamide \geq
 189 99% (Sigma-Aldrich). During measurements, each probe was dropped on the cell layer or the
 190 solid surfaces, and CA were measured immediately for 10 s. Left and right contact angles in at
 191 least 3 locations were measured, with highest and lowest values discarded. The CA was
 192 calculated as the average of these values. According to the LW-AB approach, CA were
 193 converted into surface free energies using equation 1 (Eq.1) [33], which ignores spreading
 194 pressure and highlights Lifshitz-van der Waals and Lewis acid/base surface free energy
 195 components.

$$196 \quad (1) \quad \gamma_L^t (1 + \cos \theta) = 2 \left(\sqrt{\gamma_S^{LW} \gamma_L^{LW}} + \sqrt{\gamma_S^+ \gamma_L^-} + \sqrt{\gamma_S^- \gamma_L^+} \right)$$

197 Here, γ^{LW} , γ^+ and γ^- are the Lifshitz-van der Waals, electron-acceptor (or Lewis-acid) and
 198 electron-donor (or Lewis-base) components of the surface free energy respectively; θ is the
 199 CA and the subscripts L and S denote the liquid and solid samples.

200 Equation 2 allows accessing to the Lewis acid-base components of the surface free energy:

$$201 \quad (2) \quad \gamma^{AB} = 2\sqrt{\gamma^+ \gamma^-}$$

202 ΔG_{adh}^{Total} is obtained by first determining the surface properties of micro-organisms and
 203 substrata and the tension surface of the medium. Then, the total interfacial free energy of
 204 microbial adhesion is determined by the triple relationship between the organism (NJ), the
 205 substratum (S) and the liquid (L) [34,35]:

$$206 \quad (3) \quad \Delta G_{adh}^{Total} = \Delta G_{adh}^{LW} + \Delta G_{adh}^{AB}$$

207 (4)
$$\Delta G_{adh}^{LW} = \left(\sqrt{\gamma_{NJ}^{LW}} - \sqrt{\gamma_S^{LW}} \right)^2 - \left(\sqrt{\gamma_{NJ}^{LW}} - \sqrt{\gamma_L^{LW}} \right)^2 - \left(\sqrt{\gamma_S^{LW}} - \sqrt{\gamma_L^{LW}} \right)^2$$

208 (5)
$$\Delta G_{adh}^{AB} = 2 \left[\sqrt{\gamma_L^+} \left(\sqrt{\gamma_{NJ}^-} + \sqrt{\gamma_S^-} - \sqrt{\gamma_L^-} \right) + \right.$$

209
$$\left. \sqrt{\gamma_L^-} \left(\sqrt{\gamma_{NJ}^+} + \sqrt{\gamma_S^+} - \sqrt{\gamma_L^+} \right) - \sqrt{\gamma_{NJ}^- \gamma_S^+} - \sqrt{\gamma_{NJ}^+ \gamma_S^-} \right]$$

210 **1.5 Microscopic analyses**

211 Scanning Electron Microscopy (SEM) was used to examine the aspect of the microalgal cells
 212 after freeze drying. Samples were previously metallized by a layer of gold-palladium under
 213 vacuum. SEM observations were made with a JEOL 5410 JV SEM in high vacuum mode,
 214 using 2.0 KV as accelerating voltage.

216 **1.6 *N. jeffreyi* attachment densities measurements**

217 Lyophilized microalgae were re-suspended in ASW diluted 1:1000 to give a suspension of
 218 cells with a chlorophyll content of approximately 0,3 µg/mL measured by fluorimetry with
 219 acetone 90 %. Five milliliters of cell suspension were carefully pipetted into a quadriPerm
 220 microwell plate (Sarstedt) containing three pieces of material (4 cm²) on the bottom. Two
 221 series were produced. After a set of different periods of attachment (2, 3, 5.5, 18, 24 and 48
 222 hours, and 3 and 4 days) with slow orbital agitation (20 rpm, Rotamax 120, Heidolph) in the
 223 dark, the cell suspensions were carefully aspirated. Afterward, 5 ml of ASW diluted 1/1000
 224 was pipetted into each well followed by slow shake back and forth five times to remove
 225 unattached cells. After washing, all the coupons were dried under a laminar flow hood for 1 h.
 226 Attachment densities were obtained by counting the adhered cells using a fluorescence
 227 microscope (Leica DMRB) combined with image software (Leica Application Suite v3.8).

228 The Y5 filter set was used (excitation 620-660 nm) and the λ used for emission was 700-775
229 nm. Counts were made for 20 fields (0.16 mm²) randomly taken on the surface on each of the
230 three replicate coupons. Cell settlement data are expressed as the mean number of cells
231 adhered per mm². Each standard deviation is represented by error bars and calculated with
232 raw data of three independent replicates.

