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

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Abstract:

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

Introduction:

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

Results and Discussion:

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

the agar (with a glass coverslip) does not promote the same cluster development. We find this clustering phenotype is conserved among *P. aeruginosa* strains, as all common laboratory strains tested (PA14, PAO1, MPAO1, PAK and PAO1C) develop clusters to some degree (Figure S1).

Inspection of these clusters over time shows they are transient and facilitate the restructuring of the community. Cells cluster, divide into smaller groups, and recluster numerous times within a minute. Some of these clusters move multiple cell lengths to join other clusters or the larger advancing colony within less than a second (Figure 1—top panels), thus appearing to “snap” from one region of the frame to another (Movie S1).

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

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

apparent trend changes are attributable to the original time of assay inoculation. However, the total distance traveled by snapping clusters strongly relates with water availability in these surface motility assays. The maximum snapping distance on 0.45% agar assays is 37 μm or ~9 cell lengths, while the maximum distance cells snap on 1.0% agar is 10 μm .

Table 1. *P. aeruginosa* snapping behavior dynamic traits.

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

Quantification from n=70 identified snapping events for *P. aeruginosa* growing on 0.8% agar.

*Pearson correlation between cells in cluster and distance travelled is 0.475.

**Pearson correlation between cells in cluster and duration of event is 0.039.

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

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

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

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

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

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

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

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

Figure 4. Sequence of events demonstrating eDNA release from a lysing cell within the same time span as snapping motility that occurs elsewhere in the same frame. A cell ready to lyse (top arrow, 0.00 s) ceases production of GFP (12.5 s), explodes by 16.6 s, and releases its exogenous DNA at 24.9 s. The red color indicates the DNA released by the lysing cell. A cluster begins to pull away (113.4 s) from the cluster exhibiting a high concentration of high eDNA and snaps onto a different cluster by the 116.9 s. Scale bar represents 10 μ m.

Since DNA did not serve as an attractant for snapping clusters, we next investigate the *P. aeruginosa* polysaccharides Pel and Psl, which have been shown important to *P. aeruginosa* surface motility and aggregation as a stage in biofilm development [12,13]. Similarly, the bacterium *Myxococcus xanthus* is well known to require exopolysaccharide to confer “social” TFP-mediated motility [14-16]. However, we find that the Pel and Psl polysaccharides for *P. aeruginosa* do not confer cluster development or the snapping phenotype. Elimination of either

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

Figure 5. Top (10 $\times$ magnification) demonstrates cluster formation by Pel-deficient ($\Delta pelF$), Psl-deficient ($\Delta pslABC$), and polysaccharide-deficient ($\Delta pslABC\Delta pelF$) in similar fashion as the wild-type. Bottom row (100 $\times$ magnification) shows the arrangements of single cells (mono-layer) within these clusters and advancing swarm edges. Bottom row (100 $\times$ magnification) show clusters at of single cell level. Scale bar represent 100 μm (top) and 10 μm (bottom).

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

Materials and Methods

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

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

Isogenic mutant construction. Primers and gBLOCK nucleotides used for this work are included in Table S2. Plasmid pCSM1 was constructed to allow creation of a markerless $\Delta pslABC$ mutant. pCSM1 was constructed by inserting a single gBLOCK of double-stranded DNA (IDT, Coralville, IA) containing both regions upstream of the *pslA* gene and downstream of the *pslC* gene according to the *Pseudomonas* genome database [18]; this gBLOCK was restricted using EcoRI and HindIII and ligated into the pEX18Ap vector between the EcoRI and HindIII sites and expressed in *E. coli* DH5 α .

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

Mutations were introduced using conjugational mating. Single cross-over recombinants were selected on LB plates (1.5% agar wt/vol), augmented with 100 μ g/ml carbenicillin (pNMS, pCSM1 and pCSM103) or 100 μ g/ml gentamycin (pEX18Gm $\Delta pelF$). Double crossover recombinants were then selected on 5% wt/vol sucrose LB plates. Deletions were confirmed by comparing PCR amplification of the target region with the PAO1C wildtype.

