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1 **Intermittent pili-mediated forces fluidize *Neisseria meningitidis***
2 **aggregates promoting vascular colonization**

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4 D. Bonazzi^{1†}, V. Lo Schiavo^{1†}, S. Machata¹, I. Djafer-Cherif², P. Nivoit¹, V. Manriquez¹,
5 H. Tanimoto³, J. Husson⁴, N. Henry⁵, H. Chaté^{2,6,7}, R. Voituriez^{5,7}, G. Duménil^{1*}

6
7 **Affiliations :**

8 ¹ Pathogenesis of Vascular Infections, Institut Pasteur, INSERM, Paris, France.

9 ² Service de Physique de l'Etat Condensé, CEA, CNRS, Université Paris-Saclay, France.

10 ³ Institut Jacques Monod, Paris, France.

11 ⁴ Laboratoire d'Hydrodynamique (LadHyX), Department of Mechanics, Ecole polytechnique-
12 CNRS UMR7646, 91128 Palaiseau, France.

13 ⁵ Laboratoire Jean Perrin, CNRS/UPMC, Paris, France.

14 ⁶ Computational Science Research Center, Beijing 100193, China.

15 ⁷ LPTMC, Université Pierre et Marie Curie (CNRS/UPMC), Paris, France.

16
17 *Correspondence to: guillaume.dumenil@pasteur.fr

18 † *Authors contributed equally to this work.*

22 **Abstract**

23 *Neisseria meningitidis*, a bacterium responsible for meningitis and septicemia, proliferates
24 and eventually fills the lumen of blood capillaries with multicellular aggregates. The impact of
25 such an aggregation process and its specific properties are unknown. We first show that
26 aggregative properties are necessary for efficient infection and study their underlying
27 physical mechanisms. Micropipette aspiration and single cell tracking unraveled unique
28 features of an atypical fluidized phase, with single cell diffusion exceeding that of isolated
29 cells. Quantitative description of bacterial pair interactions combined with active matter
30 physics based modeling showed that this behavior relies on type IV pili active dynamics,
31 which mediate alternating phases of bacteria fast mutual approach, contact and release.
32 These peculiar fluid properties are found to be necessary to adjust to the geometry of micro-
33 channels mimicking capillaries upon bacterial proliferation. Intermittent attractive forces thus
34 generate a fluidized phase that allows efficient colonization of the blood capillary network
35 during infection.

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48 **Introduction**

49 Mechanisms and principles driving bacterial aggregation are highly diverse as they depend
50 on the specific properties of the bacterial species involved and the conditions they live in.
51 Bacterial aggregates in the context of biofilms for instance are the focus of intense study
52 because of their clinical importance as a mechanism of antibiotic resistance. Their
53 mechanical properties are largely defined by the self-produced matrix composed of DNA,
54 polysaccharides and proteins, which behave as a hydrated polymeric meshwork. Such
55 structures display viscoelastic properties combining both viscous fluid and elastic behavior
56 (Persat et al., 2015). A second type of bacterial aggregates relies on bacterial motility. In
57 particular, *Escherichia coli* or *Salmonella typhimurium* are able to form clusters of different
58 shapes and sizes which depend on flagella-based motility and chemotactic cues (Budrene
59 and Berg, 1991).

60 Another example of motility-driven aggregation is that of *Neisseria gonorrhoeae*. In this case,
61 the retractile properties of their long filamentous organelles known as type IV pili (T4P),
62 allow them to move on surfaces, a process known as twitching motility. Combined with
63 interactions between pili this motility promotes bacterial clustering (Oldewurtel et al., 2015;
64 Taktikos et al., 2015). In addition to *Neisseria gonorrhoeae*, T4P are expressed by a large
65 number of Gram-negative bacteria including human pathogens such as *Vibrio cholera*,
66 *Pseudomonas aeruginosa* or *Neisseria meningitidis* as well as Gram-positive bacteria such
67 as *Streptococcus sanguinis*. T4P are several microns long semi-flexible fibers extending out
68 of the bacterial body. In *Neisseria spp.* pili are highly dynamic as they grow and retract at
69 speeds in the order of 1 $\mu\text{m/s}$. As they retract through an ATP-dependent process, individual
70 pili can generate very high forces, reaching up to 100 pN for single pili and ten-fold this value
71 for bundles of pili (Biais et al., 2008; Maier et al., 2002). Pilus-pilus interaction is thought to
72 promote auto-aggregation.

73 In *Neisseria meningitidis*, T4P are central virulence factors required for bacterial adhesion
74 along the endothelium and subsequent formation of intravascular aggregates. This vascular
75 colonization process is a prerequisite for vascular damage observed during infection

76 (Melican et al., 2013). *In vitro* studies have shown that aggregates formed by *N. meningitidis*
77 also depend on type IV pili but the biophysical properties of these clusters and their impact
78 on the infection process have not been characterized. Once bacteria adhere to capillary
79 endothelial cells, they proliferate and progressively occlude the vessel lumen. Growth in the
80 tubular and sometimes tortuous geometry found in capillaries imposes important mechanical
81 constraints that require specific adaptation. This interdisciplinary work explores how the
82 biophysical properties of *N. meningitidis* aggregates determines human disease progression.
83

84 **Results**

85 **Auto-aggregation enhances vascular colonization *in vivo***

86 During meningococcal infection, bacterial aggregates can be found in the lumen of
87 capillaries located in most organs including the brain, but the impact of aggregation on
88 disease progression is unknown (Mairey et al., 2006). Such aggregates also form in human
89 capillaries of a humanized mouse model based on human skin xenograft, which reproduces
90 the key histological features of *N. meningitidis* infection (Melican et al., 2013). In such a
91 model, we observed that aggregates filled the vessel lumen 6h post-infection while adjusting
92 to the complex, sometimes anastomosed capillary network over tens to hundreds of μm
93 (Figure 1A). To assess the role of aggregation, we exploited a naturally occurring variant of
94 the major pilin termed PilE_{SA} (Nassif et al., 1993). This variant occurred spontaneously
95 through a genetic recombination system present in *Neisseria* species (Rotman and Seifert,
96 2014). In contrast with the frequently occurring PilE_{SB} variant that promotes both adhesion
97 and aggregation, the PilE_{SA} variant selectively promotes adhesion but not aggregation
98 (Figure S1A). Individual bacteria from a strain expressing the SA and SB pilin variants had
99 the same ability to adhere to endothelial cells in the presence of flow and proliferated at the
100 same rate (Figures S1B and S1C). However, *in vitro*, single adhering bacteria of the PilE_{SB}
101 variant formed 3D densely packed microcolonies on the cellular surface upon proliferation,
102 while the PilE_{SA} variant led to more dispersed, 2D microcolonies formed by a single bacterial
103 monolayer (Figures S1D and S1E). *In vivo*, the aggregative capacity of bacteria did not
104 affect survival in the blood of infected animals (Figure 1B). At the initial time points of
105 infection (6h) aggregation had little effect on the ability of the bacteria to accumulate along
106 vessels, but after 24h the non-aggregative strain showed a 60-fold decrease in bacteria
107 number in the human skin compared to the aggregative one (Figure 1C). These results show
108 that bacterial auto-aggregation is important for disease progression, and underline the ability
109 of aggregates to highly deform in order to adapt to the complex geometry of the
110 microcirculation.

111

112 ***Neisseria meningitidis* aggregates display a viscous liquid behavior in suspension**

113 The above results raised the question of the physical mechanisms governing the formation
114 and dynamics of *N. meningitidis* aggregates. Interestingly, several properties of aggregates
115 formed *in vitro* were reminiscent of the behavior of liquid droplets. In liquid suspension, *N.*
116 *meningitidis* cells spontaneously formed quasi-spherical, dynamic aggregates that tended to
117 sediment to the bottom of the wells without adhering to the surface (Figures 2A and S2;
118 Movie S1). In addition, when two aggregates came in close proximity, they rapidly fused and
119 relaxed to a larger, spherical aggregate within a few minutes (Figures 2B, S3A, S3B, S3C
120 and S3D; Movie S1), suggesting that they can be described as a liquid phase endowed with
121 an effective surface tension. This viscous liquid behavior was also corroborated by wetting
122 experiments in which progressive aggregate spreading on an adhesive surface could be
123 observed by TIRF microscopy (Figures S3E, S3F and S3G). To quantify the mechanical
124 properties of this phase, we adapted a micropipette aspiration protocol to our system
125 (Guevorkian et al., 2010; Guevorkian et al., 2011): the application of a constant negative
126 pressure on a bacterial aggregate led to its deformation inside the micropipette (Figures 2C
127 and 2D, movie S2). Interestingly, in the range of applied pressures (10-100 Pa), the length of
128 the tongue increased linearly over time, indicating a purely viscous liquid behavior (Figure
129 2E). By analyzing the dynamics of the aggregates and further applying the Laplace law, we
130 could obtain estimations of the aggregate viscosity (9 Pa.s) and surface tension at rest (0.1
131 mN/m; Figures 2F and 2G). Thus, aggregates of *N. meningitidis* exhibit a liquid-like behavior
132 with no elastic component even at short time scales. As a comparison, values of viscosity
133 and surface tension for common viscous liquids can be found in Supplementary Table 1.
134 The viscosity of *N. meningitidis* aggregates is close to that of honey (2-10 Pa.s), with
135 however a surface tension that is much lower than the one of honey (50-60 mN/m).

