# Physical factors contributing to regulation of bacterial surface motility

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**Microbes routinely face the challenge of acquiring territory and resources on wet surfaces. Cells move in large groups inside thin, surface-bound water layers, often achieving speeds of 30 µm/s within this environment, where viscous forces dominate over inertial forces (low Reynolds number). The canonical Gram-positive bacterium *Bacillus subtilis* is a model organism for the study of collective migration over surfaces with groups exhibiting motility on length scales three orders of magnitude larger than themselves within a few doubling times. Genetic and chemical studies clearly show that the secretion of endogenous surfactants and availability of free surface water are required for this fast group motility. Here we show that: (i) water availability is a sensitive control parameter modulating an abiotic jamming-like transition that determines whether the group remains fluidized and therefore collectively motile, (ii) groups self-organize into discrete layers as they travel, (iii) group motility does not require proliferation, rather groups are pulled from the front, and (iv) flow within expanding groups is capable of moving material from the parent colony into the expanding tip of a cellular dendrite with implications for expansion into regions of varying nutrient content. Together, these findings illuminate the physical structure of surface-motile groups and demonstrate that physical properties, like cellular packing fraction and flow, regulate motion from the scale of individual cells up to length scales of centimeters.**

## Introduction

In their search for resources microbes contend with physically distinct environments ranging from soft surfaces to bulk Newtonian fluids and complex fluids like mucus. In bulk fluid environments, canonical microbes like *Escherichia coli* (1) and *Bacillus subtilis* (2, 3) ascend favorable chemical gradients via run-and-tumble chemotaxis (4). This mechanism of gradient ascent requires both flagellar-mediated motility and a complex system of phosphorylation-memory and chemical sensors on the bacterial surface, that together regulate the run-tumble transition frequency (5). In contrast, bacterial surface motility has different requirements and inputs, and different species have evolved distinct modalities of surface motion. For instance, in the predatory species *Myxococcus xanthus,* individual cells move in back-and-forth motions that employ two distinct sets of protein machinery for 'twitching' and 'gliding' motility, and cells assemble into larger motile groups that traverse surfaces as monolayers (6, 7). Many other species, including the opportunistic pathogens *Serratia marcescens* (8, 9) and *Proteus mirabilis* (10, 11), also form large groups of motile cells that are capable of rapidly expanding over surfaces, in some cases even against bulk fluid flow (12). Similarly, when present in sufficient numbers *Paenibacillus dendritiformis* form intricate fractal-like patterns on soft agar surfaces in response to lateral chemical gradients (13, 14). Even baker's yeast have been observed to exhibit group movement over fluid surfaces (15). Other species exhibit surface motility in response to non-chemical fields; for instance the cyanobacterium S*ynechocystis* is phototactic, responding to incident light by asymmetrically extending and retracting pili from its surface to create a biased random walk toward a light source (16, 17). Crucial to its motion, *Synechocystis* modifies the local surface environment by secreting exopolysaccharides, and only when enough cells have participated in such surface modification can the group move toward the light source. These examples demonstrate that in response to various gradients, microbes have evolved distinct sensing capabilities and modalities of motion to acquire resources and respond to selective pressures on surfaces. Despite their differences, surface motility in all of these species (and even in abiotic systems (18)) appears to be a collective phenomenon, requiring the motion of and/or surface modification by large numbers of cells (19, 20). Uncovering biochemical and genetic factors that regulate motion is crucial for understanding how each species executes surface motility, but those factors are only part of the full picture. Physical forces that produce, regulate, and guide microbial group motion on surfaces may be relevant across species and contexts, and are thus integral to our understanding of microbial ecology in natural environments and will expand the suite of design tools for engineering microbial systems.

Modeling plays an important role in these systems because it has the potential to connect experimental observations with physical forces and regulators of motion (21–29). Different models posit

qualitatively distinct physical mechanisms for group motility, with basal assumptions motivated by species-specific attributes. In *P. dendritiformis* growth and spreading of cells in dendritic patterns are modeled as a diffusion-limited conversion of nutrients into biomass at the growing tips of the dendrite (13, 30). Cellular motion is thought to rely on chemotaxis that follows nutrient gradients that are created by metabolic consumption of the colonies themselves (22, 30, 31). Together these effects recapitulate many of the classes of bacterial surface patterning observed in experiments (26, 32–36). However, within a dense and active (e.g. swarming) group whose velocity correlations rapidly decay on the length scale of a few cells (21), it is unclear how individuals could effectively modulate their tumble frequency and accumulate sufficiently persistent runs to deliberately bias their random walks and hence execute run-and-tumble chemotaxis. Indeed, previous work in *B. subtilis* (37–40) (and *Pseudomonas aeruginosa* (41), *Salmonella enterica* (42), and *Escherichia coli* (43)) shows that neither chemotaxis nor motility of individuals are necessary for rapid and outward-directed surface motility. Other models of surface motility (e.g. swarming or sliding) in *B. subtilis* or *P. aeruginosa* focus on the role of surfactants (23, 44) and/or osmotic potential (24), both of which appear to be crucial for rapid surface motility in those species (37, 45–47).

While cell density is clearly an important factor in motion, in so much as many cells are required for collective movement, and genetics implicates endogenous surfactant production as a physical requirement for collective motion, it remains unclear how density, material flow, and dendrite structure contribute to regulation of this ubiquitous behavior. In this work we combined high resolution video and time-lapse microscopy with computational image processing to clarify the relative contributions of density, flow and structure by examining the surface motility of the extensively-studied Gram-positive bacterium *B. subitilis* (5, 24, 48, 49). Within a few hours of deposition, a small central inoculum of wild-type cells can rapidly colonize the entire surface of a wet 10 cm agar plate via apparent swarming motility (50). Such group motility over soft surfaces has been shown to depend on the secretion of 'surfactin', a bacterially produced surfactant and wetting agent (22, 23, 25, 37, 51–53). Functional knockouts for surfactin production (Δ*srf*) result in a phenotype where individual cells are still motile and chemotactic in bulk fluid, but bacterial groups cannot move across surfaces (37, 40, 54). Localized secretion of surfactin is thought to generate a gradient in surface tension, and thus produce collective motion via the Marangoni force (23, 55, 56).

Building on that biophysical picture, we found evidence that the movement of *B. subtilis* over soft surfaces was regulated by cell density, whereby groups of cells are subject to a jamming-fluidization transition (57) that correlates with packing fraction (and thus water availability). These results

complement findings that the viscosity of bacterial suspensions depends on cell concentration, shear rate, and level of motility (58, 59). Further, we show that such groups operate in two distinct modalities of motion: groups can move without growth as 'islands' that translocate independent of the parent colony, or dendrites can extend with large-scale flow of material from the parent colony to the dendrite tip. We used a chemotropic assay to show that when connected to a nutrient-rich parent colony, extending dendrites can venture into nutrient-barren regions, while colonies that originate in nutrient-barren regions are not collectively motile. These data suggest that, in addition to genetically regulated production of surfactants, multiple physical and abiotic factors regulate motion independent of the chemotactic or motile abilities of the constituent cells, and that groups move differently when presented with anisotropic nutrient environments. When combined with previous work, these data suggest a model in which individual motility and subsequent swarming are mechanisms that maintain a fluidized state on which surface-tension gradient forces (Marangoni forces) and osmotically driven colony hydration can act to precipitate group motion over a surface, and that a jamming-like transition, like those found in other macroscopic granular systems (57, 60–65), may be a key regulator of whether bacterial groups are able to move.

