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1 **Clustering and rapid long-range motility behavior of bacteria by type IV pili**

2

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16

17 **Abstract**:

18 Many organisms coordinate to move and colonize over surfaces. Bacteria such as *Pseudomonas*
19 *aeruginosa* exhibit such surface motility as a precursor step to forming biofilms. Here we show a
20 group surface motility where small groups of *P. aeruginosa* use their type IV pili (TFP)
21 appendages over long- distances. Small cell clusters employ their TFP to move multiple cell
22 lengths in fractions of a second and form new multicellular groups. Given the length scale and
23 speed of displacement, cells appear to "snap" to a new position and then resume their previous
24 behavior. The same long range TFP action also leads to rapid community contraction of sparsely
25 arranged cell clusters. Cluster development and snapping motility does not require exogenous
26 DNA or extracellular polysaccharides.

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29 **Introduction:**

30 TFP function as motility appendages by an extension, tip attachment, and retraction order
31 of action [1]. These thin structures (~6 nm diameter) range from 0.5-7.0 µm in length and can
32 generate pulling forces exceeding 100 pN [1-5]. For the bacterium *Pseudomonas aeruginosa*,
33 TFP are known to confer motility phenotypes known as twitching and walking. *P. aeruginosa*
34 also employs its polar flagellum to confer motility modes called swimming and swarming. The
35 transition between flagellar-mediated motility to TFP-mediated motility is not well understood.
36 For example, while swarming requires active flagella, the absence or over expression of TFP
37 have been shown to influence the overall swarm phenotype [6]. Here we show that TFP facilitate
38 a cluster development and rapid motility phenotype in a subset of cells following flagellar-driven
39 swarming community expansion. We find that *P. aeruginosa* TFP can extend multiple cell
40 lengths (>30µm) and then retract to translocate small clusters of cells to join with other cell
41 clusters in less than one second.

42

43 **Results and Discussion:**

44 There are two modes of *P. aeruginosa* surface motility that are routinely investigated
45 using agar motility plate assays. Flagellar-mediated swarming is studied using 0.4%-0.6% agar
46 and TFP-mediated twitching is studied using 1.0% agar. Both swarming and twitching are
47 commonly assessed in these plate assays as growing and expanding *P. aeruginosa* populations
48 that develop over hours to days. Here we detail surface motility behavior that occurs on surfaces
49 between those conditions typically used to examine swarming and twitching (0.4-1.0% agar). We
50 find that as *P. aeruginosa* colonies expand from the point of inoculation, small clusters of cells
51 advance beyond the larger group. Using this approach, an open-air plate was required—covering

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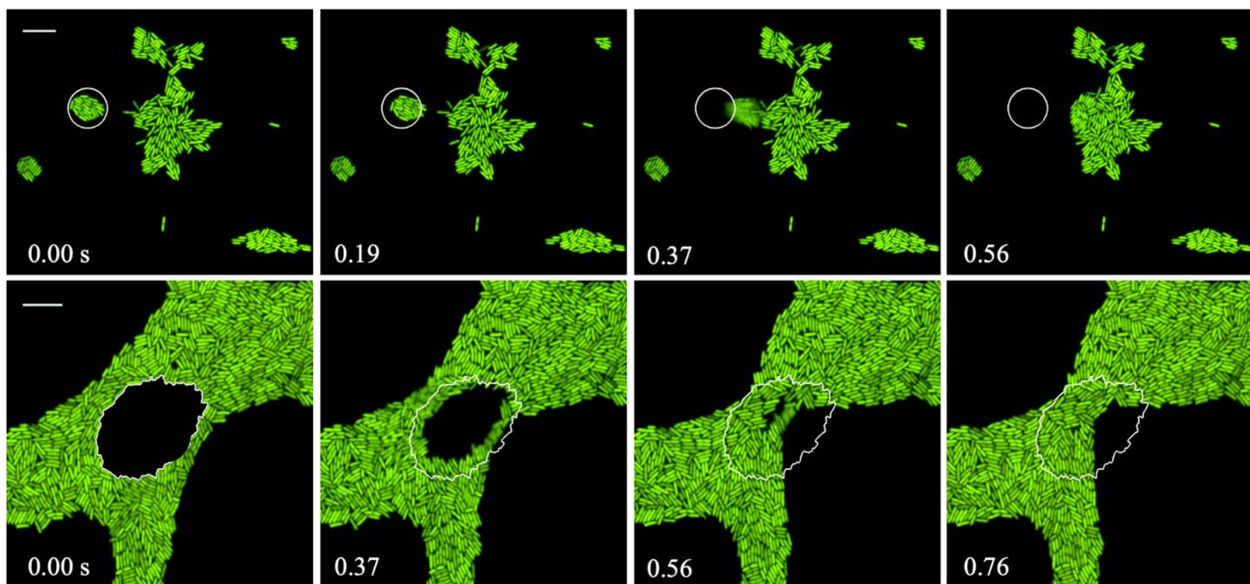
52 the agar (with a glass coverslip) does not promote the same cluster development. We find this
53 clustering phenotype is conserved among *P. aeruginosa* strains, as all common laboratory strains
54 tested (PA14, PAO1, MPAO1, PAK and PAO1C) develop clusters to some degree (Figure S1).
55 Inspection of these clusters over time shows they are transient and facilitate the
56 restructuring of the community. Cells cluster, divide into smaller groups, and recluster numerous
57 times within a minute. Some of these clusters move multiple cell lengths to join other clusters or
58 the larger advancing colony within less than a second (Figure 1—top panels), thus appearing to
59 "snap" from one region of the frame to another (Movie S1).