136

137 **Diffusion of bacteria inside the aggregates reaches values higher than individual**
138 **bacteria in suspension**

139 To gain insights into the interactions driving this viscous behavior we then moved to a
140 smaller scale and characterized the motion of single fluorescently-labeled wild-type (WT)
141 bacteria inside aggregates, 30-40 μm in diameter, using spinning disk confocal imaging at
142 high spatio-temporal resolution (Figures 3A, S4A and S4B; Movie S3). We characterized the
143 movement of individual bacteria by an effective 2D diffusion coefficient. These results
144 showed that this coefficient was small for bacteria close to the aggregate center of mass
145 (COM), and progressively increased with the distance from the center (d_{COM}) reaching
146 maximal values at the aggregate periphery. The relationship between bacterial diffusion
147 coefficient and distance to the center was confirmed over multiple aggregates (n=20) after
148 normalization for aggregate size (Figure 3B). The values of diffusion coefficients
149 corresponding to a ~ 10 μm thick outer layer ($0.3 - 0.5 \mu\text{m}^2/\text{s}$) exceeded the diffusion
150 coefficient of single freely diffusing bacteria (Figures 3B and 3C). Diffusion of individual
151 diplococci in suspension in liquid medium was determined experimentally to be $0.25 \mu\text{m}^2/\text{s}$
152 and was not affected by piliation as a non-piliated strain (*pilD*) exhibited the same motility,
153 consistently with the passive diffusive motion of a $1 \mu\text{m}$ diameter particle in water. This
154 feature stands in contrast to ordinary liquids at equilibrium, where attractive intermolecular
155 forces ensure cohesion and slow down particle motion compared to the gas phase. In the
156 case of *N. meningitidis* bacterial aggregates, we found on the contrary that individual
157 bacteria within the aggregate (liquid phase) exhibit an apparent diffusion coefficient higher
158 than non-interacting single bacteria outside of the aggregate (dispersed or gas-like phase).
159 Pursuing the analogy with the movement of particles of a classical fluid in a liquid or
160 dispersed phase, we concluded that this active system displays an anomalously fluidized
161 phase, the origin of which deserves further clarification.

162

163 **Pairwise interaction between bacteria shows cycles of attraction and release**

164 In order to elucidate the origin of the unusual properties of *N. meningitidis* aggregates, we
165 focused on the likely elementary building block of bacterial aggregation, *i.e.* the interactions
166 between two cells. To this aim, we performed time-lapse microscopy of 2-10 isolated WT

167 bacteria confined in quasi-2D circular microfabricated (width = 50 μm , height = 10 μm) agar
168 chambers (Figure 4A). Bacteria were prevented to interact with the glass substrate by
169 passivation with a hydrophobic molecule (PLL-PEG) and no evidence of twitching motility
170 could be observed in these conditions (Figures S4C and S4D). Bacteria diffused freely in the
171 chambers, until two of them rapidly moved towards each other and got in close contact
172 (Figure 4B and Movie S4). After a variable contact time, bacteria detached and eventually
173 resumed free diffusion or undertook a new phase of attraction, possibly with another cell.
174 Detection of these “active pulling” events was implemented in a semi-automatic script based
175 on the analysis of interbacterial distance and its first derivative as a function of time (Figure
176 4C). The active pulling events were T4P-dependent, since non-piliated bacterial mutants
177 (e.g. *pilD*) failed to display them. Analysis of over 100 events of interacting bacteria provided
178 a quantitative description of the elementary process initiating aggregation (Figures 4D, 4E,
179 4F and 4G). For instance, mean interbacterial distance at the beginning of a pulling event
180 was 6.9 μm but could reach up to 20 μm (Figure 4D). This is in agreement with previously
181 reported measurements of average T4P length of the same strain by immunofluorescence
182 (Imhaus and Dumenil, 2014). Furthermore, a mean approaching speed of 1.5 $\mu\text{m/s}$ (Figure
183 4E) during attraction sequences is in line with previous measurements using optical
184 tweezers (Merz et al., 2000). Another signature of the attractive phase was given by the
185 average interaction angle, defined as the angle between each single bacterial step and the
186 axis intersecting the two bacteria, which was narrowly centered around 0 during interaction
187 events underlining the high directionality of the mutual approach (Figure 4E, inset). Most
188 importantly, and for the first time, this approach allowed to quantify the time intervals
189 bacteria spent attracting each other (ON phase) or not (OFF phase) once they fall within the
190 interaction range (20 μm). Probability distribution function of the ON and OFF phase
191 durations followed an exponential decay with strikingly similar characteristic times, namely
192 $t_{ON} = 15.5$ s and $t_{OFF} = 16.3$ s (Figures 4F and 4G). Analysis at the level of individual pairs of
193 cells thus demonstrated an intermittent interaction process and provided the key parameters
194 characterizing the T4P-mediated pairwise interaction between bacteria.

195

196 **An intermittent attractive force between particle pairs is sufficient to recapitulate**
197 **aggregate properties**

198 The above results prompted us to investigate the role of intermittent attractive forces
199 between bacteria as the physical basis of the highly fluidized phase formed by *N.*
200 *meningitidis* aggregates. We hence built a 2D minimal model of active fluid in order to
201 assess how an intermittent active process of rapid attraction between bacteria could relate to
202 the overall unique material properties of aggregates (Supplementary Information model).
203 Models have been previously used to describe the interaction of single *N. gonorrhoeae* cells
204 with an abiotic surface (Marathe et al., 2014; Weber et al., 2015). Here, in order to analyze
205 aggregation properties, we rather built and parametrized the model for numerical simulations
206 focusing on the pairwise *N. meningitidis* bacterial interactions, characterized quantitatively in
207 the micro-chambers (d_{int} , v_{int} , D , t_{ON} and t_{OFF}). Bacteria were modeled as apolar circular solid
208 particles interacting via a pairwise potential with a hard-core repulsion reflecting the cell finite
209 size. The T4P-dependent attachment and active pulling were modelled via an attractive
210 force, which was stochastically turned ON and OFF for pairs of particles that were (i) within a
211 cut-off distance (defined as twice the maximal pili length) and (ii) neighbors as defined by a
212 Voronoi tessellation plane partitioning (Figure 5A). In effect, this restriction to Voronoi
213 neighbors favored interactions within a first circle of an average of 6 neighbors, which
214 corresponds to the experimentally determined average pili number per bacterium (Imhaus
215 and Dumenil, 2014). With these simple assumptions and experimentally determined
216 parameters, simulated particles in a pair could undergo a cycle during which they attached,
217 moved towards each other, remained in contact and finally detached (Figure 5B). At higher
218 particle densities, initially randomly dispersed particles progressively organize in dynamic
219 round-shaped aggregates that occasionally fused and formed a new larger and circular
220 cluster (Figure 5C). *In silico* simulated large groups of particles displayed apparent diffusion
221 coefficients in complete agreement with the experimental results of bacterial motion inside
222 aggregates, remarkably requiring no parameter adjustment (Figure 5D). Moreover, the

223 spatial distribution of diffusion coefficients was conserved, with low diffusion at the
224 aggregate center and increasing values going outward, reaching values higher than free
225 diffusion at the aggregate periphery (Figure 3K, dotted line). Aggregates of particles
226 intermittently attracting each other thus exhibit a liquid-like behavior with high diffusion
227 confirming that the active intermittent interaction is sufficient to reproduce the highly diffusive
228 fluidized phase of real aggregates. A mean field analysis (SI Model) confirmed that the
229 observed high diffusion properties can be attributed to the intermittent dynamics of the pili,
230 which act as an active source of noise in the system. Intermittent attractive forces thus
231 promote efficient aggregate formation combined with a fluidization effect due to high particle
232 diffusion.

233

234 **Intermittent attractive properties between bacteria define a phase diagram of** 235 **aggregation phenotypes**

236 To test the reach of the model we examined its main prediction: aggregate material
237 properties are controlled by the ratio of the time spent in the OFF (no attraction) and the ON
238 (attraction) phases (i.e. t_{OFF}/t_{ON}). Accordingly, aggregate simulations at constant $t_{ON} = 15$ s
239 while modulating t_{OFF} led to different states of matter (Figure 6A and movie S5): a dispersed
240 gas-like phase where no aggregates formed at high values of t_{OFF}/t_{ON} , a liquid phase with
241 dynamic aggregates with $t_{OFF}/t_{ON} \sim 1$, and finally a solid phase with frozen crystal-like
242 aggregates for low values of t_{OFF}/t_{ON} . To quantitatively determine these phase transitions, we
243 used the average particle diffusion coefficient in the aggregate to determine solid-to-liquid
244 transition, and the dynamic stability of large aggregates defined by the radius of gyration for
245 the liquid-to-gas transition (Figure 6B). Interestingly, these simulations indicate that the
246 average diffusion coefficient curve peaked at a t_{OFF}/t_{ON} ratio equal to 1, very close to the
247 value measured experimentally with WT bacteria. Importantly, particles in the external layer
248 of the simulated aggregates showed a higher effective diffusion constant compared to
249 individual particles ($0.25 \mu\text{m}^2/\text{s}$) thus recapitulating the high diffusion regime observed at the
250 periphery of WT bacterial aggregates (Figure 6B, dashed line). Properties of T4P interaction

251 corresponding to a t_{OFF}/t_{ON} ratio equal to 1 which were observed with WT bacteria thus
252 correspond to a maximum of intra-aggregate bacterial diffusion. Finally, we built up a
253 complete phase diagram in the $t_{ON} - t_{OFF}$ space by independently varying both parameters
254 (Figure 6C). Numerical simulations were in agreement with mean-field analysis and showed
255 that the actual balance between the time spent in the ON and OFF phases, the t_{OFF}/t_{ON} ratio,
256 dictates the aggregative properties of the system (SI model).