233

234 **2. Results and discussion**

235 **2.1 *N. jeffreyi* : from microscopic observations to biochemical and physico-chemical** 236 **surface characterization**

237 We used a freeze-drying treatment in this study because it allows the maintenance of
238 microalga in a “fixed” state with initial mucilage. In the experiments presented here, this fixed
239 state gives access to the initial step of attachment without having interference from polymeric
240 metabolites (biofilm mucilage), which are synthesized when colonization occurs. The idea is
241 to study the very early stage of attachment, that corresponds to a passive step, due to the net
242 force of interaction between the diatom surface and the support that arises from the balance
243 between van der Waals and Lewis acid-base forces. The diatoms are here considered as
244 colloidal particles with bound initial adhesive polymers. It has to be kept in mind that these
245 “living colloids”, upon contact with a surface, will excrete supplementary adhesive polymers,
246 allowing for strong adhesion to the surface and finally for irreversible biofilm formation. The
247 ability of benthic diatoms to form biofilm is largely due to the secretion of these mucilaginous
248 EPS from the raphe [12,36].

249 Scanning electromicroscopy (SEM) shows *N. jeffreyi* cells (Figure 1) with an intact structure,
250 indicating that freeze-drying does not damage microbial cell surfaces. The SEM also shows
251 the presence of EPS closely bound to the *N. jeffreyi* cells (Figure 1). The EPS detected by
252 SEM in our samples correspond to the bound initial adhesive polymers that are very important
253 mediators in the initial step of adhesion of diatoms to surfaces.

254 In order to extract cell bound *N. jeffreyi* EPS a cationic resin was used. Extraction protocols
255 can distinguish a range of EPS types, depending on their degree of interaction with diatom
256 cells: (1) colloidal fractions, corresponding to the EPS excreted into the medium, (2) bound
257 fractions, corresponding to the EPS surrounding the cells and (3) residual fractions,
258 corresponding to the internal EPS [26]. Total sugar and protein content assays showed that the
259 bound EPS had 2.5 times more proteins than sugars. Sulfated sugars were not detected. This
260 composition is consistent with the works of various authors who have shown that adhesive
261 EPS are composed of cross-linking proteins (probably glycoproteins), polysaccharides and
262 phenols with covalent o-linkages [37,38]. These surface polymers are directly involved in the
263 physico-chemical surface properties of microorganisms.

264 The monosaccharide composition of the polysaccharidic fraction of bound EPS from *N.*
265 *jeffreyi* was also determined. In order to check that the freeze-drying step used to fix the
266 diatom cells did not have any influence on this composition, the bound EPS extraction
267 procedure was applied to diatom cells with and without freeze-drying and the monosaccharide
268 composition of polysaccharides was determined for both samples. The results were very
269 similar whether or not the diatoms had undergone freeze-drying (Table 2). The
270 monosaccharidic composition (expressed in % w/w) was 17 % of galacturonic acid, 0 % of
271 sulfated sugars and 83% of neutral carbohydrate. The neutral monosaccharidic composition
272 was dominated by glucose (36.1 % of the total fraction) but contained other sugars such as
273 rhamnose (8.9 %), mannose (18.4 %) and galactose (19.5 %). This composition is similar to
274 the one described for two other benthic diatoms, *Cylindrotheca closterium* and *Navicula*
275 *salinarum* [38].

276 We then characterized the surface energy of *N. jeffreyi* cells using contact angle (CA)
277 measurements.

278 Diatom layers were prepared by filtering their suspensions (c.f. Materials and Methods), in a
279 way that the cells covered the filter surface homogeneously and formed a cohesive layer.

280 The measured CA from *N. jeffreyi* layers are detailed in Table 3. Based on these CA
281 measurements, equations 1 and 2 allowed the determination of the surface energy components
282 of filter alone and of *N. jeffreyi* cells (Table 4). Results show that there are significant
283 differences between the data obtained with the membrane filter alone (controls) and those
284 measured with the *N. jeffreyi* layers deposited on the same membrane, for the different
285 individual surface energy components: γ^t , γ^- and γ^+ . The CA of water with the *N. jeffreyi* layers
286 (68.6°) shows that the microalgae surface, in general, is moderately hydrophobic.

287 The layer of diatoms presented a γ^t value equal to 36.7 mJ/m², a γ^{LW} value equal to 31.8
288 mJ/m² and a γ^{AB} value equal to 4.8 mJ/m². It showed no Lewis acid character ($\gamma^+ < 1$ mJ/m²)
289 and an average Lewis basic character ($\gamma^- = 16.1$ mJ/m²). The predominance of the electron-
290 donor character is an indication of the nature of the chemical groups exposed at the surface of
291 diatoms. Indeed, Lewis basic character is often attributed to neutral or slightly charged basic
292 chemical groups such as carboxylate (COO⁻), amine (NH₂), phosphate (PO₄⁻) groups of
293 phospholipids or lipoproteins [39,40].