**Results**

*Bacillus subtilis* is a model motile Gram-positive bacterium, capable of sensing and responding to its chemical environment in bulk fluid via run-and-tumble chemotaxis (2, 5, 66). On wet surfaces wild-type *B. subtilis* rapidly move out from a central inoculum (67), apparently via collective swarming motility (50) that requires secretion of the endogenous bio-surfactant 'surfactin'. Mutants that lack the ability to produce surfactin (Δ*srf*) do not exhibit collective motility from their central inoculum (37) (and Movie S1). We inoculated small, dense (OD ~ 10 - 20) droplets of wild-type *B. subtilis* on soft (~0.5% w/v agarose) nutrient-rich surfaces. After a brief quiescent phase, groups of cells rapidly expanded over the surface in a dendritic pattern that reached the edge of the plate (~5 cm of travel) in less than 6 hours (Movie S2). Dendrites robustly moved outward away from the original point of inoculation into fresh territory at an average group motility rate of ~5 μm/s and up to ~15 μm/s (SI Fig. 1). Cells within the dendrites were highly motile, exhibiting swarming motility (37) with individual cells moving at rates up to ~30 μm/s in highly circuitous paths. Consistent with previous work (37, 38), we confirmed that mutants lacking the ability to run (tumble-only, Δ*CheY*), lacking the ability to tumble (run-only, Δ*CheB*), and mutants lacking flagella (Δ*hag*) were all able to exhibit rapid surface motility, albeit with some differences in dendrite speed and spreading pattern (SI Fig. 2, Movies S3 - S5).

118 In our early observations we noted transitions in the motile state of groups, wherein cells within
119 a *contiguous region* appeared to be either highly motile and moving as a group or immobilized on both
120 the individual and group levels. These distinct states of motion were frequently observed at the same
121 time, and cellular groups were observed transitioning between these states on the time scale of a minute,
122 too short to be accounted for by phenotypic changes.
123
124 **Movement is regulated by a jamming-like transition**
125 While surface motility of *B. subtilis* is robust to genetic manipulations of chemotaxis and motility,
126 it was very sensitive to gel stiffness as measured by agarose concentration (37). Below ~0.4% agarose by
127 weight the gel is sufficiently porous that bacteria can penetrate and swim within it, akin to canonical swim
128 plates (50, 68). Above ~0.7% agarose, limited water availability hinders surface motility (50), leading to
129 expansion across plates through the replication of sessile cells at the leading edge of growth, as seen in *P.*
130 *dentritiformis* (dendrite-like) or *E. coli* (growing circular colonies), both of which expand at much slower
131 rates (31, 69, 70). Thus, across a relatively narrow range of agarose percentages, groups of *B. subtilis*
132 exhibit three qualitatively distinct behaviors: swimming through agarose (below ~0.4%), rapid surface
133 motility (between ~0.4% and ~0.7%), and slow growth by replication (above ~0.7%). Why does the
134 transition from rapid surface motility to slow proliferative growth occur over such a narrow range of
135 agarose stiffness, and correspondingly, water availability?
136 Many previous studies of *B. subtilis* characterized spreading and attendant morphological
137 behaviors at the length-scale of colonies (mm to cm) and on the timescale of bacterial replication, imaging
138 colony morphology and spreading with minutes or hours between frames (26, 32, 37, 50). These excellent
139 studies revealed much of what is known about spreading phenotypes, their genetic mechanisms, and
140 biochemical correlates. We performed high temporal and spatial resolution imaging of spreading colonies
141 to illuminate processes that potentiate spreading. We captured images at 30 or 60 frames per second
142 with spatial resolution of 5 µm/pixel or 15 µm/pixel, respectively, both of which enabled us to see intensity
143 variations produced by the movements of cells within the swarm. We wrote a custom image analysis script
144 that measured a scalar correlate of motion as a function of position through time. Briefly, the algorithm
145 measures the mean absolute value of local intensity fluctuations at a position across a set of *N* (usually 5
146 – 7) frames and thus reports on the level of motile activity at each position through time (see Methods).
147 With this 'activity' filter, we were able to visualize on the scale of 10s to 100s of microns which parts of
148 the colony were actively motile and in which parts cells were stationary (Fig. 1A).

149 In wild-type cells, the rapid movement of dendrites and cellular groups was correlated with
150 significant increases in the quantitative measure of activity from our image analysis, indicating that
151 movement of individuals positively correlates with movement of the group. Conversely, regions whose
152 constituent cells had a low measure of activity were stationary and did not move or flow over the surface
153 (Fig. 1 and Movie S6). Frequently, stark boundaries in activity level formed within contiguous regions of
154 cells, indicating that within a genotypically and phenotypically identical population, sub-populations could
155 adopt two qualitatively distinct states of motion at the same time. We interpreted the high activity state
156 as a fluidized (low viscosity) state in which cells were swarming, and the low activity state as a 'jammed'
157 state where the network of contacts and forces between cells led to local immobility. We observed that
158 jammed regions could be fluidized if they came into contact with a fluidized region. Further, the phase
159 boundary between these regions actively fluctuated in time, translating fractions of a micron per second
160 (Fig. 1B), meaning that individual cells transitioned between motile and immotile states faster than
161 (potential) phenotypic changes in the local environment. Physical theories of jamming in granular
162 materials (61, 63, 64, 71) predict that the transition between jammed and fluidized states result from
163 small (technically infinitesimal) differences in packing fraction.
164 We took two approaches to assess the connection between packing fraction and motion. Phase
165 contrast video microscopy allowed us to visualize the cells as dark objects, while open spaces – even a
166 fraction of a cell's area – are relatively bright (SI Fig. 3A, Movie S7A). First, we reasoned that at suitably
167 high magnification (60X), the average intensity in a region is directly correlated with the amount of open
168 space (assuming the cells are in a monolayer, see next section of Results), or in other words, brighter
169 regions have lower packing fractions. We hypothesized that if packing fraction was controlling the
170 transition, there should be a positive correlation between local intensity and the local level of activity. We
171 averaged both the intensity and the activity over regions ~0.8 $\mu m^2$, and then calculated (i) the histogram
172 of spatially correlated values between those two measurements, (ii) the mean local intensity vs. the local
173 activity, and (iii) the bivariate correlation coefficient (SI Fig. 3B). The mean intensity values increased
174 monotonically with activity and the correlation coefficient was 0.34 ($p < 10^{-6}$, relative to the null-
175 hypothesis of no correlation between the two signals, SI Fig. 3B). Second, we hypothesized that there
176 should be a negative correlation between packing fraction and flow speed – with the understanding that
177 the nature of the jamming transition means that the relationship between those observables is non-linear
178 (i.e. we would expect a significant, but weak negative correlation). After removing low frequency
179 background variations, we used a fixed threshold to define which pixels were cells (dark) and which were
180 open space (light), and averaged the resulting packing fraction over square regions 12.4 $\mu m^2$ in area. We

181 then used open-source particle image velocimetry software (72) to measure the local speed of cellular
182 flow through the same pixel groups. We calculated the histogram of spatially correlated values between
183 flow speed and packing fraction, with a bivariate correlation coefficient of – 0.11 (with $p < 10^{-6}$, relative to
184 the null-hypothesis of no correlation between the two signals) (SI Fig. 3C). Thus both methods provide
185 significant support for the hypothesis that packing fraction regulates collective movement.

186 Next, we wanted to determine if available water (and its anticorrelate: packing fraction) could
187 regulate the transition between the fluidized / collectively motile state and the jammed immotile state.
188 We imaged expanding tips that were suddenly subjected to evaporation, and hence reduced water
189 availability. As water evaporated, the expanding tips exhibited a dynamic transition from a fluidized and
190 rapidly swarming state to a state where both individuals and the group were immotile (Fig. 2A/B and
191 Movie S7B). The motile-to-immotile transition did not occur at the same time across the group, and local
192 variations in density allowed some cells to 'rattle' in place, even after the group as a whole had become
193 immotile – consistent with the appearance of 'rattlers' in jammed granular systems (73, 74). These
194 observations further support the hypothesis that the transition is not a phenotypic change and that local
195 drag and frictional forces are not preventing motion. Then, to examine the reversibility of this transition,
196 we took the same plates and expanding tips, resealed them to halt evaporation, and continued imaging
197 while water from the gel rehydrated the cells. Over a few minutes, the colony re-fluidized into domains
198 of high activity sub-groups, which then coalesced until the entire tip region regained fluidity and continued
199 to expand (Fig. 2C/D and Movie S7C). This strongly indicates that re-wetting and the corresponding
200 reduction in packing fraction 'reverses' the jamming-like transition back to a fluidized and collectively
201 motile state (61, 71, 75).
202