257 To complete the model validation, we tested its predictions by genetically manipulating
258 bacteria. Slightly decreasing the t_{OFF}/t_{ON} ratio was predicted to strongly reduce intra-
259 aggregate diffusion and thus increase viscosity (Figure 6D). *N. meningitidis* expresses a
260 thick capsule at its surface composed of polymers of negatively charged sialic acid residues
261 in the serogroup C strain used here. Mutants devoid of capsule (*siaD*) were still able to form
262 aggregates but became “stickier” than their wild-type counterparts, thus displaying larger
263 contact times and a corresponding decrease in the t_{OFF}/t_{ON} ratio (Figures 6C and 6E; Movie
264 S6). The results corroborated the model predictions showing that a low t_{OFF}/t_{ON} ratio
265 decreased the intra-aggregate bacterial diffusion and flattened the spatial distribution of the
266 diffusion values (Figure 6F). Accordingly, micropipette measurements showed that non-
267 capsulated mutants form more viscous aggregates (50 Pa.s, Figure 6G). Symmetrically, a
268 slight increase in the t_{OFF}/t_{ON} ratio was predicted to fluidize the aggregate core and hence to
269 decrease the overall viscosity (Figure 6H). In particular, the spatial distribution of diffusion
270 coefficients was predicted to progressively flatten by increasing t_{OFF}/t_{ON} , with simulated
271 particles in the aggregate center being more motile while particles at the boundaries being
272 less motile compared to the simulation of the WT case (Figure 6H). To increase t_{OFF}/t_{ON} in
273 our biological system, we reduced the number of pili expressed by bacteria (Imhaus and
274 Dumenil, 2014) and hence decreased the probability of attraction. As predicted by the
275 model, bacteria at 60% piliation level compared to WT exhibited a flattened distribution of
276 effective diffusion coefficients within the aggregate (Figure 6J). Micropipette aspiration
277 showed a linear relationship between the average number of pili per cell and the viscosity of
278 the aggregate (Figure 6K). In conclusion, by experimentally varying the probabilities of

279 attraction and separation between real bacteria using genetics we could modulate the
280 physical properties of the bacterial aggregates as predicted by the model. Remarkably, the
281 specific properties of bacterial interactions in the unmodified WT *N. meningitidis* place it in
282 the phase diagram in a narrow region prone to the formation of viscous liquid aggregates
283 fluidized by high intra-aggregate diffusion.

284

285 **Fluid-like behavior of aggregates favors colonization of capillary mimics**

286 We then asked whether this intermittent attractive force generated fluidization effect of *N.*
287 *meningitidis* aggregates could have a function in their capacity to invade capillaries. The
288 viscous aggregates highlighted in our study are highly deformable and this could allow the
289 adaptation to the particular geometry of the microcirculation during bacterial proliferation. To
290 test this hypothesis, we used a bacterial mutant that forms aggregates but fails to display
291 intermittent attractive forces. The *pilT* mutant, lacking the ATPase responsible for pilus
292 retraction, is able to form contacts through its abundant pili but fails to detach (Figure 7A).
293 As predicted, aggregates of the *pilT* mutant were not round shaped, did not flow into the
294 pipette even at high aspiration pressures and individual bacteria failed to diffuse in the
295 aggregates indicating a solid material totally devoid of fluid properties (Figures 7A, 7B and
296 7C; Movie S6). To test the ability of aggregates to adjust to the capillary geometry following
297 volume increase due to bacterial proliferation, *pilT* and WT bacteria were allowed to grow in
298 10 μm -wide PDMS (polydimethylsiloxane) channels to mimic capillary dimensions. WT
299 aggregates grew in a continuous fashion while rapidly adjusting to the imposed channel
300 geometry (Figure 7D and Movie S8). Aggregate size increased over time due to cell
301 proliferation and occasional aggregate fusion with neighboring colonies (Figure 7E). In
302 contrast, the solid-like *pilT* colonies initially grew as WT until they filled up the whole channel
303 section, increased bacterial density and then stopped growing. From these results, we
304 conclude that the viscous properties of the aggregates can determine the evolution of the
305 infection process by favoring space occupancy in the particular geometry of the capillary
306 network.

307 **Discussion**

308 Analysis of human cases as well as studies conducted with a humanized animal model
309 revealed that aggregates of *Neisseria meningitidis* were able to adapt to the shape of the
310 infected vessels, reaching high aspect ratios and complex morphologies. Here we show that
311 during infection bacterial aggregation is not only a side effect of proliferation but an active
312 process due to specific physical properties of *N. meningitidis* bacteria-bacteria interactions.
313 Beyond the simple property of sticking together, the viscous-like properties of the
314 aggregates are a determining feature of the infection process. As bacteria proliferate the
315 environment of infected micro-vessels rapidly becomes a physical limit (Figure S5). The
316 ability of *N. meningitidis* bacterial aggregates to quickly adapt to the vessel geometry
317 through implementation of intermittent attractive forces could account for the exceptionally
318 rapid disease progression of meningococemia with rapid occupation of entire vascular
319 networks and loss of organ function observed during *purpura fulminans*. Further studies will
320 be necessary to evaluate whether other pathogens display similar properties and their
321 impact on disease progression. In *Neisseria gonorrhoeae* for instance, a sorting phenomenon
322 has been described upon changes in pilus posttranslational modifications highlighting a
323 complex dynamics of aggregation in this species as well (Oldewurtel et al., 2015).

324 Our study further revealed a unique type of viscous material composed of particles endowed
325 with higher diffusion coefficients in the liquid phase than in the dispersed gas phase. This is
326 in contrast with the classical description of the gas phase which is defined by a higher
327 diffusion of particles as compared to the liquid phase. This atypical high diffusion can be
328 attributed to the intermittent dynamics of the interaction between particles, which acts as an
329 additional source of noise in the system that fluidizes aggregates. In the context of infection,
330 intermittent attractive forces between bacteria provide an optimum to combine efficient
331 aggregation with a high diffusion required for low viscosity, leading to rapid adaptation to the
332 geometry of blood capillaries during meningococcal infections. In addition to a better
333 understanding of the physics behind the pathogenicity of *N. meningitidis*, our study uncovers
334 novel design principles for materials with tunable viscosity.

335

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345

346 **Author contributions**

347 This work represents the combined research directions of 2 groups (GD, RV/HC). *In vivo*
348 experiments by SM and VM. Video-microscopy experiments of aggregate dynamics and
349 microfabrication by DB. Micropipette aspiration experiments by VS, NH and JH. Image
350 analysis by DB and HT. Modeling by IDC. All authors designed experiments. DB, GD, HC
351 and RV wrote the paper.

352

353 **Materials and Methods**

354 Bacterial strains growth and mutagenesis

355 All *N. meningitidis* strains described in this study were derived from the recently sequenced
356 8013 serogroup C strain [<http://www.genoscope.cns.fr/agc/nemesys>] (Rusniok et al., 2009).
357 Strains were streaked from -80°C freezer stocks onto GCB agar plates (Difco), and grown
358 overnight in a moist atmosphere containing 5% CO₂ at 37°C. For all experiments, bacteria
359 were transferred to liquid cultures in pre-warmed RPMI medium supplemented with 10 %
360 FBS at adjusted OD₆₀₀=0.05, and incubated with gentle agitation for 2 hours at 37°C in the
361 presence of 5% CO₂. The *pilF pilF_i* strain was plated on GCB plates and then cultured in
362 liquid media both containing IPTG in a range of 0 to 100 µM concentrations, depending on
363 the level of piliation needed (Imhaus and Dumenil, 2014). Natural variants in the pilin gene,
364 namely PilE_{SA} and PilE_{SB} strains, have been previously described (Nassif et al., 1993) and
365 were fluorescently labeled by cloning the gene encoding GFP under the control of the pilE
366 gene promoter. Mutations in *pilD* (Rusniok et al., 2009), *siaD* (Brissac et al., 2012), *pilT*
367 (Pujol et al., 1999) genes have been previously described. Bacteria were fluorescently
368 labeled by cloning the gene encoding near infrared fluorescent protein iRFP (Filonov et al.,
369 2011) under the control of the pilE gene promoter.

370

371 Cell culture

372 Primary HUVECs (PromoCell) were used between passages 1 and 10 and grown at 37°C in
373 a humidified incubator under 5% CO₂ in Endo-SFM (Invitrogen) supplemented with 10%
374 heat-inactivated FBS (Invitrogen) and 40 µg/ml of endothelial cell growth supplement
375 (Harbor Bioproducts).

376

377 Xenograft model of infection

378 Introduction of human dermal microvessels into SCID/Beige mice by xenografting human
379 skin was performed as previously described (Melican et al., 2013). For immunofluorescence,
380 grafts were removed 6 hs post infection with GFP-expressing bacteria and fixed in 4%

381 paraformaldehyde (PFA). Samples were frozen in OCT (Tissuetek, Sartorius, USA) and
382 sliced into 10mm fragments. Immunofluorescence was performed using Mouse on Mouse
383 detection kit (Vector laboratories, Eurobio ABCys, France). Ulex Europaeus agglutinin
384 Lectin-Rhodamine (Vector laboratories, Eurobio ABCys, France) was used to stain human
385 vessels. Samples were mounted in Vectashield mounting reagent (Vector laboratories,
386 Eurobio ABCys, France) and imaged on a spinning-disk confocal microscope.

387

388 Growth assay

389 Bacterial pre-cultures were diluted at initial $OD_{600} = 0.05$, loaded in a 96 well plate and
390 incubated in a microplate reader (Cytation5, Biotek) under agitation at 37°C in the presence
391 of 5% CO_2 , with automated OD_{600} acquisition every 30 minutes for 18 hs.

392

393 Initial adhesion

394 Laminar flow experiments were performed as previously described (Mairey et al., 2006).
395 Briefly, HUVECs were plated on 8-mm-diameter wells on glass slides coated with fibronectin
396 (10 μ g/ml) at a density of 10^4 cells/well. Cells grown on glass slides were placed in a parallel
397 plate flow chamber and vacuum sealed. Experiments using the flow chamber were
398 performed in Endo-SFM supplemented with 2% FBS at 37°C. Bacterial pre-cultures were
399 diluted to $OD_{600} = 0.075$ and introduced into the chamber using a syringe pump (Vial
400 Medical, Becton Dickinson, or Harvard Apparatus) at a shear stress of 0.044 dynes/cm².
401 After 10 minutes of flow application, adherent bacteria were automatically detected and
402 counted for multiple fields of view in the case of the pilin variants PilE_{SA} and PilE_{SB}.