294 In the case of *N. jeffreyi*, the Lewis basic character of the diatom surface may derive, in part,
295 from galacturonic acid and proteins in bound EPS.

296 In conclusion, the results of this first part demonstrate that the diatom cells of *N. jeffreyi* are
297 moderately hydrophobic and also show an average Lewis basic character. In the second part,
298 we demonstrate that these surface properties are linked to the propensity of the diatom to
299 adhere to different solid surfaces, presenting a gradient in hydrophobic and hydrophilic
300 character.

301

302 **2.2 Initial bioadhesion of *N. jeffreyi* is strongly affected by the surface properties of the** 303 **substrata**

304 Prior to carrying out initial bioadhesion measurements, an optimization of the method for
305 diatom attachment measurement was carried out. The highest densities of attached cells were

306 reached between 24-48h periods and then remained almost constant, as seen in Figure 2.
307 Furthermore, data showed no significant difference between a 24 h- and a 48 h-adhesion time
308 and the spatial distribution of cells on the different surfaces was homogeneous until 48 hours
309 of contact between cells and surfaces with very rare visible aggregates (no more than 10 cells
310 stuck together). Thus a period of 24 h adhesion time in the dark was chosen. Cells were then
311 counted after their adhesion to different surfaces (c.f. Materials and Methods and Table 4).
312 The initial affinity was calculated by means of the initial slope of each curve before 18h
313 attachment. *N. jeffreyi* adhered with a speed of 10 and 12 cell.mm⁻².h⁻¹ on glass and
314 polyamide-nylon (PA), respectively, and 77 and 78 cell.mm⁻².h⁻¹ on stainless steel (SS316)
315 and polyethylene (PE), respectively. Finally, *N. jeffreyi* adhered most quickly on
316 polytetrafluoroethylene (PTFE), with a speed of 121 cell.mm⁻².h⁻¹.

317 To assess the effect of solid surfaces properties on the diatom-substratum interaction, physical
318 properties of the surfaces were measured. The surface energies, calculated from measured CA
319 (Table 3), of the five substrata used in this study are listed in Table 4. The obtained γ^{tot} values
320 were in the range of 14.7-56.4 mJ/m², γ^{LW} in the range of 15.9-40.4 mJ/m², γ^+ in the range of
321 0.1-1.3 mJ/m², and γ^- in the range of 1.9-54 mJ/m². As expected, the results for the five
322 different surfaces showed reasonably good agreement with the literature data, within
323 experimental error [41]. The present data indicate that the five substrata form a gradient with
324 decreasing surface hydrophobicity and increasing hydrophilicity. PTFE has the lowest γ^{tot} and
325 γ^{LW} values, equal to 17.1 ± 0.9 mJ/m² and 15.9 ± 0.6 mJ/m² respectively, and negligible γ^+ and
326 γ^- . Next, PE has a γ^{tot} equal to 32.5 ± 2.7 mJ/m², then, SS316 with γ^{tot} of 40.6 ± 0.4 mJ/m², PA
327 with γ^{tot} of 42.4 ± 0.9 mJ/m² and glass with γ^{tot} of 56.2 ± 0.3 mJ/m².

328 To assess the influence of solid surface properties on the adhesion of diatom *N. jeffreyi*, the
329 mean cell densities of attached cells on the five different solids were quantified. The
330 attachment densities of diatoms decreased with an increase in the total surface energy γ^{tot} of
331 the substratum (Table 4). When γ^{tot} is over 40 mJ/m², diatom adhesion is minimized. There

332 were significant differences between attachment densities on the tested surfaces. The values
333 of attachment densities ranged from 2160 ± 110 cells/mm² at 17.1 mJ/m² to 225 ± 29
334 cells/mm² at 56.2 mJ/m² after 24 h attachment. The effect of the total surface energy on the
335 adhesion of the diatom *Navicula closterium* MMDL533, using a series of more or less
336 silanized glass slides as model surfaces, has been measured by other authors [34]. Our data
337 profiles are similar to those of Li and co. [34] who examined initial attachment after 5.5h. We
338 confirm here the preference of *N. jeffreyi* for hydrophobic surfaces.