203 **Motile groups are pulled by the front in discrete layers**

204 In our bright-field imaging it appeared that motile dendrites frequently traversed surfaces in
205 discrete layers of cells, first traversing the surface as dense monolayers, then double layers, and so forth,
206 up to 4 discrete layers (see Movies S6, S8 and S9). To confirm that groups migrated in discrete layers we
207 measured the surface height of expanding dendrites with high *Z*-resolution using an interferometric
208 profilometer (see Methods). In Fig. 3A/B we show the height field and a 1D profile for an expanding mono-
209 layer dendrite – its height across the expansion region was almost exactly 1 µm, the thickness of a single
210 *B. subtilis* cell. As dendrites slowed and/or encountered space constraints, the layer thickness jumped to
211 higher integer values, as shown in Fig. 3C/D whose dendrites transition between 0 and 3 layers of cells (4
212 layers visible in Movies S6 and S10). Looking back to the jamming data in Figs. 1 and 2, multiple discrete

213 layers are visible in the interior of the colony. Frequently, the fastest dendrites were advancing as
214 monolayers (the fastest moving groups in Movies S6, S8, and S9, notably tips in S10 appeared multi-
215 layered). In addition to being valuable *in situ* data of motile-group structure, this also meant that our
216 imaging of rapid motile groups (and attendant activity filtering) was characterizing the motion of all cells
217 in *Z*, as opposed to there being motile and immotile cells at different *Z* positions (e.g. a biofilm blocking
218 view of motile cells). This discrete structuring is noteworthy because relevant models of surfactant-driven
219 spreading take cell-layer thickness as a continuous variable, whereas these data demonstrate that
220 (frequently, though not strictly) motile groups move in monolayers with possible transitions to other
221 discrete heights, especially when near the jamming transition.

222 The boundary shape between bare agarose and dense cellular monolayers also contained
223 information. While the shape of the meniscal boundary can be influenced by surface pinning and
224 depinning (76), this suite of imaging data showed that such boundaries have different shape statistics near
225 and far from the advancing front. At the advancing front, the boundary curvature is smooth (low variation)
226 with an overall positive curvature whereas behind the front retraction of material causes curvature to
227 vary widely in a so-called 'lacunar' structure (SI Fig. 4A). Further, within the regions whose boundaries
228 were lacunar, voids appeared with similar distributions of boundary curvature to the external boundary
229 (SI Fig. 4B). Consistent with previous work on 'viscous fingering' (77) and models of surfactant-driven flow
230 (23, 24), these data support the hypothesis that the advancing front has positive pressure (pushes
231 outward, covers new territory) while the boundary behind the front experiences negative pressure (pulls
232 inward, retracts and reduces coverage), both consistent with cellular groups primarily being front-pulled
233 by gradients in surface tension, not pushed from the back by proliferation. Consequently, on one occasion
234 we observed a dendrite tip contacting its own surfactant field (78), resulting in an almost immediate
235 (within ~ 15 s) cessation of motion (SI Fig. 5 and Movie S11) despite the number of cells in the tip increasing
236 even after self-contact. Also consistent with a front-pull mechanism, much of the same imaging data
237 showed independent islands of cellular monolayers, completely detached from their parent colony,
238 traversing the surface independently, with characteristic positive curvature at the front and negative
239 curvature regions in the back (Movies S6, S8, S12 and S13).

240 While high numbers of cells are required for group motility, these data indicate that proliferation
241 alone is not generating outward pressure to drive group motion. To definitely determine this, we
242 measured the area of dense monolayer islands of cells as they moved over time. In Fig. 4, we show one
243 such island traversing an agarose surface over a distance many times its length. Rather than increasing
244 due to cell division and growth, the island area decreased over time as such islands tend to leave a trail of

245 cells immobilized on the surface by the meniscus. Therefore, during surfactin-driven surface motility
246 groups of cells are capable of large-scale motion without growth in the population and without connection
247 to their parent colony. Rather, boundary curvature analysis and the existence of independent islands
248 provide evidence that cells are pulled by the front, frequently in monolayers at a density near the
249 threshold for jamming.
250
251 **Material flows from parent colony to dendrite tip**
252 While our data were consistent with outward front-pressure pulling the group away from the
253 parent colony, multiple time-lapse experiments showed apparent rapid flow of discrete cellular layers
254 from parent colony to the expanding dendrite tip (e.g. see Movie S10). To confirm that these observations
255 were indeed flow of material over the scale of millimeters to centimeters, we performed our typical wild-
256 type expansion assay, but doped the initial cellular deposition with 1 μm fluorescent polystyrene beads,
257 at a ratio of ~ 1 bead per 100 cells (see Methods). We imaged the motion of the tracer beads at 30 fps,
258 which allowed us to see the trajectories of individual beads on the time scale of minutes (Movie S14, SI
259 Fig. 6A). The swarming motility of wild-type cells caused the motion of individual beads to be erratic, akin
260 to a non-thermal random walk with drift (79) especially when flow speed was slow in comparison to
261 cellular swim speeds (Movie S15, SI Fig. 6B). This erratic motion prohibited flow tracking in wild-type
262 groups, however, as we had confirmed earlier (38), mutants of *B. subtilis* unable to perform flagellar self-
263 propulsion (Δ*hag*) exhibit rapid surface motility with speeds and morphologies similar to wild-type cells
264 (see SI Fig. 2). This strain did not cause severe bead agitation and thus we were able to perform *in situ*
265 flow measurements using those mutants (Fig. 5 and Movie S16).
266 Using open-source cross-correlation software, we measured bead flow via particle image
267 velocimetry (72). Similar to the flow characteristics of a typical incompressible fluid, the flow speed of
268 dense cellular monolayers decreased in regions with wide meniscal boundaries and significantly sped up
269 at points of constriction (see inset Fig. 5). The similarity between incompressible 2D flow and flow of dense
270 cellular monolayers is consistent with the fact that cells are effectively solid steric objects densely packed
271 in (incompressible) water. Average flow speeds, and following the motion of individual beads, showed
272 that material originating in the parent colony frequently made its way to the expanding tip (Fig. 5 and
273 Movie S16). Thus, while our island data showed that motile groups need not be connected to the parent
274 colony and that cellular proliferation was not required for group motility, these data indicate that when
275 connected back to the parent colony, cells and material (e.g. nutrients) can be supplied to the expanding
276 tip of a dendrite.

277 Having confirmed that our bright-field data was indeed revealing large scale flow, we examined
278 how the speed of tip expansion into fresh territory compared with the speed of cellular flow along
279 established dendrites connecting the tip to the parent colony (SI Fig. 7). Consistent with the bead-flow
280 data, kymograph analysis showed that when expanding dendrites are connected to their parent colony,
281 cells (and surrounding fluid) flow many centimeters from the colony into the expansion zone. Such flows
282 along an established dendrite are significantly faster (~ 10 μm/s) than the speed of tip movement (~ 3
283 μm/s) into fresh territory, as they must be for material to reach the tip from the colony. At this point it is
284 unclear how groups create these differentials in speed along a dendrite, but we offer a potential
285 mechanism in the Discussion.
286
287 **Motile groups use material flow to traverse nutrient poor regions**
288 We knew from previous work (37, 38) that neither chemotaxis nor flagellar-mediated motility is
289 required for group surface motility, but that surface motility was potently modulated by (e.g.) the
290 presence of monovalent ions (K+ (25)). This suggested that groups might respond to their chemical
291 environment indirectly via changes in metabolism and/or gene expression. For instance, differences in
292 nutrient concentration might affect the rate of synthesis and/or secretion of surfactin, and thereby affect
293 surfactin-mediated motility. Further, our large-scale flow data suggested that differences across the
294 chemical landscape that affect group surface motility could be ameliorated by flow of material from other
295 regions.
296 We wanted to test whether motile groups had differential responses to exogenously presented
297 nutrient gradients, and specifically whether groups would avoid negative nutrient gradients. We created
298 agarose plates with two distinct halves – one half containing the same rich defined medium (RDM) used
299 earlier, the other half containing (potassium-free) NaCl buffer osmotically matched with the RDM to
300 maintain the same osmotic potential (see Methods). A thin impermeable barrier separated the two
301 halves, initially keeping the nutrients on one side. Once the agarose set, we poured a thin (~ 1 mm) layer
302 of osmotically matched NaCl buffer with agarose, and thus linked the two regions into a contiguous
303 surface with uniform mechanical properties that allowed cells to move freely between them. The nutrient-
304 rich half also contained a red fluorescent tracer dye (rhodamine) that served as an approximate reporter
305 of nutrient diffusion over the barrier. With or without cells, these plates showed diffusion of the dye from
306 the nutrient rich side into the side devoid of nutrients, meaning that there was a negative concentration
307 gradient on the surface from the nutrient rich to the nutrient poor side. We reasoned that if wild-type
308 cells were responding (potentially indirectly) to the outward-facing chemical gradients on a standard plate