403

404 Bacteria proliferation on HUVECs

405 Time-lapse experiments were performed in Endo-SFM supplemented with 10% FBS at 37°C
406 5% CO_2 . HUVECs were seeded into Ibidi μ -slides with Ibidi-treated plastic bottom (Ibidi) at a
407 density of 10^4 cells/channel. Bacterial pre-cultures were diluted to $OD_{600} = 0.02$ (MOI = 200),
408 loaded into the channels for 15 minutes at 37°C 5% CO_2 to allow bacterial adhesion and

409 finally washed 3X with fresh medium. Time-lapse movies of GFP expressing PiiE_{SA} and
410 PiiE_{SB} bacterial strains proliferating on HUVECs were acquired over 4hs with a 20x phase
411 objective. High resolution z-stacks of bacterial colonies were performed with a 100X
412 objective (Z-step = 0.2 μ m).

413

414 Fusion of bacterial aggregates

415 Bacterial aggregates from a 2h pre-culture in RPMI + 10% FBS were loaded in a μ -dish
416 chamber (Ibidi). Mineral oil was added on top to avoid evaporation. A micropipette was
417 introduced in the chamber and used to put in close proximity aggregates with similar sizes to
418 facilitate the fusion. 2D fusion time-lapses were acquired in transmission at 0.2 fps for 2
419 minutes. To characterize the fusion dynamics, the temporal evolution of the contact surface
420 between aggregates was quantitated via kymographs.

421

422 Wetting of bacterial aggregates

423 Glass substrates of CellView cell culture slides (Greiner, REF. 543979) were plasma
424 activated and coated with 100 μ g/ml FITC-conjugated lectin from *Triticum Vulgaris* (Sigma-
425 Aldrich, L4895) to allow bacterial adhesion. Bacterial aggregates from a 2h pre-culture in
426 RPMI + 10% FBS were loaded and fast time-lapse acquisition in TIRF mode was started to
427 selectively visualize the temporal evolution of the aggregate contact area with the substrate
428 over 20 minutes.

429

430 Micropipette aspiration

431 Micropipette aspiration was largely performed as previously described (Guevorkian et al.,
432 2010). Pipettes were fabricated by pulling borosilicate capillaries (1mm/0.78mm O/I
433 diameter, Harvard Apparatus) with a laser-based puller (P-2000, Sutter Instruments). and
434 sized to the desired diameter and fire-polished by using a microforge with a heated glass
435 ball, to generate a smooth glass surface. Finally, the pipette tip was slightly bent by heating
436 it with a flame, in order to minimize the angle between the pipette and the bottom of the

437 observation chamber. A liquid suspension of bacterial aggregates was transferred to a tissue
438 culture treated plastic Ibidi μ -dish (Biovalley, France) with walls cut to facilitate the access of
439 the micropipette. Aggregates were then suspended in 600 μ l of RPMI + 10% FBS and the
440 open end was sealed with mineral oil to prevent evaporation. The micropipette was
441 eventually introduced into the chamber. A range of pressures ($\Delta P = 10$ -100 Pa) was attained
442 by vertically displacing a water reservoir, connected to the pipette, with respect to the
443 observation chamber. Movies of the progression of aggregates inside the pipette were
444 recorded in bright field with a frame rate of 10 images/s. Image processing was performed
445 using Image J software (Schneider et al., 2012).

446

447 Live-cell imaging

448 Acquisitions were performed on a Ti-inverted spinning disk microscope (Nikon) at 37°C, with
449 5% CO₂ atmosphere, using 20X (NA 0.75), 40X (NA 1.3) and 100X (NA 0.5-1.3) objectives.
450 Images were acquired using Metamorph (Molecular Devices). The excitation source was
451 either transmission illumination or the 491, 561, 642 laser line. Images were acquired with a
452 EMCCD camera (Evolve, Photometrics). TIRF images were acquired with an ORCA C8484-
453 03G01 Hamamatsu camera.

454

455 Single bacteria tracking in aggregates and analysis

456 Plastic bottom microchannels (Ibidi) were passivated upon plasma activation and 30 min
457 incubation with 0.1 mg/ml PLL(20)-g[3.5]-PEG(2) (Surface Solutions) solution in 10mM
458 HEPES pH 7.4. Bacteria from 2h pre-cultures in RPMI + 10% FBS and various
459 concentrations of IPTG if needed, were then loaded in microchannels (Ibidi) and allowed to
460 settle for 15 minutes at 37°C. Streaming movies of 10-20 s (time frame 30 ms) of iRFP
461 expressing bacteria were acquired with a 100X objective at the confocal middle plane using
462 aggregates of similar sizes (30-40 μ m in diameter) for the different conditions. Movies were
463 post-processed with a standardized protocol on Fiji software, comprising background
464 subtraction, Gaussian blurring and masking to define the aggregate contour. Single bacteria

465 tracking was performed also on Fiji with Trackmate plugin. Analysis of bacterial tracks was
466 performed with a custom-built script in Matlab: briefly, average distance from the aggregate
467 center of mass and mean square displacements (MSD) were calculated over each single
468 bacterial track. Diffusion coefficients were extracted with a linear fit on the initial portion
469 (10%) of the MSD curve. Aggregate size was normalized to 1 in order to obtain a cumulative
470 distribution of diffusion coefficients over multiple aggregates (n=20). Data were binned to
471 extract average values and corresponding standard deviations in different aggregate
472 regions.

473

474 Micro-chambers fabrication

475 Masters containing round micropillars of different sizes were fabricated using conventional
476 positive photolithography techniques. The designs of the brass master molds were created
477 using Clewin software. Briefly, a silicon wafer (Neyco S.A. France; diameter = 4-inch,
478 thickness = 525) was spin-coated with TI Prime adhesion promoter (Microchemicals) and
479 resist AZ® 9260 (Microchemicals). Predesigned features were patterned through a mask
480 aligner (MJB4, Süss Microtec ReMan GmbH, Germany) equipped with an I-Liner filter
481 (365nm) and a chuck for 4-inch wafers. The non-exposed resist was removed by solvent
482 AZ® 400K in water solution, air dried and passivated by overnight exposure to
483 Trichloro(1H,1H,2H,2H-perfluorooctyl)silane vapors (Sigma Aldrich, ref: 448931). A 10:1
484 mixture of PDMS Sylgard 184 or RTV614 silicone elastomer and curing agent was poured
485 onto the brass master and cured at 65°C for 3 h. PMDS chips were cleaned with isopropanol
486 before a 30-s exposure to air plasma for activation. Agarose micro-chambers were prepared
487 by pouring molten 2% agarose containing FITC-Dextran (MW 15 kD; Life Technologies) onto
488 the PDMS chip. The agar pad was then stripped off, flipped and resized to a final 5 mm X 5
489 mm thick pad. Cells at OD600~0.3 were diluted 20-fold, and 2 µL of bacterial suspension
490 were loaded onto the agarose pad. As soon as the agar surface appeared dry, the pad was
491 flipped onto a plastic bottom Fluorodish (Ibidi) previously passivated upon plasma activation
492 and 30 min incubation with 0.1 mg/ml PLL(20)-g[3.5]-PEG(2) (Surface Solutions) solution in

493 10mM HEPES pH 7.4. Streaming movies of single iRFP-expressing WT bacteria diffusing in
494 the micro-chambers were acquired with a 40X objective at 200ms time frame for 3-5
495 minutes.

496

497 Analysis of bacterial interactions

498 Single bacteria tracking was performed on Fiji with Trackmate plug-in and post-processed
499 with a custom-made Matlab script. We defined the 2 states of interactions between bacterial
500 pairs as follows: (i) Attraction phase (or ON): this combines the active period where cells
501 approach towards each other in a highly ballistic manner with a high speed, and the
502 following contact period where bacteria remain in close proximity (cell-cell distance below
503 threshold of 3 μm); (ii) No interaction phase (or OFF): corresponds to the time period where
504 bacteria do not interact, e.g. they diffuse freely and are not in close proximity (cell-cell above
505 threshold of 3 μm).

506 The approach phase was defined by characterizing the time-evolution of cell-cell distance as
507 follows. First, we compute the relative cell-cell position $\vec{X}_3(t)$ and distance $L(t)$ as:

508

$$509 \quad \vec{X}_3(t) = \vec{X}_1(t) - \vec{X}_2(t)$$

$$510 \quad L(t) = |\vec{X}_3(t)|$$

511

512 where $\vec{X}_1(t)$ and $\vec{X}_2(t)$ denote the 2D positions at instant t of bacterium 1 and 2 respectively.

513 Their time derivatives, relative velocity $\vec{V}(t)$ and rate of distance change $\dot{L}(t)$, were defined
514 as:

515

$$516 \quad \vec{V}(t) = \frac{(\vec{X}_3(t + \tau/2) - \vec{X}_3(t - \tau/2))}{\tau}$$

$$517 \quad \dot{L}(t) = \frac{(L(t + \tau/2) - L(t - \tau/2))}{\tau}$$

518

519 where $\tau = 6$ frames = 1.2 s.

520 The approach phases are characterized by a fast, ballistic approach of two bacteria, where
521 cell-cell relative distance decreases rapidly. These phases were automatically detected by
522 adopting two criteria:

523 (1) Approach phases correspond to a sharp decrease of $\dot{L}(t)$ to large negative values
524 followed by its increase back to ~ 0 ; therefore, they should contain a local minimum of $\dot{L}(t)$.