339 In another earlier study, marine fouling diatoms *Navicula perminuta* were found to adhere
340 more strongly to hydrophobic surfaces than to hydrophilic surfaces. This behavior was
341 ascribed to the physicochemical properties of their extracellular adhesives [14]. *Navicula*
342 *perminuta* cells were also shown to adhere more strongly to hydrophobic materials thanks the
343 hydrophobic segments of their EPS [42]. A similar conclusion was obtained for the diatom
344 *Amphora* [17], whose cells were found to attach more strongly to hydrophobic surfaces.
345 However, in another study, it was shown that *Navicula* diatom cells adhered with comparable
346 strength to a hydrophobic elastomer and a hydrophilic mineral [36]. This result was explained
347 by the presence of either different EPS macromolecules, different segments on these
348 macromolecules, or even different regions on the same macromolecule being likely to mediate
349 adhesion of *Navicula sp.* [36]. More generally, hydrophobic regions of adhesive exopolymers
350 correspond to hydrophobic polypeptides and lipids, whereas hydrophilic regions correspond
351 to hydrophilic saccharides on glycoproteins or polysaccharides [10]. In the case of *N. jeffreyi*,
352 bound EPS were found to include 2.5 times more proteins than sugars, which is in accordance
353 with the hydrophobic character of the diatom.

354 In general, diatom adhesion is weaker on hydrophilic surfaces when compared to hydrophobic
355 surfaces [6,17,42], in good agreement with the results obtained here.

356 The current study also addresses the question of whether the composition of EPS is similar
357 between initial and biofilm EPS for the same type of the diatom species and under the same

358 growth conditions. Numerous studies have been carried out to evaluate the differences in
359 biochemical metabolites in planktonic and biofilm cells of bacteria [10, 43]: differences
360 in carbohydrate profiles for EPS of planktonic and biofilm cells of marine diatom *Amphora*
361 *rostrata*, grown in batch culture, were highlighted. It has also been reported that, for some
362 diatoms, the adhesive properties of their EPS are unrelated to the amount of exopolymer
363 produced [10], suggesting that the chemical composition of EPS does not vary over time for a
364 particular type of diatom species grown under particular conditions. The results of the present
365 study confirm that diatom adhesion is strongest to hydrophobic surfaces. It should be noted
366 that the data obtained in this study used freeze-dried diatoms with initial mucilage after
367 passive attachment in the dark, while all other studies used diatoms with biofilm mucilage
368 after active settlement in the light. The data appears to suggest that the adhesive properties of
369 bound EPS remain constant between initial and biofilm EPS.

370 When considering the relationship between attached diatom cell density and the different
371 surface energy components of the five substrata (Table 4), it appears that the best correlations
372 are observed between cell density and van der Waals component, on the one hand, and
373 electron acceptor component on the other hand. In the case of the electron donor component, a
374 correlation was obtained between cell density and the four substrata PTFE, PA, PE and SS316
375 (the point corresponding to the glass substratum was not aligned with the others).

376 Finally, we tested whether it is possible to predict how a diatom can adhere to a substratum by
377 calculating the free energy of adhesion between the microalgae and the solid surfaces.

378 We used a Lifshitz van der Waals acid-base (LW-AB; [30]) thermodynamic approach to
379 determine the free energy of adhesion ΔG_{adh}^{Total} of *N. jeffreyi* to the five different support
380 materials (c.f. Materials and Methods). This parameter is of crucial importance and may allow
381 the prediction of the initial adhesion of microorganisms, as the adhesion process will be
382 favored if the process itself causes the thermodynamic function to decrease ($\Delta G_{adh}^{Total} < 0$).

383 Using equations (3), (4) and (5), the values of the total interfacial free energy of adhesion of
384 *N. jeffreyi* to the five studied substrata and its components (ΔG_{adh}^{LW} and ΔG_{adh}^{AB}) were calculated
385 and are presented in Figure 3. When considering the relationship between attached diatom cell
386 density and the different contributions of the free energies of adhesion to the five substrata, it
387 appears that the best correlations are observed between cell densities and ΔG_{adh}^{Total} on the one
388 hand (Fig. 3) and ΔG_{adh}^{AB} on the other hand (Fig. 3), for all the substrata except for glass which
389 is not aligned with the others. In the case of ΔG_{adh}^{LW} , its contribution to ΔG_{adh}^{Total} is insignificant
390 (Fig. 3). The negative values of ΔG_{adh}^{Total} and ΔG_{adh}^{AB} actually lead to a strong adhesion of *N.*
391 *jeffreyi* to PA, PE, PTFE and SS316 surfaces. The adhesion test reveals a close correlation
392 between the surface hydrophobicity and ΔG_{adh}^{Total} and the attachment of *N. jeffreyi*: the more
393 hydrophobic the substratum is, the more strongly *N. jeffreyi* adheres. For glass, the positive
394 values of ΔG_{adh}^{Total} and ΔG_{adh}^{AB} unexpectedly correspond to a weak but significantly positive
395 adhesion of *N. jeffreyi* to the hydrophilic surface, at a similar level to PA, for which a ΔG_{adh}^{Total}
396 value equal to -28 mJ/m^2 was calculated (Fig.3). This adhesion to glass may be due to
397 possible local attractive electrostatic interactions, which are not explicitly included in the
398 thermodynamic approach used in the present study.