60
61 **Fig. 1**. *P. aeruginosa* snapping and community contraction in less than 1 second. Time-lapse
62 sequences of representative *P. aeruginosa* snapping and community contraction events are
63 shown. Top row: advancing cluster (identified with white circle at the 0 s) prepares to snap by
64 enhacing cluster arrangement (0.19 s), begins moving (0.37 s), and joins another cluster (0.56 s).
65 Bottom row: A contraction snapping event. The open area surrounded by cells (traced in white—
66 spanning 469.87 μm^2) is covered within 0.76 s. Scale bar represents 10 µm.
67

68 In all, we track cells in 115 of these snapping clusters in various assays and specifically
69 quantify 70 snapping events in experiments containing 0.8% agar (Table 1). These snapping
70 clusters contain an average of 17.5 cells and travel a maximum distance of 17.8 µm, or ~4 cell
71 lengths. Cluster size does not correlate with distance traveled or snapping duration. Likewise, no

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72 apparent trend changes are attributable to the original time of assay inoculation. However, the
73 total distance traveled by snapping clusters strongly relates with water availability in these
74 surface motility assays. The maximum snapping distance on 0.45% agar assays is 37 µm or ~9
75 cell lengths, while the maximum distance cells snap on 1.0% agar is 10 µm.
76
77 **Table 1.** ***P. aeruginosa*** **snapping behavior dynamic traits.**

Trait	Average (± standard deviation)
Cells in cluster*,**	17.5 ± 9.60
Cluster area at t=0 (μm^2)	67.2 ± 39.4
Cell length	4.2±0.75
Distance travelled (µm)*	11.4 ± 3.00
Duration (sec)**	0.478 ± 0.183
Cluster velocity (µm/sec)	26.8 ± 10.5

78 Quantification from n=70 identified snapping events for *P. aeruginosa* growing on 0.8% agar.
79 *Pearson correlation between cells in cluster and distance travelled is 0.475.
80 **Pearson correlation between cells in cluster and duration of event is 0.039.
81

82 Larger less ordered groups also exhibit this long-distance rapid cell movement as a means
83 of community contraction. A representative example is shown in Figure 1 (bottom panels) where
84 an open area surrounded by cells (traced in white—spanning 469.87 μm^2) collapses within 0.76
85 seconds.

86 Snapping behavior requires *P. aeruginosa* TFP. Isogenic mutants deficient for TFP fail to
87 form the small aggregates and no sub-second rapid behavior is detected (Figure 2). Further,
88 functional TFP retraction is required as the retraction-deficient *pilU* strain also does not form
89 clusters and exhibits behavior like the *pilA* strain. When one considers the size of PilA
90 monomers [7] and TFP assembly [8] required to mediate snapping, nearly 17,000 PilA
91 monomers are required to assemble TFP capable of the average event measured in our
92 experiments. Accordingly, the pilus required to mediate the maximum distance event (of 37 µm)
93 contains a staggering 35,200 PilA monomers.

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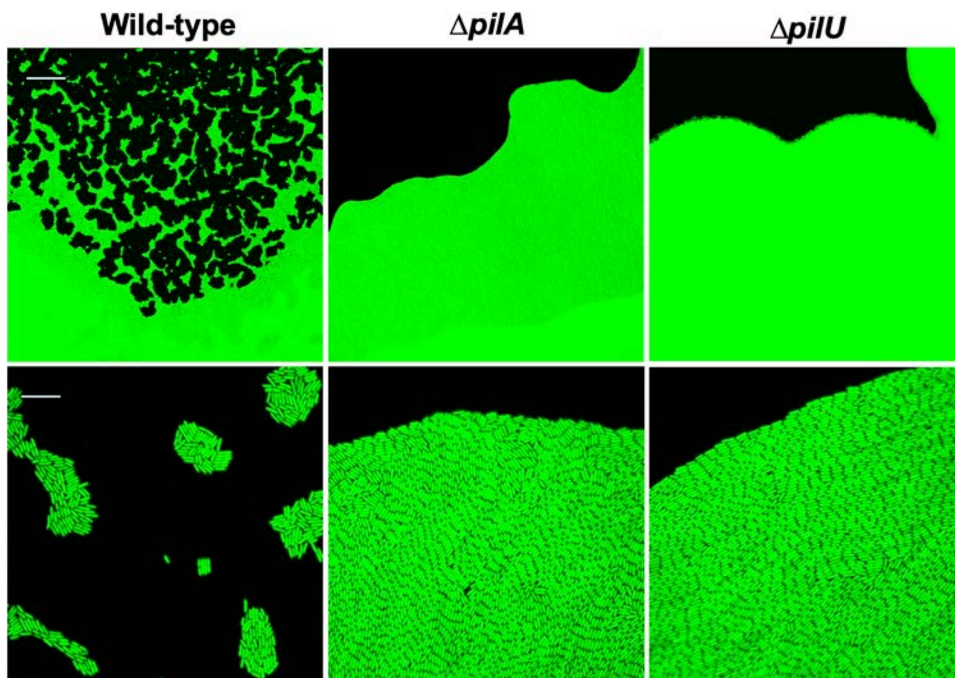