525 (2) $\dot{L}(t)$ at the local minimum should be smaller than a threshold value, $Thr1$. $Thr1$ was set
526 to be $-2.5 \mu\text{m/s}$. This principle is necessary to distinguish true approach phases from other
527 random non-directed motions.

528 The beginning and the end of a single approach phase were defined as the time points
529 where $\dot{L}(t)$ exceeds a second threshold $Thr2$. $Thr2$ was set to be $-1 \mu\text{m/s}$.

530 The approach dynamics was characterized by computing the approach speed and
531 orientation. The approach speed was defined as the norm of relative velocity:

532

$$533 \quad \vec{V}_{INT}(t) = |\vec{V}(t)|$$

534

535 The approach orientation θ_{INT} was defined as the orientation of $|\vec{V}(t)|$:

536

$$537 \quad \theta_{INT} = \tan^{-1}(\vec{V}(t) - \vec{X}_3(t))$$

538

539 Note that if two bacteria are approaching straightly, θ_{INT} is 0 and approach speed is equal to
540 $\dot{L}(t)$. Average speed and velocity orientation were defined as temporal averages for the
541 entire period. Finally, analyses of all interaction events between bacterial pairs were pulled
542 together to generate probability distributions of each parameter. Mean values of interaction
543 distance, time of interaction (t_{ON}) and time of non-interaction (t_{OFF}) were derived by
544 exponential fits, while mean values of interaction speed and interaction angle were derived
545 by Gaussian fits.

546

547 Notations:

548 $\vec{X}_1(t), \vec{X}_2(t)$: 2D position of bacteria 1 and 2 at time x_1 , vector

549 $\vec{X}_3(t)$: relative position of x_1 and x_2 , vector

550 $L(t)$: relative distance between two bacteria, scalar

551 $\vec{V}(t)$: relative true speed

552 $\dot{L}(t)$: change rate of $L(t)$ = projection of $\vec{V}(t)$ for cell-cell axis

553

554 Microchannels experiment

555 Microchannels were a kind gift from Matthieu Piel's lab, and were prepared as previously
556 described(Heuze et al., 2011). Briefly, a 10:1 mixture of PDMS Sylgard 184 or RTV614
557 silicone elastomer and curing agent was poured onto the epoxy replica and cured at 65°C
558 for 3h, to prepare microchannels of different sizes (12 μm X 8 μm , 5 μm X 4 μm). Channel
559 inlets were punched with a 2.5-mm puncher before bonding. The PDMS chamber and an
560 Ibidi μ -slide 2 well with glass-bottom dish (80287, Biovalley) were exposed to 45 s oxygen
561 plasma for bonding. The binding was left to strengthen in a 65 °C oven for 1 h. Finally,
562 chambers were exposed for 10 min to UV light for sterilization (UVO cleaner, Jelight), and
563 incubated with RPMI + 10% FBS for at least 1 h at 37 °C and 5% CO₂ before cell loading.
564 Bacteria from 2 hs pre-cultures in the same medium were re-suspended at a final OD 0.1,
565 vortexed and then injected in the chamber. Finally, the chamber was immersed in medium to
566 prevent evaporation. Time-lapse microscopy was performed in parallel on WT and *pilT*
567 strains in two separated wells over 10h and temporal evolution of aggregate size was
568 quantified.

569

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627 **Supplementary Materials contain:**

628 SI Model

629 Table T1

630 Figures S1-S5

631 Movies S1-S8

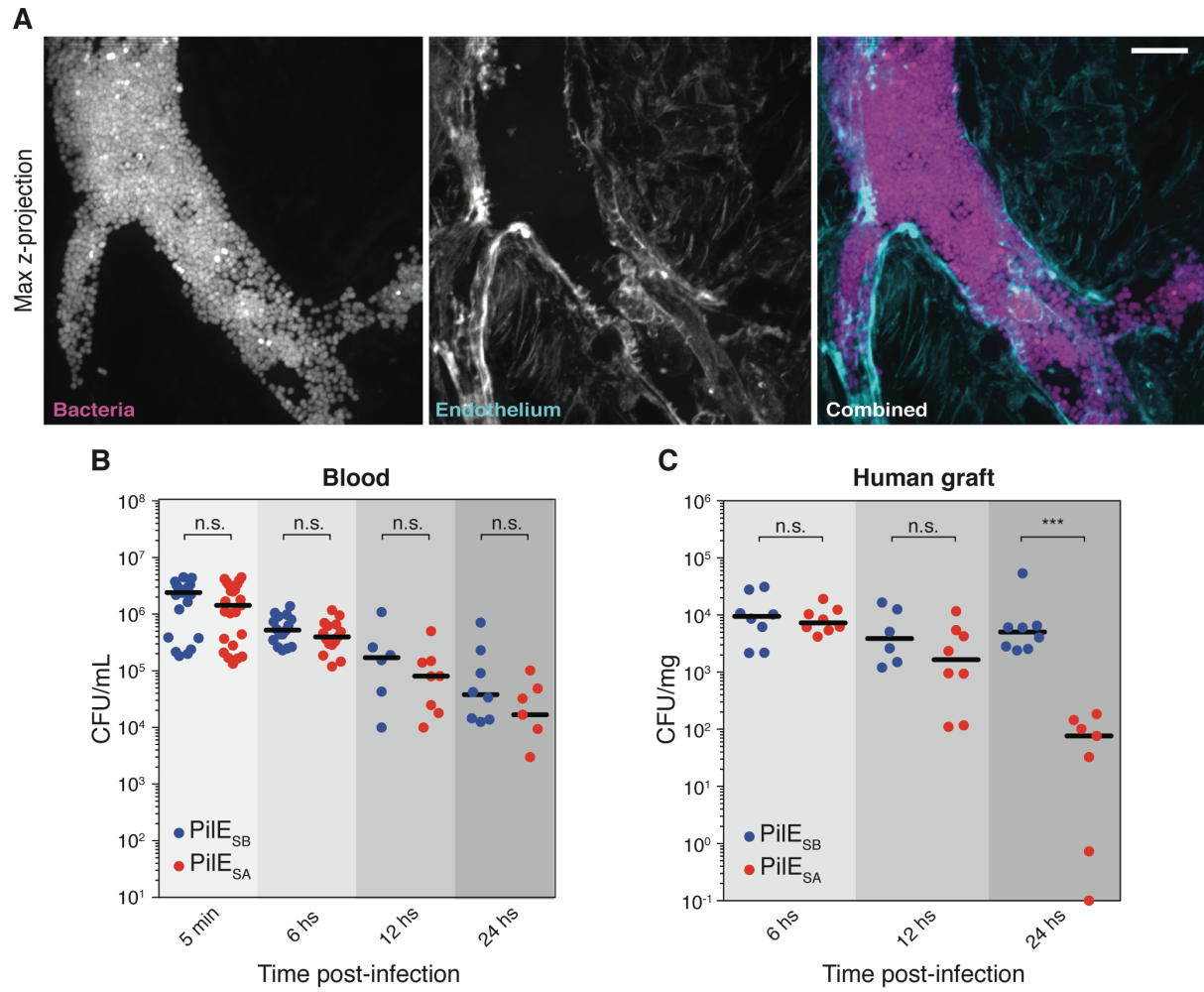


Fig. 1

632 **Figure 1: Bacterial auto-aggregation enhances vascular colonization *in vivo*. (A)**
633 Maximal z-projection from confocal slices of an infected vessel at 6h post-infection in a
634 xenograft model of infection showing the human endothelium surface (Rhodamin-labelled
635 *Ulex Europaeus* Agglutinin), GFP-expressing *Neisseria meningitidis* aggregates and
636 corresponding merged image. Scale bar, 10 μ m. **(B)** Bacterial colony forming unit (CFU)
637 counts from the blood of mice grafted with human skin and infected with the *N. meningitidis*
638 pilin variants PilE_{SB} (indicated in blue) and PilE_{SA} (indicated in red). These counts represent
639 the number of bacteria circulating in the blood at a given time-point. **(C)** Bacterial CFU
640 counts from skin biopsies taken from grafted human skin of mice infected with the *N.*
641 *meningitidis* pilin variants PilE_{SB} (indicated in blue) and PilE_{SA} (indicated in red). These
642 counts indicated the number of bacteria attached to the capillary endothelium at a given time
643 point. Each data point represents one infected mice. Black bars indicate average values for
644 each condition. n.s. = not significant; ***: P-value = 0.0003.