399 Thus, the thermodynamic analysis for hydrophobic substrata such as PTFE, PA, PE and
400 SS316 gives a good prediction of initial diatom cell attachment. This thermodynamic model is
401 a potentially very interesting tool for predicting the initial adhesion of diatoms on all types of
402 hydrophobic or moderately hydrophobic surfaces.

403

404 **Conclusion**

405 In the present paper, the initial interaction between diatom cells and different substrata, with
406 very different hydrophobic and hydrophilic surface properties was studied. Diatom cells were
407 grown on a shaker so that they did not form a biofilm, and then freeze-dried, in order to have

408 diatoms with their initial mucilage only. A chemical attraction occurred between these diatom
409 cells and the substrata, which was predicted by the free energy of adhesion between the two
410 components. The free energy was calculated from the surface energy of both diatom cells and
411 surface substrata, using a thermodynamic approach. In general, the more hydrophobic the
412 surface, the more strongly *N. jeffreyi* adheres to it. We observed very weak attachment to
413 surfaces with a total surface energy superior to 42 mJ/m². This paper constitutes an original
414 study of the transitory physico-chemical attraction between diatom cells containing bound
415 initial EPS and the substratum. This leads to an initial contact between the two components,
416 which was called “the first kiss” by Wetherbee and represents “an active commitment by
417 raphid diatoms to attach and activates adhesion mechanisms specifically designed for
418 subsequent binding to the substratum” [12]. One previous study about physico-chemical surface
419 properties of microalgae has been performed in 2013 [20] and showed interesting correlations
420 between surface properties and the cell-cell interactions, estimated by their propensity to form
421 colonies. The present study provides information for a better knowledge of cell-surface
422 interaction for a particular species of diatom. Both cell-cell and cell-surface interactions are
423 very important parameters for diverse biotechnological applications including algal biomass
424 production and marine biofouling prevention.

425

426 **Competing interests**

427 The authors declare that they have no competing interests.

428

429 **Author’s contributions**

430 GLK conducted supports and diatom surface energy measurements, diatom adhesion
431 measurements, SEM observations and IR measurements, she drafted the manuscript, GP
432 carried out EPS extraction, sugar and protein assays and drafted the manuscript, MNBF
433 supervised supports and diatom surface energy measurements and revised the manuscript,

434 JMZ carried out determination of sugar composition of bound EPS by GC/MS, MB is in
435 charge of diatom culture, TM supervised the bound EPS extraction and their subsequent
436 analysis, the study was coordinated by MG, who also contributed to the data analysis and
437 revised the manuscript.

438

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445 [laoide.net](http://traduction.lefevere-laoide.net)).