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

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

Surface motility assays. Surface motility plate assays were composed of modified fastidious anaerobe broth (FAB) media that included 0.1% casamino acid (wt/vol) and no ammonium sulfate ((NH₄)₂SO₄) [6,17]. Surface motility was evaluated over a range of Noble Agar concentrations between 0.4%-3.0% (wt/vol). Plate assays were spot inoculated by pipetting 1µm log-phase cells onto the center of the 60mm Petri dish and quickly transferred to a laminar hood for 3-5 minutes with lids partially open, promoting rapid absorption of the inoculation droplet into the motility agar. All plates were inverted and incubated at 30°C in a humidity-controlled (85% RH) incubator for 15-30 h. Prior to imaging, motility plates were equilibrated to room temperature for 45-60 minutes.

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

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

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269 The PA14 $\Delta pelF$ strain and pEX18Gm $\Delta pelF$ plasmid were provided by Matthew Parsek,
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Supplementary Materials:

Tables S1-S2

Figure S1

Movies S1-S2

Table S1. Strain list

Strain or plasmid	Relevant Characteristics	Source or reference
<i>P. aeruginosa</i>		
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]
$\Delta pilA$	PAO1C $\Delta pilA$; markerless	[6,21]
$\Delta pilA$ -GFP	$\Delta pilA$ miniTn7 gfp2; Cm ^r , Ap ^r , Gm ^r	[6]
$\Delta pilU$	PAO1C $\Delta pilU$; markerless	[6]
$\Delta pilU$ -GFP	$\Delta pilU$::miniTn7 gfp2; Cm ^r , Ap ^r , Gm ^r	[6]
$\Delta rhlAB$	PAO1C $\Delta rhlAB$; markerless	This study
$\Delta rhlAB$ -GFP	$\Delta rhlAB$::miniTn7-gfp2; Cm ^r , Ap ^r , Gm ^r	This study
$\Delta fliC$	PAO1C $\Delta fliC$; markerless	This study
$\Delta fliC$ -GFP	PAO1C $\Delta fliC$::miniTn7-gfp3; Tc ^r , Km ^r , Sm ^r	This study
Δpsl	PAO1C $\Delta pslABC$; markerless	This study
Δpsl -GFP	$\Delta pslABC$::miniTn7-gfp2; Cm ^r , Ap ^r , Gm ^r	This study
$\Delta pelF$	PAO1C $\Delta pelF$; markerless	This study
$\Delta pelF$ -GFP	$\Delta pelF$::miniTn7-gfp2; Cm ^r , Ap ^r , Gm ^r	This study
$\Delta psl\Delta pel$	PAO1C $\Delta pslABC\Delta pelF$; markerless	This study
$\Delta psl\Delta pel$ -GFP	$\Delta psl\Delta pel$::miniTn7-gfp2; Cm ^r , Ap ^r , Gm ^r	This study
PA14-GFP	wildtype PA14::miniTn7 gfp2; Cm ^r , Gm ^r	[22]
PA14pelF	PA14 $\Delta pelF$	[23]
PAO1-GFP	wildtype PAO1::miniTn7 gfp2; Cm ^r , Gm ^r	This study
MPAO1-GFP	wildtype MPAO1::miniTn7 gfp2; Cm ^r , Gm ^r	This study
PAK-GFP	wildtype PAK::miniTn7 gfp2; Cm ^r , Gm ^r	This study
<i>Plasmids</i>		
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	<i>rhlAB</i> allelic replacement vector in pEX18Gm; Gm ^r	This study
pEX18Gm $\Delta pelF$	pEX18Gm:: $\Delta pelF$ allelic replacement vector; Gm ^r	[23]
pCSM1	<i>pslABCD</i> allelic replacement vector in pEX18Ap; Amp ^r	This study
pCSM103	<i>fliC</i> allelic replacement vector in pEX18Gm; Gm ^r	This study