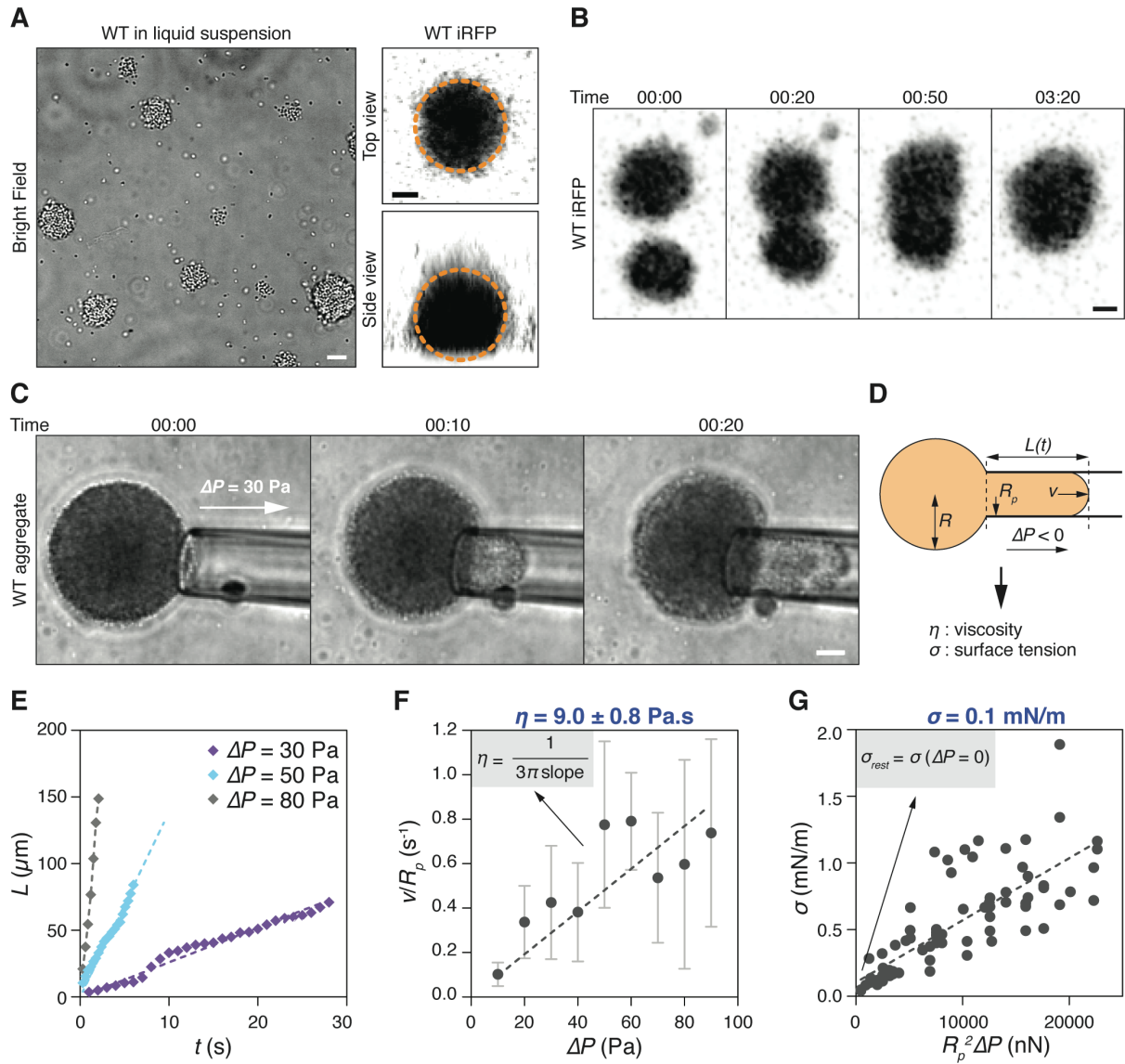


Fig. 2

645 **Figure 2: *Neisseria meningitidis* aggregates display a viscous liquid behavior in**
646 **suspension. (A)** On the left, Bright-Field image of WT bacterial aggregates. On the right,
647 top and side confocal views of a WT iRFP bacterial aggregate. The quasi-spherical
648 aggregate shape in both views is highlighted by a dotted orange line. The plastic substrate
649 was treated with PLL-PEG to minimize adhesion events. Scale bars, 10 μm . **(B)** Confocal
650 time-lapse of a fusion event between WT iRFP bacterial aggregates. Indicated times are in
651 min:sec. Scale bar, 10 μm . **(C)** Bright-field temporal evolution of a WT aggregate
652 progressing into a micropipette upon aspiration ($\Delta P = 30 \text{ Pa}$). Indicated times are in min:sec.
653 Scale bar, 10 μm . **(D)** Schematic representation of micropipette aspiration and definition of
654 physical parameters to estimate aggregate viscosity and surface tension. R : aggregate
655 radius; R_p : pipette radius; $L(t)$: tongue length at instant t ; ΔP : applied negative pressure; v :
656 tongue progression speed in the pipette; η : aggregate viscosity; σ : aggregate surface
657 tension. **(E)** Aggregate progression inside the pipette (tongue length, L) as a function of time
658 t upon application of 3 different pressures: 30, 50 and 80 Pa, indicated with violet, light blue
659 and grey dots respectively. Dotted lines indicate corresponding linear fits used to estimate
660 tongue progression speed. **(F)** Tongue progression speed to pipette radius ratios for a range
661 of applied pressures (10-100 Pa). Dots and error bars indicate corresponding average
662 values and standard deviations for each applied pressure, ΔP . N=80 WT aggregates. A
663 linear fit to extract aggregate viscosity is represented by a dotted line. **(G)** Surface tension as
664 a function of the applied force $R_p^2 \Delta P$. Dots indicate single surface tension estimations for
665 each aspiration event. Surface tension at rest is extrapolated with a linear fit (dotted line) at
666 corresponding null force. N=80 WT aggregates.

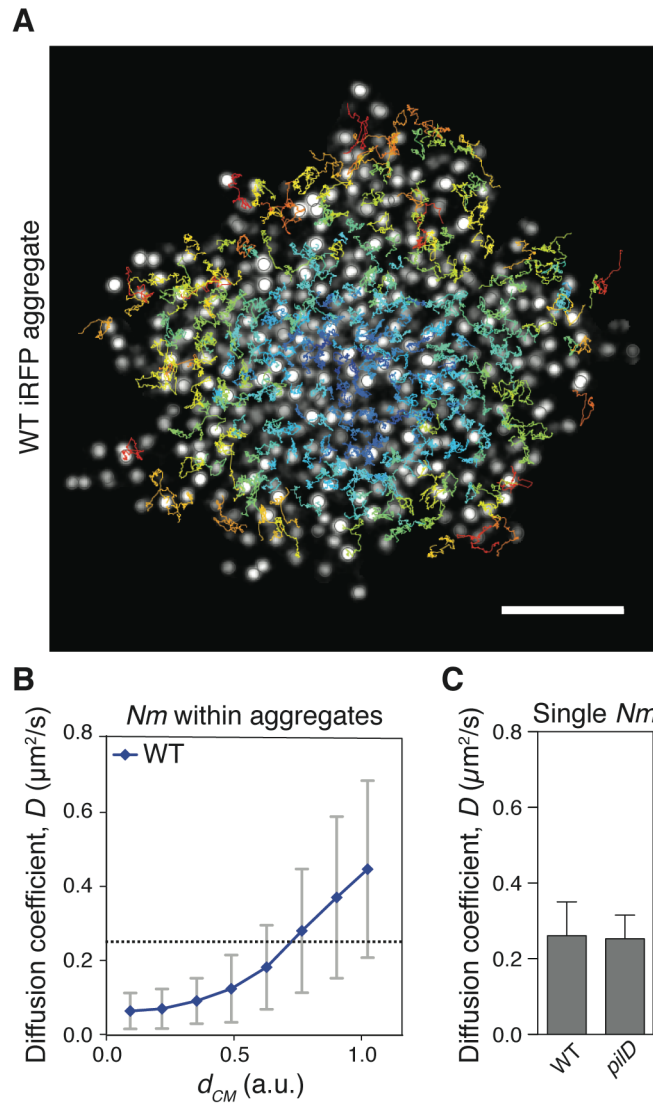


Fig. 3

667 **Figure 3: Diffusion of bacteria inside the aggregates reaches values higher than**
668 **individual bacteria in suspension. (A)** Single confocal slice of a WT iRFP aggregate in the
669 middle plane, and overlay of single bacterial tracks over a 10 s period with 30 ms time step.
670 Tracks are color-coded depending on their mean instantaneous speeds. Scale bar, 10 μm .
671 **(B)** Average effective diffusion coefficients and corresponding standard deviations of
672 individual bacteria as a function of their normalized distance from the aggregate center of
673 mass, d_{CM} (0, center; 1; periphery). N=20 WT aggregates of similar size. Dotted line
674 indicates the average diffusion coefficient of individual isolated WT bacteria. **(C)** Average
675 diffusion coefficients and corresponding standard deviations of individual bacteria for WT
676 and the non-piliated mutant *pilD*.