95
96 **Figure 2.** Advancing colony phenotype of *P. aeruginosa* on motility assay plates containing 0.8%
97 agar. Top (10× magnification) demonstrates cluster development by wild-type beyond the
98 advancing swarm zone. TFP-deficient (Δ*pilA*) and TFP retraction-deficient (Δ*pilU*) strains make
99 no clusters. Bottom row (100× magnification) shows arrangements of single cells, and Δ*pilA* and
100 Δ*pilU* appear highly ordered and tightly packed. Scale bars represent 100 μm (top) and 10 μm
101 (bottom).
102

103 A lag time is seen after plate assay inoculation before snapping is observed. We also see
104 that a functional flagellum is a required precursor to this snapping motility phenotype. On 0.5%
105 agar assays that promote robust swarming, the lag time for cluster development and snapping is
106 1.5-2 days (as the plates dry out). However, using an agar range of 0.8%-1.0% promotes cluster
107 formation and snapping within a few hours post inoculation. Cells missing their polar flagellum
108 (*fliC*) are widely dispersed and fail to develop the small clusters that form beyond the advancing
109 large population on these surface assays (Figure 3). Thus, it appears that flagellar-mediated
110 swarming can serve as an initial step to establish the needed population of cells to subsequently
111 form cell clusters that will snap together using their TFP.

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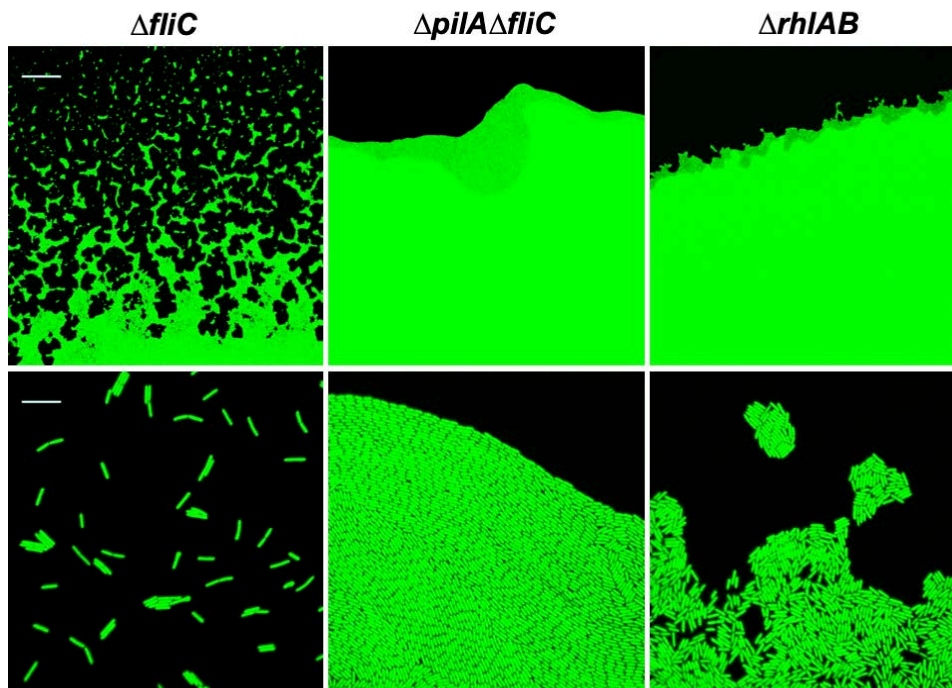
112 Having established TFP as the appendages that mediate snapping, we investigate the
113 additional biological factors required. We find that snapping also requires the surfactant
114 rhamnolipid. Cells from a rhamnolipid-deficient strain (Δ*rhlAB*) can move away from the
115 uniformly expanding *P. aeruginosa* colony and form some clusters, but these bacteria never
116 exhibit snapping motion (Figure 3).
117



118

119 **Figure 3.** Colony edge and clustering phenotype of *P. aeruginosa* mutants on 0.8% agar. Top
120 (10× magnification): Flagella-deficient (Δ*fliC*) are mostly sparsely distributed at the single-cell
121 level, while the appendage-deficient strain (Δ*pilA*Δ*fliC*) do not form cells clusters. Rhamnolipid-
122 deficient (Δ*rhlAB*) cells make a reduced number of smaller clusters closer to the colony edge.
123 Bottom row (100× magnification) show arrangements of single cells (mono-layer). Images were
124 obtained using confocal microscopy twelve hours after inoculation. Top (10× magnification)
125 Scale bars represent 100 μm (top) and 10 μm (bottom).
126