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600

601 Figure Legends

602

603 Figure 1: Scanning electron micrographs of *Navicula jeffreyi*. White arrow highlights the
604 bound EPS.

605

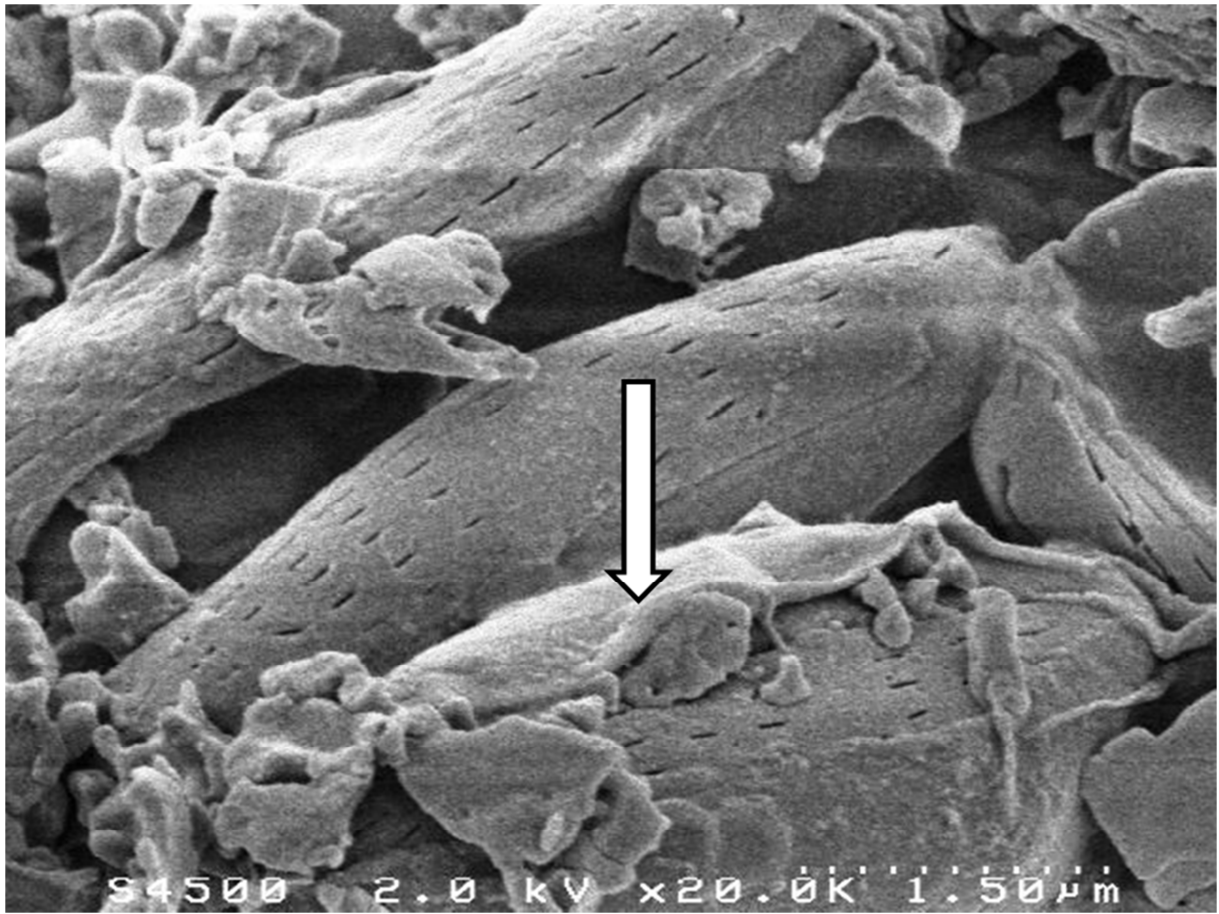
606 Figure 2: Kinetic study of the adhesion of *Navicula jeffreyi* on five different substrata (Glass,
607 PTFE, PE, PA and SS316; see Materials and Methods).

608

609 Figure 3: The values of the total interfacial free energy of adhesion of *N. jeffreyi* to the five
610 studied substrata and its components are given. Total (ΔG_{adh}^{Total} , black dots), acid/base (ΔG_{adh}^{AB} ,
611 grey dots) and Lifshitz van der Waals (ΔG_{adh}^{LW} , empty dots) interfacial free energy of adhesion
612 of *Navicula jeffreyi* on five different substrata.

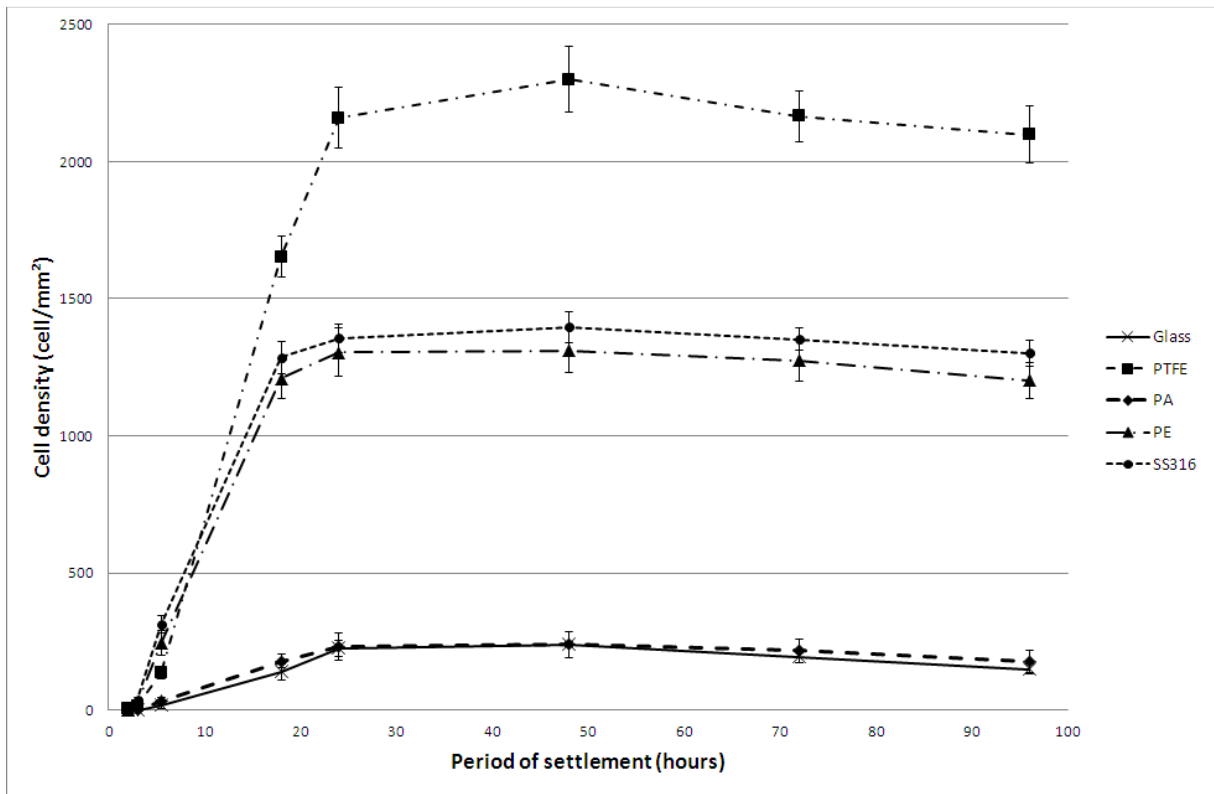
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614 Figure 1