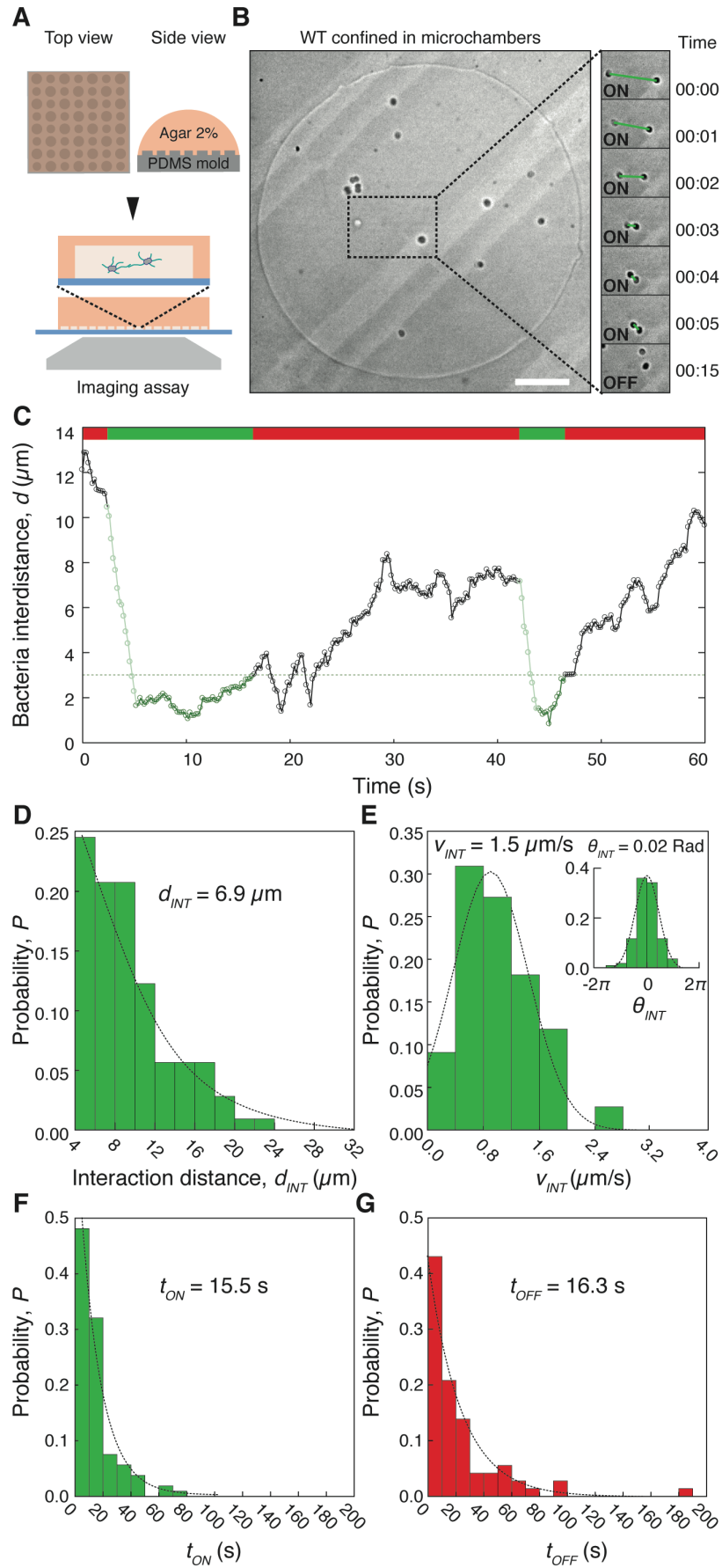


Fig. 4

677 **Figure 4: Pairwise interaction between bacteria shows cycles of attraction and**
678 **release. (A)** Schematic of agar chamber fabrication and experimental setup to observe the
679 interaction of a controlled number of bacteria. **(B)** On the left, Bright-field view of WT
680 bacteria confined inside agar chambers. On the right, zoom-in view of a time-lapse
681 sequence showing an event of attraction (indicated by a green line) and release between
682 two bacteria. Time is indicated in min:sec. **(C)** Interbacterial distance measured as a function
683 of time for the pair highlighted in Figure 3B. The attraction and contact phases appear in
684 light and dark green respectively, while the non-interacting phases appear in red. An
685 arbitrary threshold of 3 μm for the interbacterial distance was chosen to define the end of the
686 ON phase. **(D)** Probability distribution histogram of measured interaction distances. A mean
687 value is extracted with an exponential fit. **(E)** Probability distribution histogram of measured
688 approaching speeds and angles (inset). Mean values are extracted with a gaussian fit. **(F)**
689 Probability distribution histogram of measured interaction times (t_{ON}). A mean value is
690 extracted with an exponential fit. **(G)** Probability distribution histogram of measured non-
691 interacting time while within interaction range (t_{OFF}). A mean value is extracted with an
692 exponential fit.

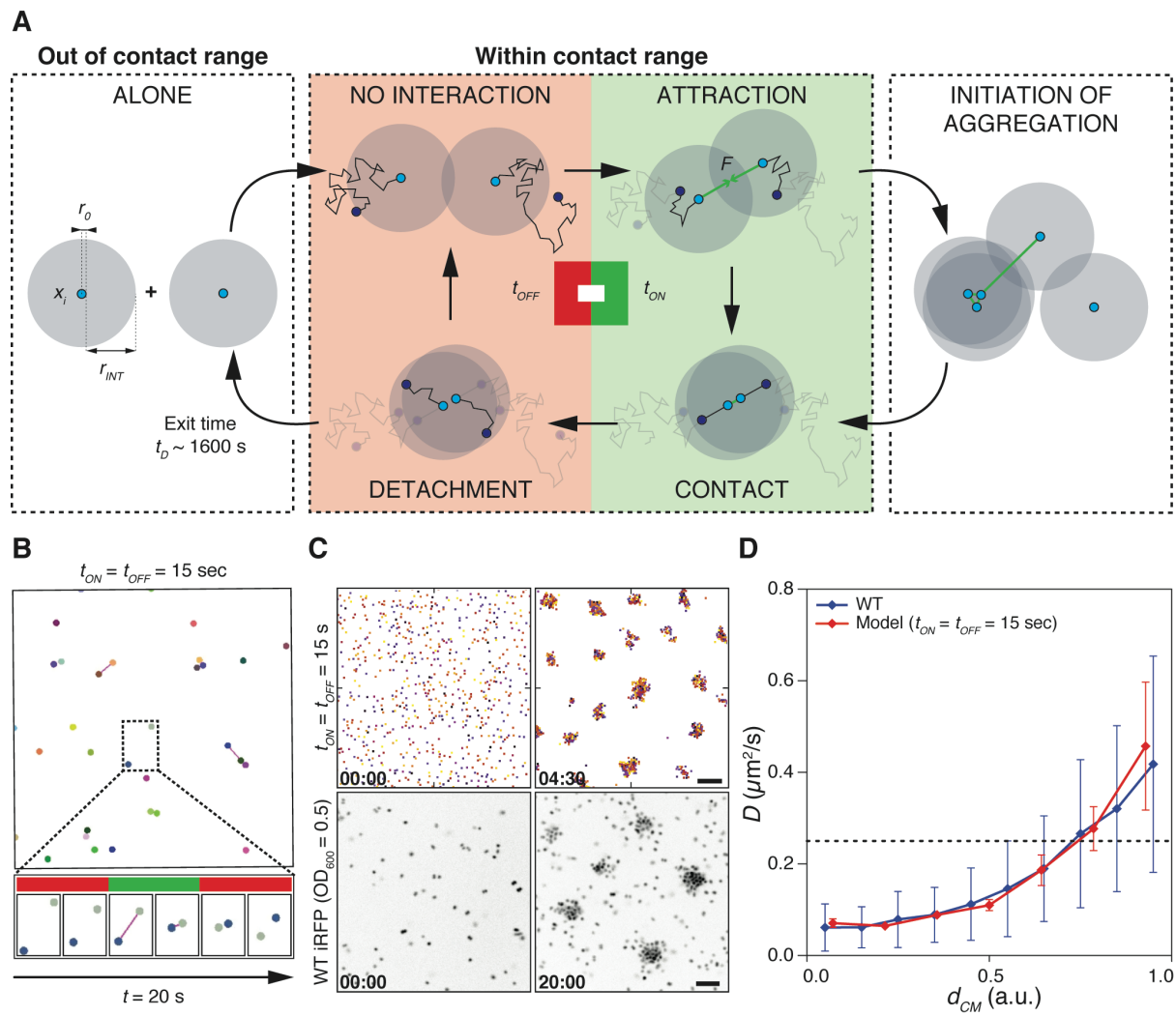


Fig. 5

693 **Figure 5: An intermittent attractive force between particle pairs is sufficient to**
694 **recapitulate aggregate properties. (A)** Schematic of the main physical principles
695 implemented in the computational model of intermittent interaction. Individual particles are
696 defined by a hard core (r_0) and an interaction range (r_{INT}). When the pair is within the
697 interaction distance r_{INT} , it has a given probability of interaction (t_{ON}). During the interaction
698 phase, an equal attractive force (F) is exerted on both particles bringing them together and
699 keeping them in contact; finally, the force is released, particles detach and move away from
700 each other. The exit time t_D corresponds to the time necessary for two particles to be out of
701 range after contact by diffusion only. In the case of bacteria, $t_D \sim 1600$ s considering $D = 0.25$
702 $\mu\text{m}^2/\text{s}$ and a $20 \mu\text{m}$ maximum contact range. **(B)** On the top, view of simulated interactions
703 between particles for $t_{ON} = t_{OFF} = 15$ s. On the bottom, zoom-in view on a simulated time-
704 lapse showing interacting and non-interacting phases between two particles over 20 s,
705 indicated in green and red respectively. **(C)** On the top, simulation of aggregate formation
706 dynamics starting from an initial random distribution, for $t_{ON} = t_{OFF} = 15$ s and high particle
707 density. On the bottom, aggregate formation dynamics of WT iRFP bacteria at $\text{OD}_{600} = 0.5$.
708 Time is indicated in min:sec. Scale bars, $10 \mu\text{m}$. **(D)** *In vitro* and *in silico* effective diffusion
709 coefficients of individual bacteria inside WT aggregates as a function of normalized distance
710 from the aggregate center of mass (d_{CM}), indicated in blue and red respectively. Simulated
711 aggregates correspond to $t_{ON} = t_{OFF} = 15$ s. Dots and error bars correspond to average
712 values and standard deviations. The dotted line indicates the experimentally determined
713 diffusion coefficient of isolated bacteria.