127 We next examine the potential role of DNA as a marker and promoter of snapping
128 motility as previous studies have shown the ability of *P. aeruginosa* TFP to track to exogenous
129 DNA as a component of biofilm development [9,10]. We find that snapping precedes the

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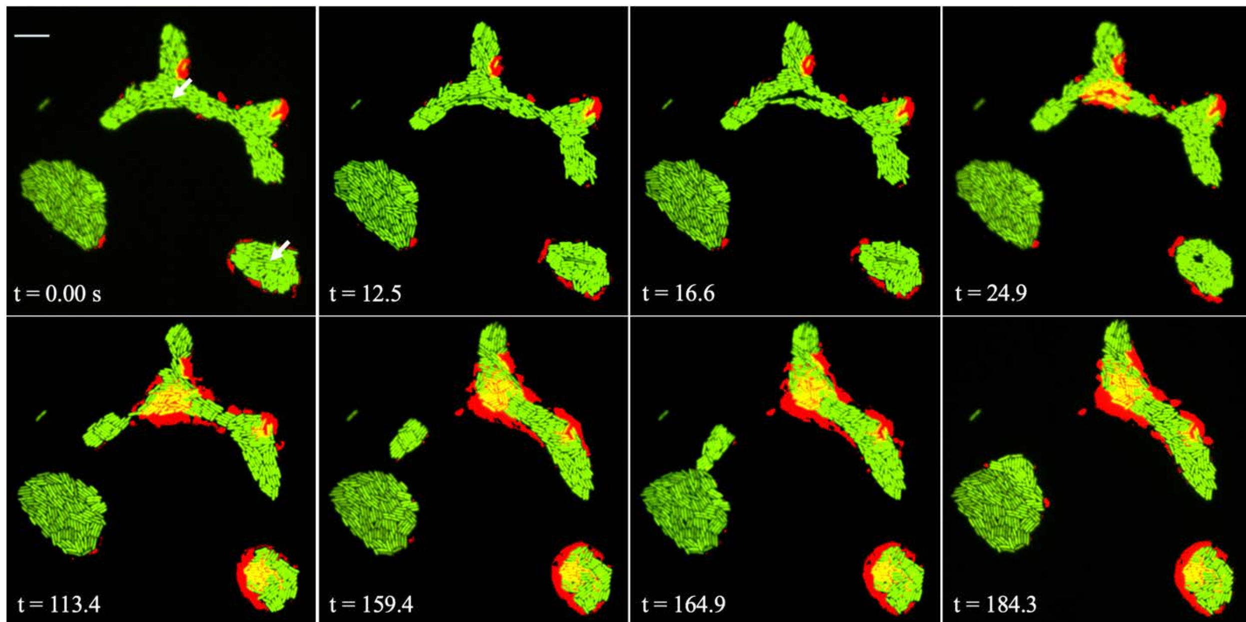
130 presence of detectable DNA in these small cell groups. Snapping is observed to occur in the
131 same experiment as "explosive cell lysis" [11]. Figure 4 shows snapping of a small cluster in a
132 region with no exogenous DNA while a cell simultaneously explodes to release DNA in a
133 separate region (sequence included as Movie S2). Thus, we conclude that snapping does not
134 require TFP-DNA interaction and is distinct from TFP actions mediated by exogenous DNA.



135
136 **Figure 4.** Sequence of events demonstrating eDNA release from a lysing cell within the same time
137 span as snapping motility that occurs elsewhere in the same frame. A cell ready to lyse (top arrow,
138 0.00 s) ceases production of GFP (12.5 s), explodes by 16.6 s, and releases its exogenous DNA at
139 24.9 s. The red color indicates the DNA released by the lysing cell. A cluster begins to pull away
140 (113.4 s) from the cluster exhibiting a high concentration of high eDNA and snaps onto a different
141 cluster by the 116.9 s. Scale bar represents 10 µm.
142
143 Since DNA did not serve as an attractant for snapping clusters, we next investigate the *P.*
144 *aeruginosa* polysaccharides Pel and Psl, which have been shown important to *P. aeruginosa*
145 surface motility and aggregation as a stage in biofilm development [12,13]. Similarly, the
146 bacterium *Myxococcus xanthus* is well known to require exopolysaccharide to confer "social"
147 TFP-mediated motility [14-16]. However, we find that the Pel and Psl polysaccharides for *P.*
148 *aeruginosa* do not confer cluster development or the snapping phenotype. Elimination of either