309 (i.e. with initially isotropic nutrients) or to the absence of required potassium ions on the split-plate, they
310 would react to the reverse nutrient gradient on these split plates and either avoid the nutrient poor region
311 or show reduced motility toward it.

312 To test this, we simultaneously inoculated such a split-plate in two positions from the same
313 isogenic population of wild-type cells, one colony on the nutrient rich side, one on the nutrient poor side
314 (Fig. 6A/B). The colony inoculated on the nutrient poor side did not exhibit collective motility and was
315 effectively stationary, while the colony on the nutrient rich side rapidly covered the nutrient rich zone and
316 simultaneously ventured into the nutrient poor zone. Initially, dendrites that expanded into the nutrient
317 poor zone covered the plate less densely than dendrites that never left the nutrient rich zone (Fig. 6B),
318 but tip speeds were similar in both zones. Ultimately, cells originating from the nutrient-rich zone
319 colonized the entire plate (both zones, see Movie S17). The difference in initial coverage between the
320 zones supports the hypothesis that groups do exhibit a (mild) chemotropic response to gradients in
321 nutrients, ions, or potentially their own secreted waste products. Interestingly, these data show that
322 connection to a parent colony in a nutrient rich zone allows cells to explore nutrient-poor regions, even if
323 those regions lack critical chemical species (K+ ions in wild-type (25)). This also complements findings that
324 bacteria can transport other bacteria as cargo during surface motility (80). We did not observe any
325 independently moving islands of cells in the nutrient-poor region. Our flow data offer a qualitative
326 mechanism for this 'scouting' ability, that large-scale flow of material from parent colony to the extending
327 tip brings metabolic resources, presumably to fuel, in part, surfactin production, thus allowing Marangoni
328 forces to continue to pull the group outward via positive tip pressure.
329

330 **Discussion**

331 Our data support a model in which water availability – a proxy for bacterial packing fraction – is a
332 sensitive control parameter for an abiotic jamming transition in the granular material that is densely
333 packed, expanding bacterial populations. We hypothesize that motility plays a contributory role,
334 encouraging a fluidized state via motility-dependent viscosity (59, 81). When sufficient fluid is extracted
335 from the substrate by endogenous osmolyte secretion and cells are in a fluidized state, groups flow over
336 the surface driven and guided by surface-tension gradients (Marangoni forces). Conversely, when water
337 availability decreases, either by (e.g.) evaporation or decreasing osmotic potential, packing fraction
338 increases and cells transition to a jammed and immotile state. It is worth noting that while our data are
339 consistent with a jamming-fluidization transition, we cannot use our current data to *prove* that we are
340 meeting the technical requirements of such a transition. Jamming is defined by a non-crystalline network

341 of steric contacts and forces between particles that are 'isostatic', that is, they do not have free modes of
342 motion (other than rattlers (73)), but we are unaware of a modality of microscopy that permits live, *in situ*
343 visualization of the force and contact network in a dense bacterial group (stationary or collectively motile).
344 Nonetheless, the potential existence of an abiotic jamming transition that regulates group motility
345 through the dense packing of nematic actors offers a mechanism for why small increases in agarose gel
346 stiffness (~0.05%) switch groups from rapid surface migration to slow steric growth. It also clarifies why
347 the secretion of both surfactants and osmolytes is required for collective motility (37, 50); the former
348 being crucial for force generation and the latter for maintaining fluidization. Thus we hypothesize that
349 colonies that are not able to achieve the fluidized state – for any reason – are constrained to expand on
350 the order of 100 times slower over surfaces using the forces generated by cell wall growth and cell division
351 (i.e. the mode of sessile growth one typically associates with single colonies growing on a petri dish).
352 Our examination of boundary structure (SI Fig. 4) was consistent with outward-facing pressure at
353 the expanding front and inward-facing pressure behind the front. However, many data sets showed
354 persistent flow from parent colony, or other regions behind the front, toward the advancing front, without
355 the appearance of lacunar structure (e.g. Movies S2 and S10). We speculate that these differences in
356 boundary structure, and correspondingly flow, might reflect differences in the magnitude of force being
357 generated at the front and/or changes to the effective viscosity of the cellular suspension. Differences in
358 force magnitude might arise from varying rates of surfactin secretion and/or processes that affect the
359 spreading or degradation of surfactin. Differences in effective viscosity of the cellular suspension could
360 arise due to changes in cellular density (including but not limited to jamming), cellular aspect ratio (9, 82),
361 internal shear arising from individual motility (59, 81), or processes that affect the rate of fluid extraction
362 (e.g. via osmolytes) or loss (e.g. via evaporation) from the substrate. Similarly, we hypothesize that the
363 difference in tip speed vs. flow speed along an established dendrite (SI Fig. 7) – which allows material to
364 be brought from parent colony to dendrite tip – might reflect different energetic costs for motion in those
365 two scenarios. A tip advancing into fresh territory uses surface tension differences to generate force, but,
366 given our layering data, it must also pay a line-energy cost along the meniscus on each side of the dendrite,
367 which to first order would appear like a constant cost in energy per unit length of movement (equivalent
368 to a constant reduction in overall force). Conversely, assuming that cells flowing within an established
369 dendrite are using the same mechanisms for force generation, they move within the same meniscal
370 boundaries established by the tip, thus do not pay this energetic cost, and thus have a larger effective
371 force pulling them outward.

372 The existence of large scale flow from parent colony to dendrite tip presents a number of
373 biophysical questions and possibilities. How far into nutrient poor zones can expanding dendrites
374 venture? How do those distances depend on the available nutrients or the position of the parent colony?
375 How do surface properties like local water availability and topography (83, 84) affect flow and
376 colonization? How do structures on the surface guide and/or impeded collective motility? The fact that
377 we observed islands of cells moving independently on nutrient-rich surfaces, whereas flow from a parent
378 colony was required for exploration of nutrient-poor zones, suggests that those modalities of motion
379 (island vs. flowing dendrite) depend on environmental conditions. For instance, an island that ventures
380 into a nutrient poor region might quickly deplete its resources, and thus decrease secretion of osmolytes
381 and surfactants required for motion (54). Likewise, the transport of materials and nutrients from a
382 common pool (parent colony) into discrete tendrils far from the common pool (80) spur game-theoretic
383 questions of how those resources are divided, whether phenotypic changes are playing a role, and – like
384 other collectively motile and reproductive systems (e.g. *Dictyostelium* (85, 86)) – how the specific
385 genomes of individuals propagate in space and time relative to their nearly isogenic kin.
386 Whereas individual cells in bulk fluids propel themselves and direct their motion according to the
387 algorithms of chemotaxis, these data and previous work strongly suggest a fundamentally different set of
388 rules govern group surface motility. Specifically, that collective secretion of surfactants and osmolytes
389 drives and directs motion, that large-scale flow plays a critical role in exploration of territories with
390 differing nutrient concentrations, and that bacterial packing fraction – modulated through multiple
391 mechanisms – is a sensitive control parameter that regulates transitions between fluidized (collectively
392 motile) and jammed (immotile) states of the group. Thus continued understanding and modeling of
393 microbial communities on surfaces must consider how these physical factors – not fully described by
394 genetics and biochemistry – contribute to population dynamics.
395
396 **Methods**
397 *B. subtilis culture storage and preparation*
398 Isogenic cultures of *B. subtilis* strains were grown to OD 0.5, mixed 50% in glycerol aliquots,
399 individually snap frozen and stored at - 80 C. For each experiment, 200 µL aliquots were removed and
400 thawed, then diluted in 10 ml LB medium and grown in a shaking incubator at 37 C for 4 hours until mid-
401 log phase, then pelleted by centrifugation at 4000 g for 10 mins. The supernatant was removed, and the
402 bacterial pellets were then resuspended in 200 µL LB, yielding a culture with an approximate density OD
403 10, then 1.5 µL of that culture was deposited on 0.5% agarose plates with Teknova EZ-RDM media (a rich