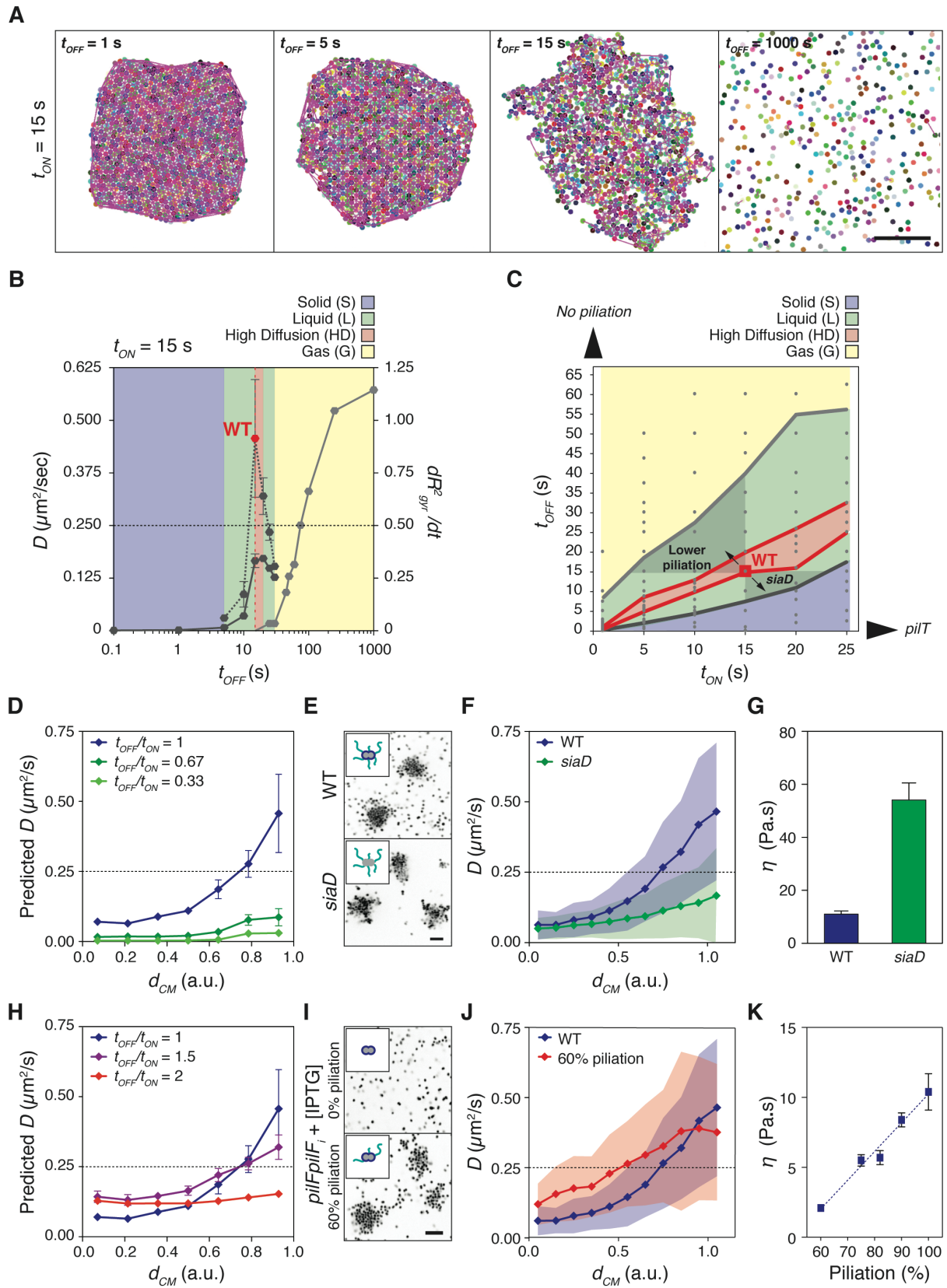


Fig. 6

714 **Figure 6: Intermittent attractive properties between bacteria define a phase diagram of**
715 **aggregation phenotypes. (A)** Views of *in silico* aggregation states for a fixed value of $t_{ON} =$
716 15 s and four different values of t_{OFF} : 1, 5, 15 and 1000 seconds. Scale bar, 10 μm . **(B)**
717 Theoretical phase diagram of particle behavior as a function of t_{OFF} for a fixed value of $t_{ON} =$
718 15 s. Solid (blue) to liquid (green) transition is defined with the average diffusion coefficient
719 of all particles in the aggregate (left axis, dark grey line). Liquid (green) to gas (yellow)
720 transition is defined with the aggregate expansion rate, calculated as the derivative of
721 square gyration radius (right axis, light grey line). The high diffusion zone (red) corresponds
722 to a regime where the average diffusion coefficient of particles at the aggregate periphery
723 (left axis, dark grey dotted line) exceeds the diffusion coefficient of isolated bacteria (black
724 dotted line). Location of WT bacterial aggregates is indicated with a red dot and red dotted
725 line. **(C)** Theoretical phase diagram of particle behavior as a function of t_{ON} and t_{OFF} . The
726 color code is the same as in 4B. Grey dots indicate all simulated conditions to assess phase
727 transitions. Light grey regions illustrate model predictions for lower (bottom right region,
728 corresponding to *siaD* mutant) or higher (top left region, corresponding to underpiliated
729 mutants) t_{OFF}/t_{ON} ratios. Non-piliated mutants are located at the extreme top left of the
730 diagram, corresponding to infinite t_{OFF}/t_{ON} ratio. Hyperpiliated mutant with non-retractile T4P
731 (*pilT*) is located at the extreme bottom right of the diagram, corresponding to null t_{OFF}/t_{ON}
732 ratio. **(D)** Simulated diffusion coefficient as a function of the position in the aggregate with
733 decreasing t_{OFF}/t_{ON} ratios (1, 0.67 and 0.33). **(E)** On the top left, schematic of WT and non-
734 capsulated *siaD* mutant. On the right, corresponding views of iRFP-expressing aggregates
735 for each strain. Scale bar, 10 μm . **(F)** Experimental measurement of the diffusion coefficient
736 of individual bacteria as a function of their position in the aggregate for WT (blue) and *siaD*
737 (green). Average values and corresponding standard deviations are indicated with dots and
738 filled regions respectively. **(G)** Viscosity of the *siaD* (green) mutant compared to WT (blue)
739 as determined by micropipette aspiration. **(H)** Simulated diffusion coefficient as a function of
740 the position in the aggregate with increasing t_{OFF}/t_{ON} ratios (1, 1.5 and 2). **(I)** On the top left,
741 schematic of the *pilF* inducible strain with amounts of inducer generating 0% (top) and 60%

742 (bottom) of piliation relative to WT. On the right, corresponding view of iRFP-expressing
743 bacteria for each condition. Scale bar, 10 μm . **(J)** Experimental measurement of the diffusion
744 coefficient of individual bacteria as a function of their position in the aggregate for WT (blue)
745 and *pilF* inducible strain at 60% of piliation (red). Average values and corresponding
746 standard deviations are indicated with dots and filled regions respectively. **(K)** Viscosity of
747 the *pilF* inducible strain treated with various levels of inducer and thus different levels of
748 piliation as determined by micropipette aspiration.

749

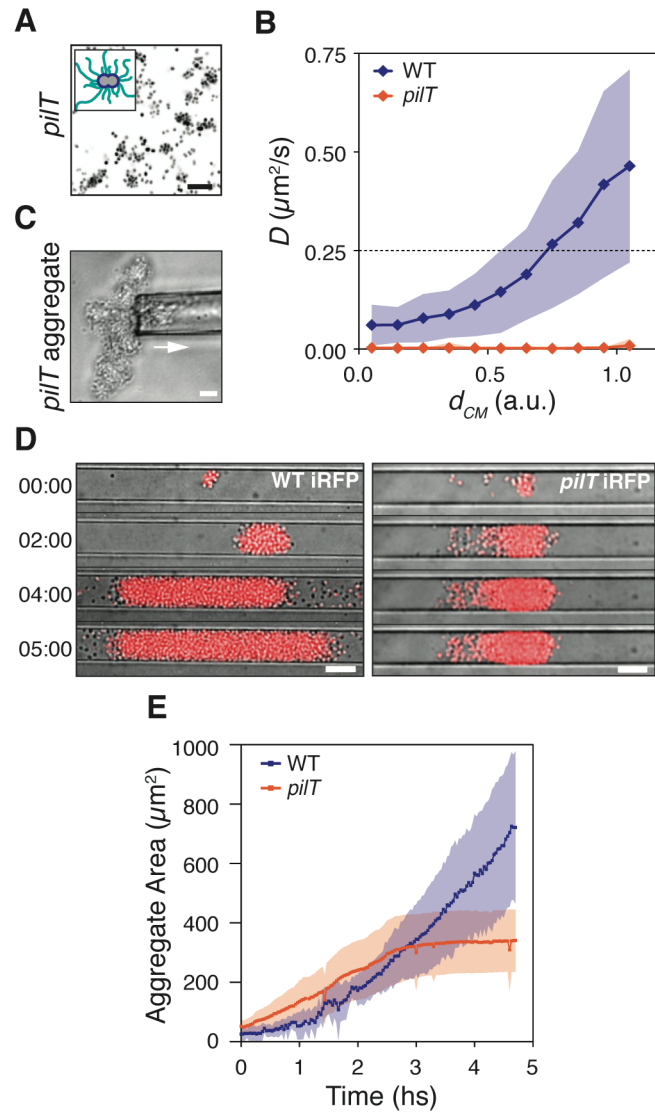


Fig. 7

751 **Figure 7: Fluid-like behavior of aggregates favors colonization of capillary mimics. (A)**
752 Schematic of the *pilT* mutant and corresponding view of iRFP-expressing mutant
753 aggregates. Scale bar, 10 μm . **(B)** Experimental measurement of the diffusion coefficient of
754 individual bacteria as a function of their position in the aggregate for WT (blue) and *pilT*
755 (orange). Average values and corresponding standard deviations are indicated with dots and
756 filled regions respectively. **(C)** Bright-Field view of a *pilT* aggregate subjected to micropipette
757 aspiration but unable to deform and progress as a fluid. Scale bar, 10 μm **(D)** Time-lapse
758 combined view of proliferating WT and *pilT* aggregates inside 10 μm wide PDMS
759 microchannels. Bright-Field: grey; iRFP: red. Time indicated in hs:min. Scale bar, 10 μm . **(E)**
760 Quantification of the surface of aggregates formed by WT (blue) and *pilT* (orange) inside
761 microchannels following proliferation over a 5-hour period. Average values and
762 corresponding standard deviations are indicated with dots and filled regions respectively.