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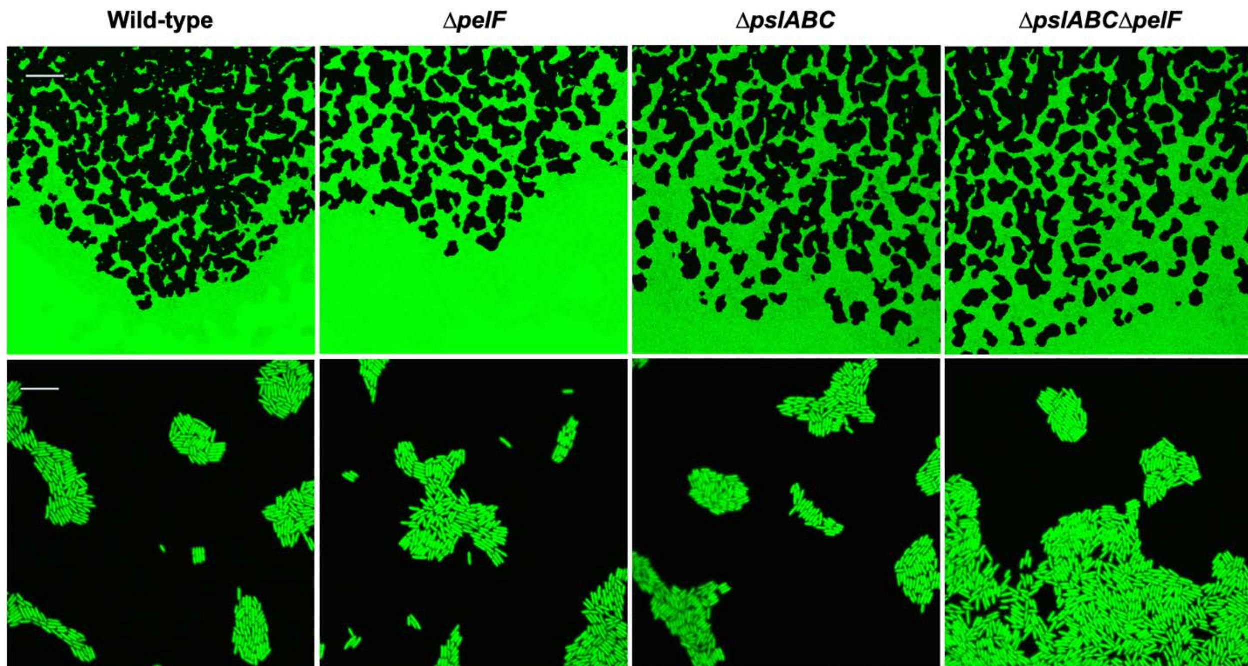
149 or both the Pel and Psl polysaccharides actually leads to increased cluster development and
150 equivalent snapping events to that we observe for the wildtype (Figure 5).



151
152
153 **Figure 5**. Top (10× magnification) demonstrates cluster formation by Pel-deficient (Δ*pelF*), Psl-
154 deficient (Δ*pslABC*), and polysaccharide-deficient (Δ*pslABC*Δ*pelF*) in similar fashion as the wild-
155 type. Bottom row (100× magnification) shows the arrangements of single cells (mono-layer)
156 within these clusters and advancing swarm edges. Bottom row (100× magnification) show clusters
157 at of single cell level. Scale bar represent 100 μm (top) and 10 μm (bottom).
158

159 Here we detail TFP-dependent snapping in the bacterium *P. aeruginosa*. Our probing of
160 cluster development on semi-solid surfaces suggests that snapping occurs readily as small groups
161 of *P. aeruginosa* cells rapidly restructure the surrounding community within seconds.

162

163 Materials and Methods

164 ***Bacteria strains and growth medium.*** Strains of *Pseudomonas aeruginosa* used in this study are
165 included in Table S1. Cultures were streaked from frozen (-80°C) stocks onto LB agar plates
166 (1.5% wt/vol) and incubated at 37°C overnight. Isolated colonies selected and grown

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167 planktonically in 6 mL FAB with 30 mM glucose [6,17] at 37°C with shaking at 240 rpm for 12-
168 15 hrs.

169 ***Isogenic mutant construction.*** Primers and gBLOCK nucleotides used for this work are
170 included in Table S2. Plasmid pCSM1 was constructed to allow creation of a markerless
171 Δ*pslABC* mutant. pCSM1 was constructed by inserting a single gBLOCK of double-stranded
172 DNA (IDT, Coralville, IA) containing both regions upstream of the *pslA* gene and downstream
173 of the *pslC* gene according to the *Pseudomonas* genome database [18]; this gBLOCK was
174 restricted using EcoRI and HindIII and ligated into the pEX18Ap vector between the EcoRI and
175 HindIII sites and expressed in *E. coli* DH5α.

176 Similarly, plasmids pNMS and pCSM103 were constructed to allow creation of a markerless
177 Δ*rhlAB* and Δ*fliC* mutants. pNMS utilized a single gBLOCK containing both regions upstream
178 of the *rhlA* gene and downstream of the *rhlB* gene and pCSM103 contained regions upstream
179 and downstream of the *fliC* gene. These amplified gBLOCK products were ligated into the
180 pEX18Gm plasmid [19] following SLiCE protocols [20].

181 Mutations were introduced using conjugational mating. Single cross-over recombinants were
182 selected on LB plates (1.5% agar wt/vol), augmented with 100μg/ml carbenicillin (pNMS,
183 pCSM1 and pCSM103) or 100μg/ml gentamycin (pEX18GmΔ*pelF*). Double crossover
184 recombinants were then selected on 5% wt/vol sucrose LB plates. Deletions were confirmed by
185 comparing PCR amplification of the target region with the PAO1C wildtype.