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

Primers	Sequence (5' → 3')
PelF-up	ACCAGCAGGATGCGTTTGTGA
PelF-down	TGGTACTGGGAAGTGGCCTA
rhlAB-F	CACGCTGAGCAAATTGTTTCG
rhlAB-R	CGAAGATCTACGCCAATGAAGG
rhlAB gBLOCK	CAGGAAACAGCTATGACCATGATTACGAATTCGCGAGCCTCGTTCCAGAGCATCCG GCTCTGGTCTGAACAGGCTGTCTGCTCCAGACCACCATTTCCGAGGAGCGCAGGCCGT TGAGGATCGCCGGATCCACGGCCCCGTAGTTCTGCATCTGGTATCGCTCCAGCCAG GCCTTGGGATAGGTGCCATGGACCTCGGTCTTCGGCCGGGTGAAGGGAATCGTGTG GCGCACGCCATAGGCGTAGTAATCGAAGCCCAGGCGCCGCACTTCCTTTTCCAGGA CGCGAACACGCCCTGGCTGTCTGCTGGATCGGCTGCATCTCGCTACGCAAACCGTCC CACCACAGCAAAAAGCCTCCGTCATTTCCTCATTGCAGTAAGCCCTGATCGATAAAA TGCATCACAGCAGAATTGGCCCCGGGTATGACGCTGTCAAGCGCGCAGCCTTGCCG ATACGGCAAAATCATGGCAACCCTATCTGTTATGCCAGCACCGTTCAGGACCGCAT TTCACACCTCCCAAAAATTTTCGAACAGGCAAAACAGCTATCGCTGCCACGGGTATC CCGGCATTACGTAGAGTTTCGTTCTTATTGTTTCGAACGGCAGACAAGTAAGTACGCG GCCATCCGCGCGGACCAGCCACGGCGGATGCGTACCCCTTCAGGCGAGGGGCTTGT GTGGGTCTTGCAGATCGGCCTGCGCAAACGATTGGCGTCCGTGTTACGCGTAGCC GATGAACACTTTTTAGCCAATTCGAAAGCTAACGGTAAGTGCAGATTTTCACAGGA CGGGCGGCCGCTGCGCATAAGCCCCTGCCCCGCCGCGATAAGGCGTGCCAGAGC GGCCGGCGGGGAACAGCGAGGGGTGGCGGAAAAGGCATGAAAAAACGGGGCGC CAGGCCCGGTTTCCGGTGTGCAAAACGCTCTGGCGTCAGGCTTTTGGCAGGGTACG CCGCGCTGGCCCTGGTATTAAGCTTGGCACTGGCCGTCGTTTTACAACGTC
fliC-F	TGCTTTGCTATCGCGACAGTC
fliC-R	GGCCATAATCCATCTCCGTCAA
fliC GBlock	TGCTTTGCTATCGCGACAGTCTCCCGGTCACGCGGGTGAAGAACCCTTCCAGGGACT GGCTGGAGGCGATCCGCGCGCACCTGGCCGATGCAGACGCCAACGCCGCGCCGGC GCGCAGTTGCGCGAGGCGGTGCGCCGCGACTGGATGCTCGAAGGCGCGCATCTCGA GGTATGGGCGGACGCTGGCTGCCGACTGAATCCGGGTTTTTCTCGAACGAGGCC GGTGGCGCAAGCCATCGGCCTTTTTTCATGCCCCGCGTGCCCTGTTGCACGGGAGGG CTAAAGAAAATCGCCGGGGGGTTCGATGCAATGGGTGTGCGAACTTCCACCCTCTGC CGGACCAACCAGGGGGCGGTTTCAGGACCGATATTGGCGAGTCCTCTTCGAAGCATGT AACCCACTGAAGAGGAAGAGAAAAAGAAAATGTTGATTTTTTCTCTAAAGCTCCGC CGGGAAACGCCGATAAACACCATGAACGCGAATTCTTGGGGCACCTGAGCAAGCAG GCCGAGAGATCGCAAGCTCAGGTAACCGAAATAGGTCCTTTGGAGGAAATCACCAT GGCCCGCTAAGCCCGGAACGGTCACTACGCGGTACTGGGAGGAAGGGGTGACCC TTCCTCCCTTTTCCCTTTGCGAGGCATGAGAAATGGACGTCGGAATATCACTTCCC TTTCTACGTTCAAGACCGGCAGGGCCCCGGAGGCCAGCGCGATATCTTTGCGCCGC GCAGCGCAGCGGATGGCAGCGGCAAACCGTTGCCGGAAGTGACGGCTTCCCGGGAG GCCAGCGAATCTCGCGATGACCTGGGGCTCGCCGTCAGCGACATCCAGTCTTTCGTG CAGAGCGTCAAGCGCAACTTGAACCTCAGCATCGACGACTCCTCTGGCCAGGTCGT GGTCAAGGTGATCGACGGTGACTCGGGCGAAGTCGTGCGGCAAATCCCTTCCGAAG AAGTACTAAAACCTTGCCGCCCGGCTCGACGACGTACACAGTGTCCTGTTTCGAAACCC GCGCCTGACGCGCGGGTTGAACGACTTGGCATGGTGCTTGCCCTATCGAAGGGATA GGGCAGTACATGCACGGTTTTTTTACGCGAGATGGATTATGGCC