404 defined medium), then dried for 10 mins in air and sealed with parafilm. For bead flow experiments, 1 µm
405 fluorescent polystyrene beads (Bangs Laboratories, Dragon Green) were diluted into the inoculation
406 culture and vortexed before deposition.
407 Depending on the type of time lapse experiment, plates were then either incubated for 1 hr then
408 imaged or imaged immediately at 37 C. For culture profilometry (height measurements), cultures were
409 incubated for 2 - 3 hrs at 37 C, then unsealed within 10 minutes of imaging to minimize evaporation and
410 jamming. For the multilayer height measurements (Fig. 3B), cultures were unsealed, transitioned to
411 immotility, and were then imaged between 60 to 80 minutes later.
412
413 *Plate preparation*
414 Plates were created by mixing 100 mL Teknova EZ-RDM and 0.5% agarose by weight and
415 autoclaving for 15 minutes at 121 C. Media was cooled to 50 C in an incubator before being poured into
416 4 plates (25 ml each). Each plate was cooled and solidified under flame in open air for 10, 15, or 30 minutes
417 before immediate inoculation, creating a range of initial gel hydrations to examine the effects of water
418 availability on colony morphology. For the split-plate experiment (Fig. 6) zero-nutrient buffer was made
419 from de-ionized water, 0.5% agarose w/v and NaCl concentration matched to the calculated osmotic
420 potential of the rich defined medium.
421
422 *Microscopy and Imaging*
423 Transmitted-light and oblique-illumination microscopy were performed using a Nikon SMZ-25
424 stereo zoom microscope, with a P2-SHR Plan Apo 1x objective, and a Prior ES111 OptiScan stage. High
425 speed images were taken using an Andor Zyla 5.5 CMOS camera. Phase contrast microscopy was
426 performed using a Nikon Eclipse Ti-E inverted microscope with an CFI S Plan Fluor ELWD 20x Ph1 ADM
427 objective (Fig. 2) or CFI S Plan Fluor ELWD 40X Ph2 ADM objective (SI Fig. 3). High speed images were
428 taken using an Andor iXon Ultra 888 EMCCD camera. Culture height images were captured using a Zygo
429 NewView 7300 optical 3D profilometer in the University of Oregon's CAMCOR facility. Low-frequency
430 variations in plate height were computationally removed by subtraction of a fitted a polynomial surface
431 to the background.
432 Activity overlays were generated using custom Matlab© scripts which calculated the per-pixel
433 sum over the absolute differences between 5 or 7 consecutive frames. Kymographs were generated using
434 custom Matlab scripts to identify and interpolate contours, then sum over cross-sectional slices of
435 intensities along the contours over time. All scripts and Matlab code are available on request.

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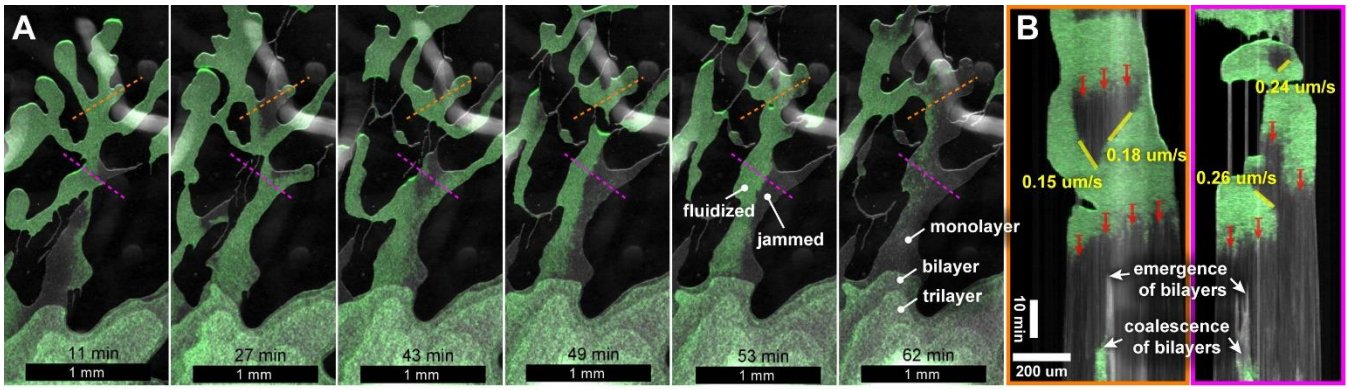



**Figure 1. Isogenic cells exist in motile and immotile phases within the same colony. (A)** Time-lapse imaging of wild-type *B. subtilis* spreading dendritically on a nutrient-rich soft-agarose surface. We applied a computational image filter that uses local intensity fluctuations over time to report on the degree of movement. Green regions are high motile activity and stationary regions are left gray. Transient motile regions and fluctuating boundaries between motile and stationary regions emerged and dissipated on timescales shorter than (potential) phenotypic changes (see Movie S1). **(B)** Kymographs along the colored dashed lines in (A) (time is downward). Transitions from large-scale motility (green) to immobilized (jammed) states are denoted by the red arrows. Regions could transition from jammed back to fluidized if they came in contact with a fluidized region (see boundary movements in yellow). At later times, once the entire population had become immotile, cells grew 'upward' into bilayers that frequently coalesced. See Movie S6.