186 ***Constitutive fluorescent strain construction.*** Plasmids harboring a Tn7 region containing green
187 fluorescent protein under control of a constitutive strong promoter were incorporated into *P.*
188 *aeruginosa* strains by conjugational mating. Either plasmid pBK miniTn7-gfp2 (harboring
189 gentamycin resistance) or pBK miniTn7-*gfp3* (harboring kanamycin and streptomycin

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190 resistance) were incorporated adjacent to *glmS* on the *P. aeruginosa* chromosome using helper
191 plasmid pUX-BF13 and mobilization plasmid pRK600. Transgenic cells were selected on LB
192 agar supplemented with 100µg/mL gentamycin or 250µg/mL kanamycin and 250µg/mL
193 streptomycin and confirmed by PCR detection of the chromosomal insertions and microscopy
194 inspection for fluorescence.

195 ***Surface motility assays.*** Surface motility plate assays were composed of modified fastidious
196 anaerobe broth (FAB) media that included 0.1% casamino acid (wt/vol) and no ammonium
197 sulfate ($(NH_4)_2SO_4$) [6,17]. Surface motility was evaluated over a range of Noble Agar
198 concentrations between 0.4%-3.0% (wt/vol). Plate assays were spot inoculated by pipetting 1µm
199 log-phase cells onto the center of the 60mm Petri dish and quickly transferred to a laminar hood
200 for 3-5 minutes with lids partially open, promoting rapid absorption of the inoculation droplet
201 into the motility agar. All plates were inverted and incubated at 30°C in a humidity-controlled
202 (85% RH) incubator for 15-30 h. Prior to imaging, motility plates were equilibrated to room
203 temperature for 45-60 minutes.

204 ***Microscopy and imaging.*** Imaging was carried out using a Nikon Eclipse Ti-E Inverted
205 microscope equipped with Plan Fluor 10X DIC LN1 and 100X LU Plan Fluor objectives and
206 Andor DU-888 X-10006 camera with Emission wavelength = 535, Excitation wavelength = 470.

207 ***Image processing and analysis.*** Images were processed using Nikon NIS-Element software and
208 further analyzed using Fiji image processing package (3). Using tools in these software
209 packages, size of clusters, distance and speed travelled by a cluster were determined.

210

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269 The PA14Δ*pelF* strain and pEX18GmΔ*pelF* plasmid were provided by Matthew Parsek,

270 University of Washington-Seattle.

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273 **Supplementary Materials:**

274 Tables S1-S2

275 Figure S1

276 Movies S1-S2

277

278

279 **Table S1. Strain list**

Strain or plasmid	Relevant Characteristics	Source or reference
<u>*P. aeruginosa*</u>		
Wild type (wt)	wildtype PAO1C	[6,21]
wt-GFP	PAO1C::miniTn7 gfp2; Cm^r, Gm^r	[6,21]
wt-mCherry	PAO1C::mini-Tn7-Gm-$P_{A1/04/03}$-mCherry	[6]
Δ*pilA*	PAO1C Δ*pilA*; markerless	[6,21]
Δ*pilA*-GFP	Δ*pilA* miniTn7 gfp2; Cm^r, Ap^r, Gm^r	[6]
Δ*pilU*	PAO1C Δ*pilU*; markerless	[6]
Δ*pilU*-GFP	Δ*pilU*::miniTn7 gfp2; Cm^r, Ap^r, Gm^r	[6]
Δ*rhlAB*	PAO1C Δ*rhlAB*; markerless	This study
Δ*rhlAB*-GFP	Δ*rhlAB::* miniTn7-*gfp2*; Cm^r, Ap^r, Gm^r	This study
Δ*fliC*	PAO1C Δ*fliC*; markerless	This study
Δ*fliC*-GFP	PAO1C Δ*fliC::*miniTn7-*gfp3*; Tc^r, Km^r, Sm^r	This study
Δ*psl*	PAO1C Δ*pslABC*; markerless	This study
Δ*psl*-GFP	Δ*pslABC::*miniTn7-*gfp2*; Cm^r, Ap^r, Gm^r	This study
Δ*pelF*	PAO1C Δ*pelF*; markerless	This study
Δ*pelF*-GFP	Δ*pelF::*miniTn7-*gfp2*; Cm^r, Ap^r, Gm^r	This study
Δ*psl*Δ*pel*	PAO1C Δ*pslABC*Δ*pelF*; markerless	This study
Δ*psl*Δ*pel*-GFP	Δ*psl*Δ*pel::*miniTn7-*gfp2*; Cm^r, Ap^r, Gm^r	This study
PA14-GFP	wildtype PA14::miniTn7 gfp2; Cm^r, Gm^r	[22]
PA14*pelF*	PA14 Δ*pelF*	[23]
PAO1-GFP	wildtype PA01::miniTn7 gfp2; Cm^r, Gm^r	This study
MPAO1-GFP	wildtype MPA01::miniTn7 gfp2; Cm^r, Gm^r	This study
PAK-GFP	wildtype PAK::miniTn7 gfp2; Cm^r, Gm^r	This study
<u>Plasmids</u>		
pEX18Ap	Suicide gene replacement vector, Ap^r	[19]
pRK600	Mobilization plasmid, Cm^r	[24]
pUX-BF13	Helper plasmid, Ap^r	[25]
pBK-miniTn7-*gfp2*	mini Tn7-*gfp2*; Cm^r, Ap^r,Gm^r	[26]
pBK-miniTn7-*gfp3*	mini Tn7-*gfp3*::Cm^r, Ap^r, Km^r, Sm^r	[26]
pNMS	*rhlAB* allelic replacement vector in pEX18Gm; Gm^r	This study
pEX18GmΔ*pelF*	pEX18Gm::Δ*pelF* allelic replacement vector; Gm^r	[23]
pCSM1	*pslABCD* allelic replacement vector in pEX18Ap; Amp^r	This study
pCSM103	*fliC* allelic replacement vector in pEX18Gm; Gm^r	This study