pslABC-F TACGGCCAGTACCTGAACTACCA

pslABC-R TTCCGGCCCCGATCTTCATCAA

pslABC
gBLOCK

GCTTGAATTCGGCAAACGTCCGCCAAGCCCCCAGGTCGGACCGGCACGCTCGAAC
GGCCAAACCTGCGTCATGGAACCGCAGGCGCATCCTGCCCAGCCAGCCCGCAGCGCC
AGTGGACAGGCGGGTCGTCGAACCGGCGGCTGGCCACCTGGCCGAGCGGCCTGCCC
TCACCTTTTCGCCCCGCTTCGCTTCCCAGGCCAGAGCGCTCGCGGATTGGCGGCGTCA
GATTTCTCTGCTACTGTTTGGATAAAAAGTTTGGCGCCAGAAATACGTCAATAAATT
GACTAAAAAACTTACCCAGACTACGGATATTTCCCTGGGAATGCTAAGATAGCTA
TCACAAAGCCACTATCGACGAATGAACCTATTCGACGGGAAAATGACTAAACCGCG
TGGCAAATGAAAAATAGTCACTAAATTGACGCTTCACCGCCTTGCTCTTCCCTATCC
ACTCAATGGACTGCCCCGTGATCGGCAGAGCAAACAACACATCATGACCTACAGGAA
GTGCTCCCTCATGAAACGCACCCTCCTCATGCTCGCCATGCTCGCCCTGGCCGCATG
CAACACCCCCGCACGGATTCCCGCACCGGACAGCGACACCGTGGACAGCGGCAAGC
GTGCCCTGGAAGAACTCGCCAGGCTACCGCCGGCGATGGAGCGGGTGCGCGTCGGG
GACACCCTGCGGATCGTCCGCGATGCCGGGGAGATGCCGACCCTCTCGGCGTTCAA
CGTCGCCACCATCTATGAACTGACGCTGTACACCGTGCTCAACGACGGCAGCATCTA
CTATCCGTTTCATCGGTTCGCATCCAGGCCGCGCACCGCACGCCGAGGAAATCGCCA
ACGAGCTGACCACCAAGCTCGCGCCGATCTACCGCGAGCCGCGGGTACGGTGAAC
ATCAACCAGGCGCCGGGCAATACGGTGTTTCGTCGGCGGCGCGGTGCGCAACCCGTC
GGCCGTGCCGATCCCCGCCGCCAACAACATGAAGCTTGCTT

Fig. S1. Cluster formation is conserved among wild-type *P. aeruginosa* strains on 0.8% agar. Scale bar represent 100 μm .

Supplemental Movie Captions:

Movie S1. *P. aeruginosa* snapping motility. The cluster in the upper right of frame undergoes some cell-cell rearrangement and then snaps to the larger cluster from right-to-left. (5.3 frames per second)

Movie S2. *P. aeruginosa* rapid community contraction. The open area surrounded by cells (spanning 469.87 μm^2) is covered within 0.76 s.