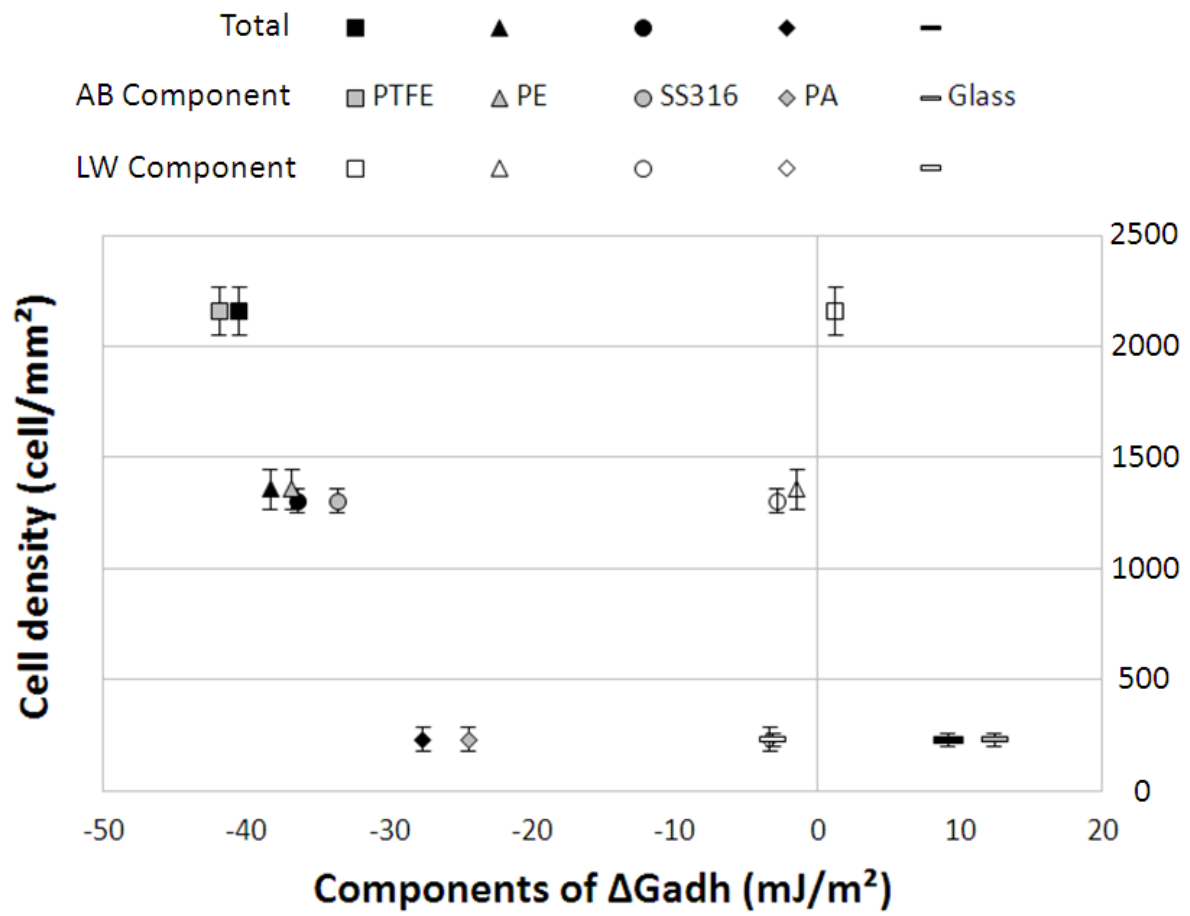
615

616 Figure 2



617

618 Figure 3



619

620 Table 1: Surface tension components of the different test solvents used in the contact angle
 621 measurements: total (γ^t), Lifshitz-van der Walls (γ^{LW}), electron-acceptor (γ^+) and electron
 622 donor (γ^-) components.