280

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281 **Table S2. Primers and gBLOCK sequences**

Primers	Sequence (5' → 3')
PelF-up	ACCAGCAGGATGCGTTTGTA
PelF-down	TGGTACTGGGAACTGGCCTA
rhlAB-F	CACGCTGAGCAAATTGTTCG
rhlAB-R	CGAAGATCTACGCCAATGAAGG
rhlAB gBLOCK	CAGGAAACAGCTATGACCATGATTACGAATTCGCGAGCCTCGTTCCAGAGCATCCG GCTCTGGTCGAACAGGCTGTCGCTCCAGACCACCATTTCCGAGGAGCGCAGGCCGT TGAGGATCGCCGGATCCACGGCCCCGTAGTTCTGCATCTGGTATCGCTCCAGCCAG GCCTTGGGATAGGTGCCATGGACCTCGGTCTTCGGCCGGGTGAAGGGAATCGTGTG GCGCACGCCATAGGCGTAGTAATCGAAGCCCAGGCGCCGCACTTCCTTTTCCAGGA CGGCGAACACGCCCTGGCTGTCGTGGATCGGCTGCATCTCGCTACGCAAACCGTCC CACCACAGCAAAAAGCCTCCGTCATTCCTCATTGCAGTAAGCCCTGATCGATAAAA TGCATCACAGCAGAATTGGCCCGGGGTATGACGCTGTCAAGCGCGCAGCCTTGCCG ATACGGCAAAATCATGGCAACCCTATCTGTTATGCCAGCACCGTTCAGGACCGCAT TTCACACCTCCCAAAAATTTTCGAACAGGCAAACAGCTATCGCTGCCACGGGTATC CCGGCATTACGTAGAGTTCGTTCTTATTGTTCGAACGGCAGACAAGTAACTCAGCG GCCATCCGCGCGGACCAGCCACGGCGGATGCGTACCCCTTCAGGCGAGGGGCTTGT GTGGGTCTTGCAGATCGGCCTGCGCAAACGATTGGCGTCCGTGTTCACGCGTAGCC GATGAACACTTTTTAGCCAATTCGAAAGCTAACGGTAACTGCCAGATTTCACAGGA CGGGCGGCCGCCTGCGCATAAGCCCCTGCCCGCCCGCGATAAGGCGTGCCAGAGC GGCCGGCGGGGAACAGCGAGGGGTTGGCGGAAAAGGCATGAAAAAACCGGGCGC CAGGCCCGGTTTCCGGTGTCGAAACGCTCTGGCGTCAGGCTTTTGGCAGGGTCACG CCGCGCTGGCCCTGGTATTAAGCTTGGCACTGGCCGTCGTTTTACAACGTC
fliC-F	TGCTTTGCTATCGCGACAGTC
fliC-R	GGCCATAATCCATCTCCGTCAAA
fliC GBlock	TGCTTTGCTATCGCGACAGTCTCCCGGTCACGCGGGTGAAGAACCGTTCCAGGGACT GGCTGGAGGCGATCCGCGCGCACCTGGCCGATGCAGACGCCAACGCCGCGGCCGGC GCGCAGTTGCGCGAGGCGGTGCGCCGCGACTGGATGCTCGAAGGCGCGCATCTCGA GGCATGGGCGGCAGCCTGGCTGCCGGACTGAATCCGGGTTTTTCTCGAACGAGGCC GGTGGCGCAAGCCATCGGCCTTTTTTCATGCCCGGCGTGCCCTGTTGCACGGGAGGG CTAAAGAAAATCGCCGGGGGGTCGATGCAATGGGTGTCGGAACTTCCACCCTCTGC CGGACCAACCGGGGGCGGTTCAGGACCGATATTGGCGAGTCCTCTTCGAAGCATGT AACCCACTGAAGAGGAAGAGAAAAAGAAAATGTTGATTTTTTCTCTAAAGCTCCGC CGGGAAACGCCGATAAACACCATGAACGCGAATTCTTGGGGCACCTGAGCAAGCAG GCCGAGAGATCGCAAGCTCAGGTAACCGAAATAGGTCCTTTGGAGGAAATCACCAT GGCCCGCTAAGCCCGGGAACGGTCACTCACGCGGTACTGGGAGGAAGGGGTGACCC TTCCTCCCTTTTCCCTTTGCGAGGCATGAGAAATGGACGTCGGAAATATCACTTCCC TTTCTACGTTCAAGACCGGCAGGGCCCCGGAGGCCAGCGGCGATATCTTTGCGCCGC GCAGCGCAGCGGATGGCAGCGGCAAACCGTTGCCGGAAGTGACGGCTTCCCGGGAG GCCAGCGAATCTCGCGATGACCTGGGGCTCGCCGTCAGCGACATCCAGTCTTTCGTG CAGAGCGTCAAGCGCAACTTGAACTTCAGCATCGACGACTCCTCTGGCCAGGTCGT GGTCAAGGTGATCGACGGTGACTCGGGCGAAGTCGTGCGGCAAATCCCTTCCGAAG AAGTACTAAAACTTGCCGCCCGGCTCGACGACGTACACAGTGTCCTGTTCGAAACCC GCGCCTGACGCGCGGGTTGAACGACTTGGCATGGTGCTTGCCCTATCGAAGGGATA GGGCAGTACATGCACGGTTTTTTGACGGAGATGGATTATGGCC