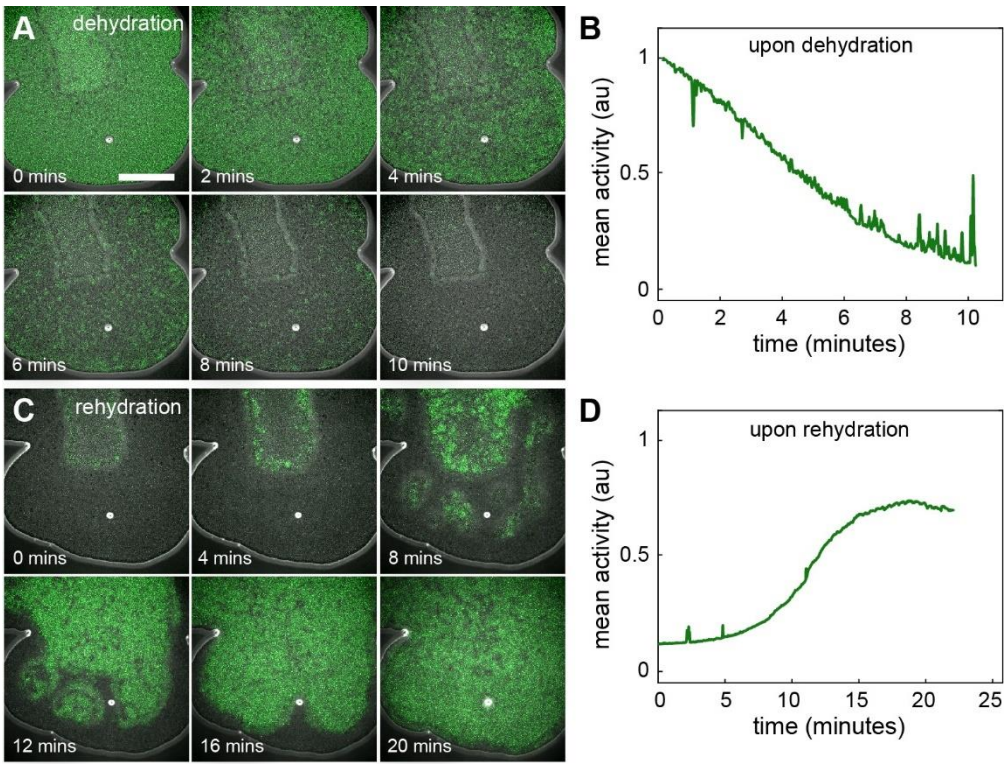



**Figure 2. Water availability controls a jamming-like transition**. **(A)** Time-lapse imaging of the tip of a wild- type *B. subtilis* dendrite on a nutrient-rich low-agarose surface. At $t$ = 0 the plate was unsealed and its surface was exposed to air. Subsequent evaporation reduced water availability at the surface, causing both collective motion (green color) and the computational measure of mean motile activity **(B)** to drop as the cellular group entered the jammed state. Across the entire dendrite tip collective motility halted. **(C - D)** The same plate was then immediately resealed and imaged over 20 minutes. The punctuated drop in evaporation rate allowed cells to retain more water from the gel. Fluidized domains of motile cells subsequently emerged and coalesced until the entire dendrite was, once again, collectively motile. In both sets of images, the rectangular structure toward the top is a cellular bilayer (see Fig. 3). Noise in the activity measurement (B) was (in part) due to manual refocusing required by the retraction of the surface as water evaporated. Scale bar is 200 µm. See Movies S7A and S7B.

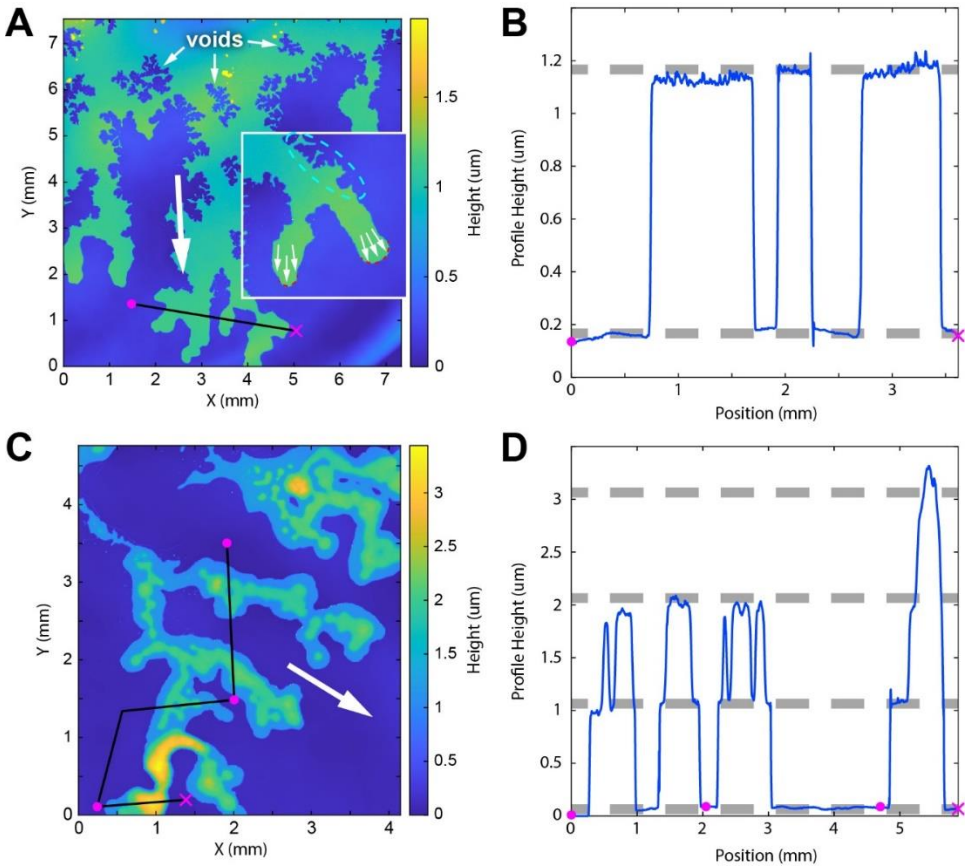



**Figure 3. Dendrites expand in discrete layers.** The left-hand column shows height images of wild-type *B. subtilis* dendrites on soft agarose taken using an interferometric profilometer (see Methods); the image backgrounds have been computationally flattened. **(A)** Advancing dendrites flowed downward (white arrow) and the fronts of those dendrites exhibited overall positive curvature (red dashed lines, inset) indicating outward pressure. Behind the advancing front, boundaries between agarose and cells exhibited a 'lacunar' structure (e.g. inset, cyan dashed ellipse), where the mode of the curvature distribution was negative (SI Fig. 3). Voids appeared behind the front, consistent with negative pressure in those regions overcoming meniscal forces. Both voids and lacunar structure are consistent with viscous fingering and cells de-wetting and being pulled to the front. **(B)** A one-dimensional profile of the surface height along the black line in (A). Here the advancing front is a densely packed cellular monolayer, whose height is one cell thickness as shown by the horizontal grey dashed lines (1 µm apart). **(C)** Same imaging modality as in (A), now after dendrites have jammed on the surface and then grown for ~ 1 hr. The original flow direction is indicated by the white arrow. **(D)** A one dimensional profile of the surface height along the black line in (C). Within contiguous cellular regions, bacterial film height is discretized into layers one cell thick; here the profile shows transitions from monolayer, to bilayer, to trilayer. Discretization ceased above four layers, when presumably cells move in any orientation, rotating about their long axis within the entire vertical structure (see also Movies S6, S8 and S9).

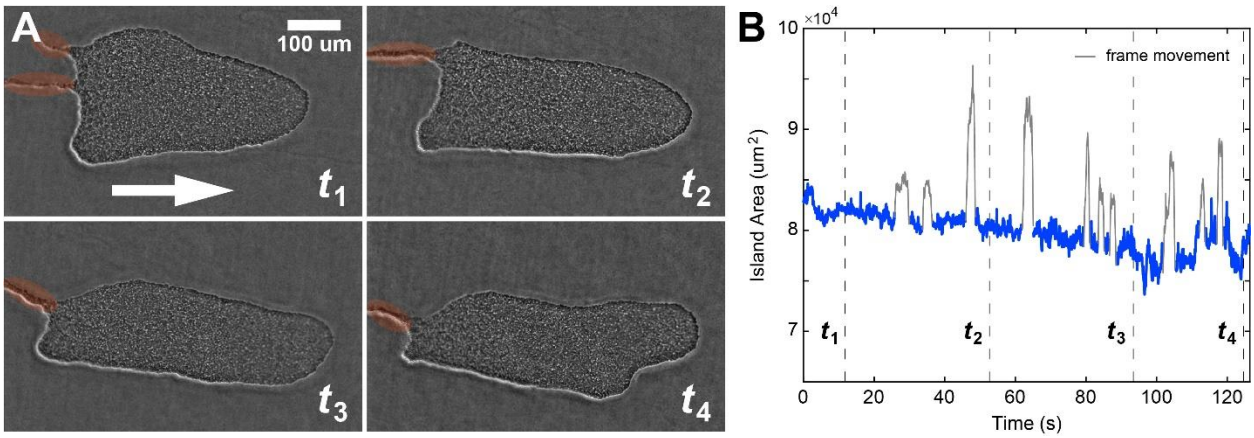



**Figure 4. Groups can move independently without growth. (A)** Four snapshots of an independent monolayer 'island' of wild-type *B. subtilis* cells moving to the right (white arrow) on soft agarose. Over the course of imaging, the group moved at an average speed of ~ 6 µm/s and the total amount of movement was ~ 700 µm, necessitating movement of the viewing frame (grey data in B). Common to our observations, such islands leave a trail of cells immobilized on the surface behind them (orange highlighted regions). **(B)** The area of the island decreased over time (time labels correspond between A and B). When combined with our data showing movement in monolayers, these data demonstrate that movement does not require proliferation. See Movie S13.