623

Test liquids	Purity	Surface energy (mJ/m ²)			
		γ_L^t	γ_L^{LW}	γ_L^+	γ_L^-
Water	MilliQ	72.8	21.8	25.0	25.0
Diiodomethane	> 98 %	50.8	50.8	0.0	0.0
Formamide	> 99 %	58.0	35.6	2.3	39.6

624

625 Table 2: Monosaccharide composition (% w/w) of polysaccharidic fraction of bound EPS
 626 from cultures of *N. jeffreyi* (end of the exponential growth phase), after extraction through
 627 Dowex-resin, with and without a freeze-drying step before extraction. Values are mean \pm SD
 628 of three samples from a culture of *N. jeffreyi*, the variability within true sample replicates of
 629 the biochemical analysis was less than 5%.

630

Monosaccharide	with freeze-drying	without freeze-drying
Galacturonic acid	17 \pm 3	15 \pm 4
Sulfated sugars	0 \pm 0.2	0 \pm 0.3
Neutral sugars	83 \pm 8.9	85 \pm 10.7
Glucose	36.1 \pm 4	34.5 \pm 3
Rhamnose	8.9 \pm 0.9	11.2 \pm 3
Mannose	18.4 \pm 2.1	21.3 \pm 4.1
Galactose	19.5 \pm 1.7	17.9 \pm 0.8

631

632

633 Table 3: Contact angle measurements of Stainless Steel AISI 316L (SS316),
 634 Polytetrafluoroethylene (PTFE), Polyamide-nylon 6 (PA) and Polyethylene (PE), Glass,
 635 membrane filters alone and *Navicula jeffreyi* layers previously deposited on cellulose
 636 triacetate membrane filters. The results presented are the average of at least 8 measurements
 637 done with each probe liquid for each surface, cell layer or solid substrata.
 638

Sample	Contact angle (°)		
	Water	Diiodomethane	Formamide
SS316	78.7 ± 0.7	44.5 ± 1	53.6 ± 0.6
PTFE	110.9 ± 2.0	83.2 ± 1.2	99.4 ± 1
PA	70.9 ± 2.5	38.5 ± 1.5	52.4 ± 1.9
PE	99.7 ± 4	85.7 ± 1.5	58.7 ± 2.7
Glass	10 ± 1.6	40.5 ± 3	10 ± 0.1
Membrane Filter	56.2 ± 1.8	44.0 ± 0.5	49.9 ± 1.7
<i>Navicula jeffreyi</i>	68.6 ± 2.9	54.3 ± 2.1	56.2 ± 6.1

639

640

641 Table 4: Surface energy of membrane filters alone, *Navicula jeffreyi* layers previously
 642 deposited on cellulose triacetate membrane filters, the five selected substrata calculated from
 643 equation (1) and cell density mean values measured on ten different fields for the five
 644 substrata.

645

646

Sample	Surface energy (mJ/m ²)					Cell density (cell.mm ²)
	γ^t	γ^{LW}	γ^{AB}	γ^+	γ^-	
Membrane Filter	39.8 ± 1.0	37.5 ± 0.3	2.2 ± 3.4	0.1 ± 0.1	28.6 ± 1.2	
<i>Navicula jeffreyi</i>	36.7 ± 4.1	31.8 ± 1.1	4.8 ± 2.9	0.4 ± 0.1	16.1 ± 2.1	
PTFE	17.1 ± 0.6	15.9 ± 0.6	1.2 ± 0.3	0.2 ± 0.1	1.9 ± 0.5	2160 ± 110
PE	32.5 ± 2.7	29.3 ± 1.5	3.2 ± 1.2	0.8 ± 0.3	3.4 ± 1.6	1356 ± 88
SS316	40.6 ± 0.4	37.3 ± 0.8	3.3 ± 0.3	0.6 ± 0.1	5.0 ± 0.6	1305 ± 52
PA	42.4 ± 0.9	40.4 ± 0.8	2.0 ± 0.2	0.1 ± 0.0	11.5 ± 1.5	231 ± 51
Glass	56.2 ± 0.3	39.3 ± 1.6	16.8 ± 1.9	1.3 ± 0.3	54.1 ± 0.2	225 ± 29

647

648