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pslABC-F	TACGGCCAGTACCTGAACTACCA
pslABC-R	TTCCGGCCCGATCTTCATCAA
pslABC gBLOCK	GCTTGAATTCGGCAAACGTCCGCCAAGCCCCCCAGGTCGGACCGGCACGCTCGAAC GGCCAACCTGCGTCATGGAACCGCAGGCGCATCCTGCCCAGCCAGCCCGCAGCGCC AGTGGACAGGCGGGTCGTCGAACCGGCGGCTGGCCACCTGGCCGAGCGGCCTGCCC TCACCTTTCGCCCCGCTTCGCTTCCCAGGCCAGAGCGCTCGCGGATTGGCGGCGTCA GATTTCCTCGTCTACTGTTTGGATAAAAGTTTGGCGCCAGAAATACGTCAATAAATT GACTAAAAAAACTTACCCAGACTACGGATATTTCCCTGGGAATGCTAAGATAGCTA TCACAAAGCCACTATCGACGAATGAACCTATTCGACGGGAAAATGACTAAACCGCG TGGCAAATGAAAAATAGTCACTAAATTGACGCTTCACCGCCTTGCTCTTCCCTATCC ACTCAATGGACTGCCCGTGATCGGCAGAGCAAACAACACATCATGACCTACAGGAA GTGCTCCCTCATGAAACGCACCCTCCTCATGCTCGCCATGCTCGCCCTGGCCGCATG CAACACCCCCGCACGGATTCCCGCACCGGACAGCGACACCGTGGACAGCGGCAAGC GTGCCCTGGAAGAACTCGCCAGGCTACCGCCGGCGATGGAGCGGGTGCGCGTCGGG GACACCCTGCGGATCGTCCGCGATGCCGGGGAGATGCCGACCCTCTCGGCGTTCAA CGTCGCCACCATCTATGAACTGACGCTGTACACCGTGCTCAACGACGGCAGCATCTA CTATCCGTTCATCGGTCGCATCCAGGCCGCGCACCGCACGCCGCAGGAAATCGCCA ACGAGCTGACCACCAAGCTCGCGCCGATCTACCGCGAGCCGCGGGTCACGGTGAAC ATCAACCAGGCGCCGGGCAATACGGTGTTCGTCGGCGGCGCGGTGCGCAACCCGTC GGCCGTGCCGATCCCCGCCGCCAACAACATGAAGCTTGCTT

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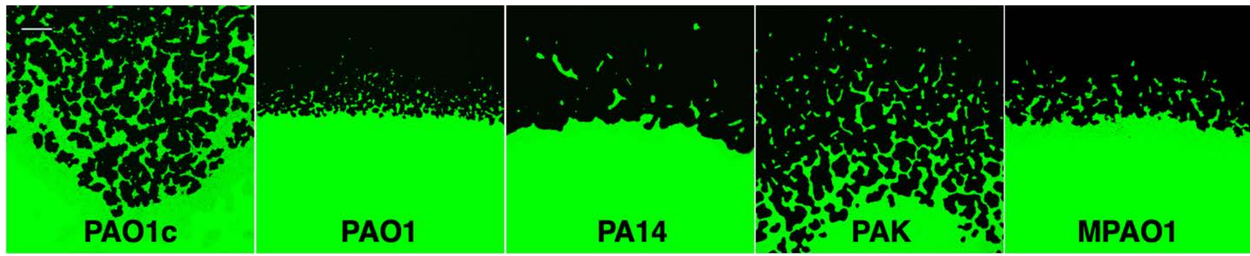
283 

284 **Fig. S1.** Cluster formation is conserved among wild-type *P. aeruginosa* strains on 0.8% agar. Scale
285 bar represent 100 μm.
286
287
288
289 **Supplemental Movie Captions:**
290
291 Movie S1. *P. aeruginosa* snapping motility. The cluster in the upper right of frame undergoes
292 some cell-cell rearrangement and then snaps to the larger cluster from right-to-left. (5.3 frames
293 per second)
294
295 Movie S2. *P. aeruginosa* rapid community contraction. The open area surrounded by cells
296 (spanning 469.87 μm^2) is covered within 0.76 s.
297
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299
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