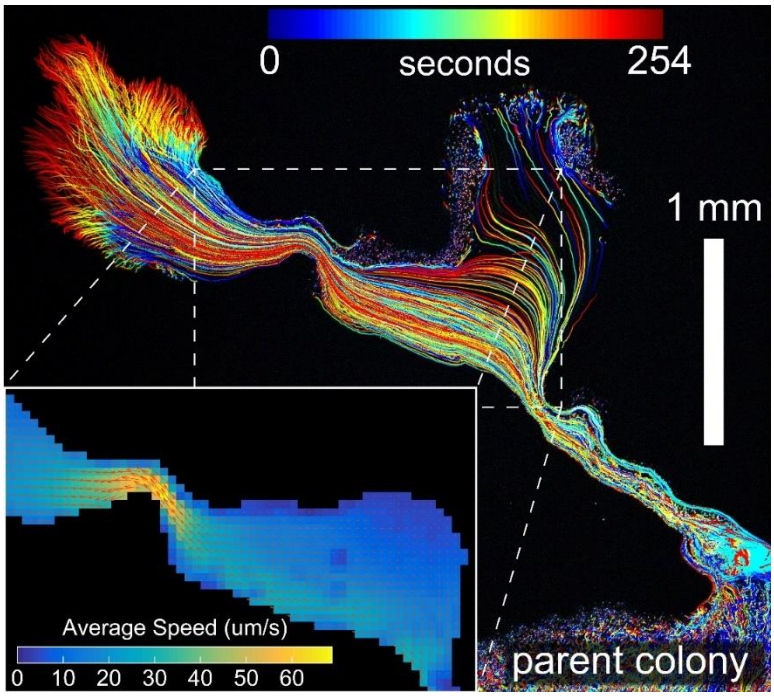
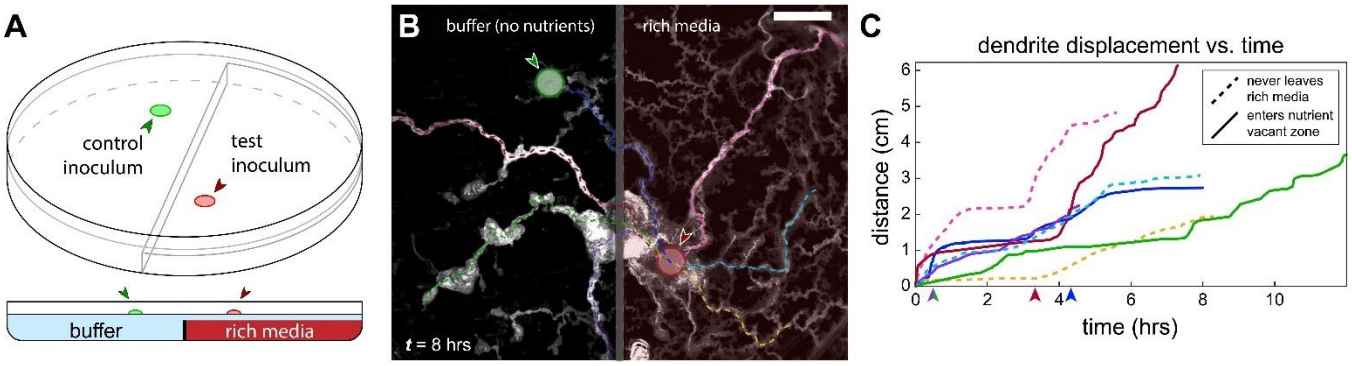



**Figure 5. Dendrite expansion corresponds with large-scale flow.** Fluorescent 1 µm tracer beads were diluted with the cellular suspension and deposited onto the agarose surface. We examined the motion of beads in Δ*hag* mutant cells (lacking flagella); this minimized bead agitation due to individual cellular motility (see SI Fig. 5 and Movies S14 and 15 for wild-type). The beads were imaged in an expanding dendrite millimeters long on the time scale of minutes; the positions of individual beads are shown colored by time. Here the dendrite is connected to its parent colony, material flow moved beads from the parent colony into the dendrite. The accompanying movie (Movie S16) shows individual beads starting in the colony and moving to the dendrite tip, demonstrating that flow generated by pulling at the front can move material long distances compared to cell size, from the colony to the tip of a growing dendrite. The inset shows the average flow velocity (orange arrows) and average flow speed through a region with varying width. Similar to a 2D incompressible fluid, constrictions accelerated flow, whereas flow slowed at wider points along the dendrite.

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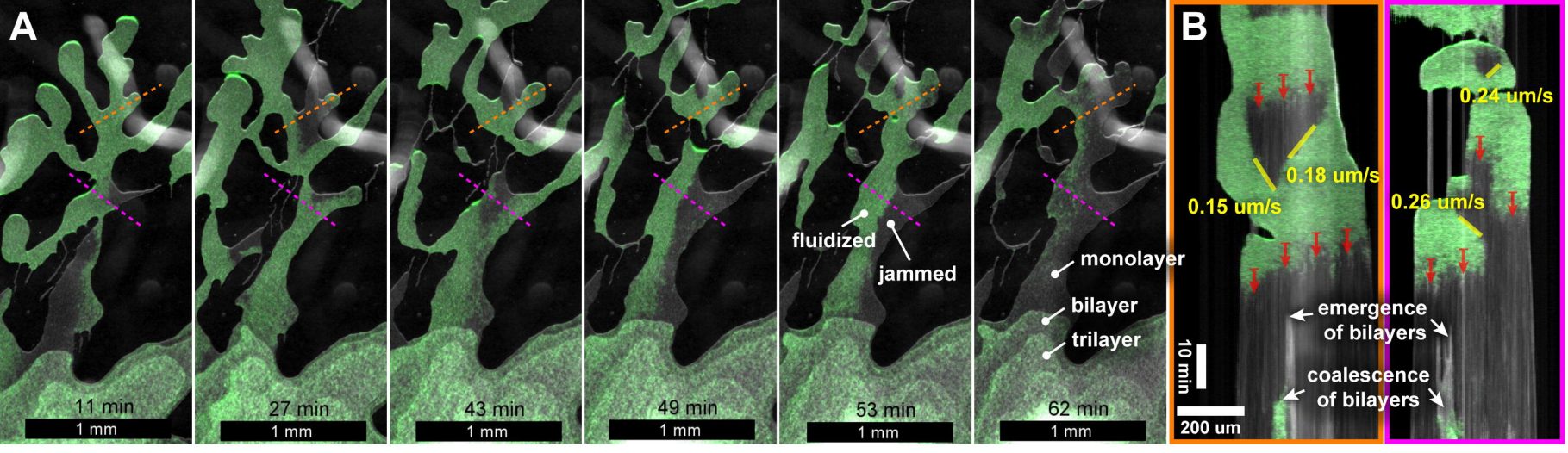
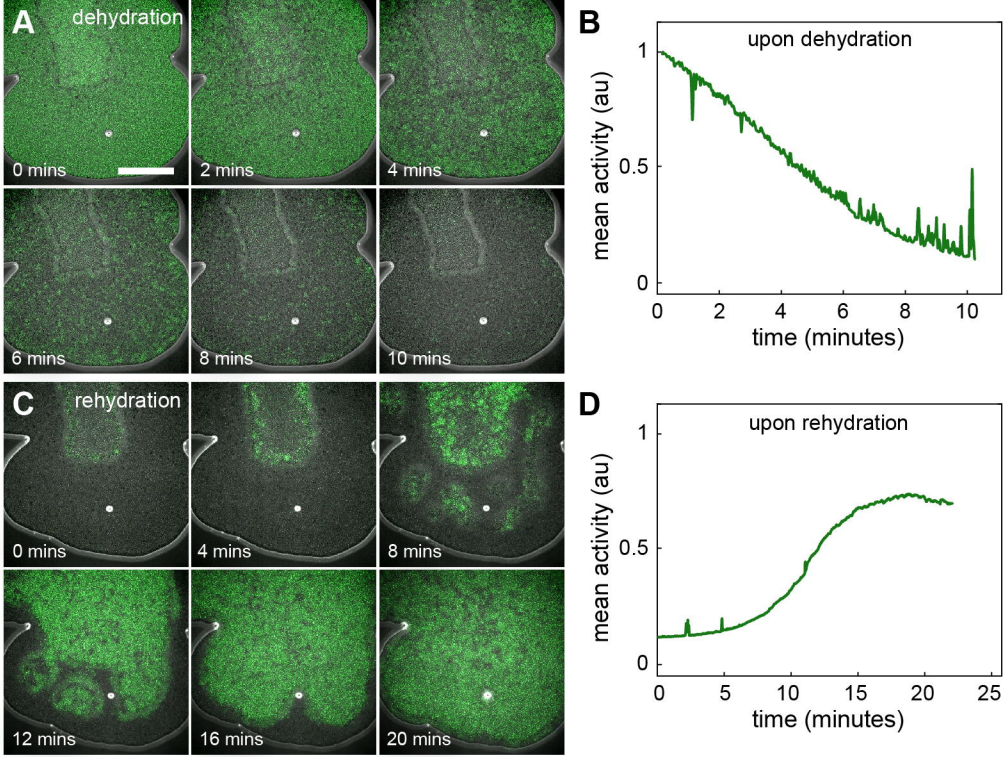
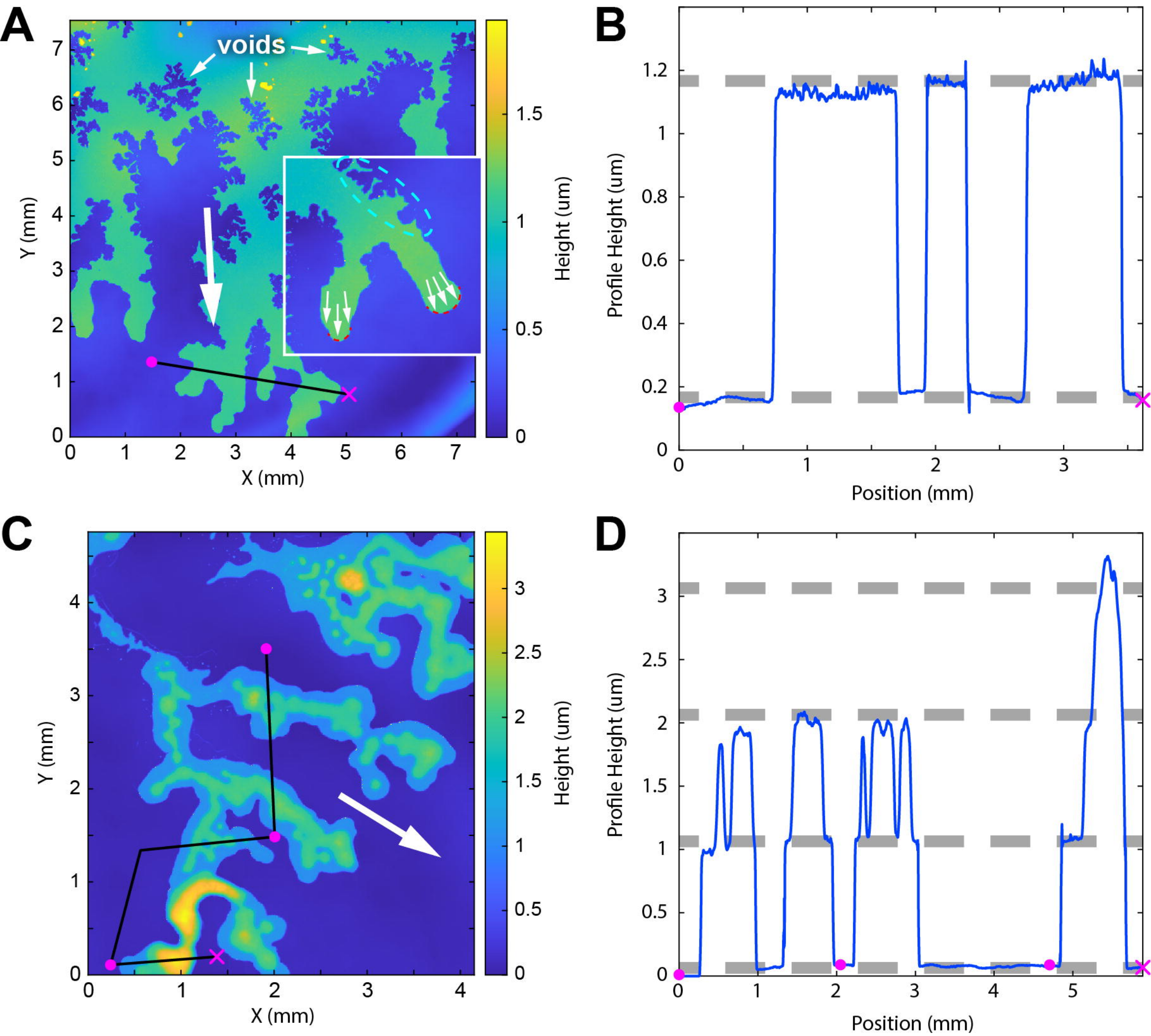
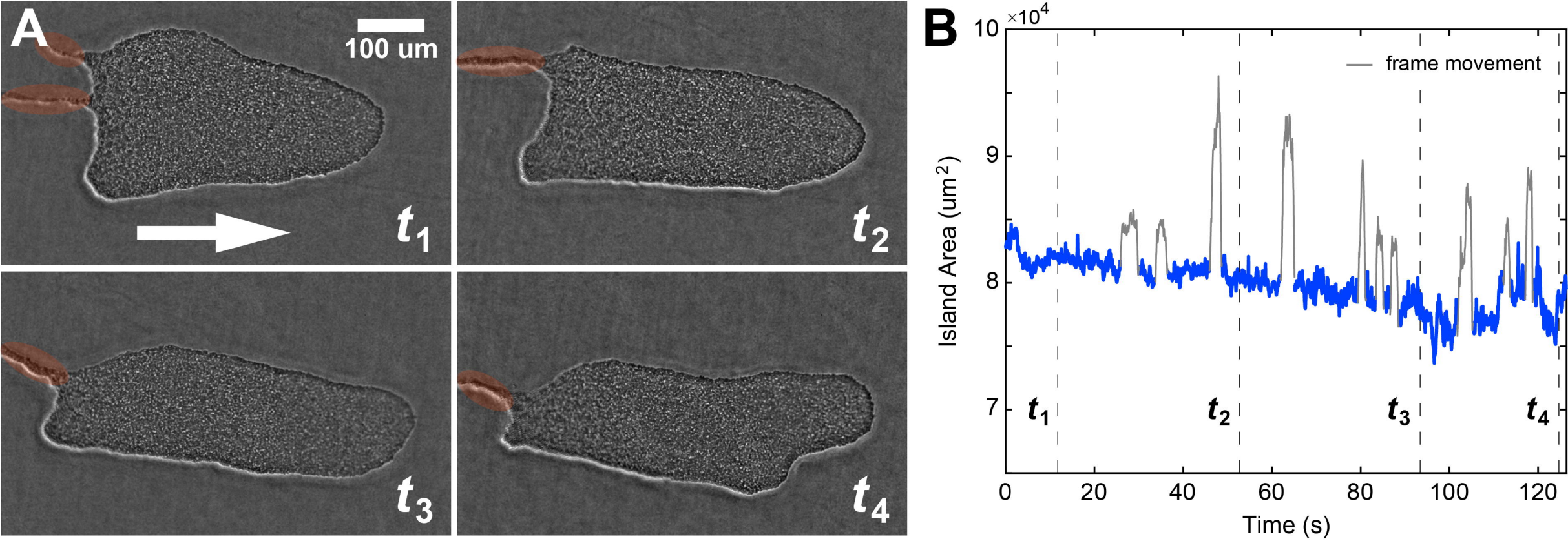
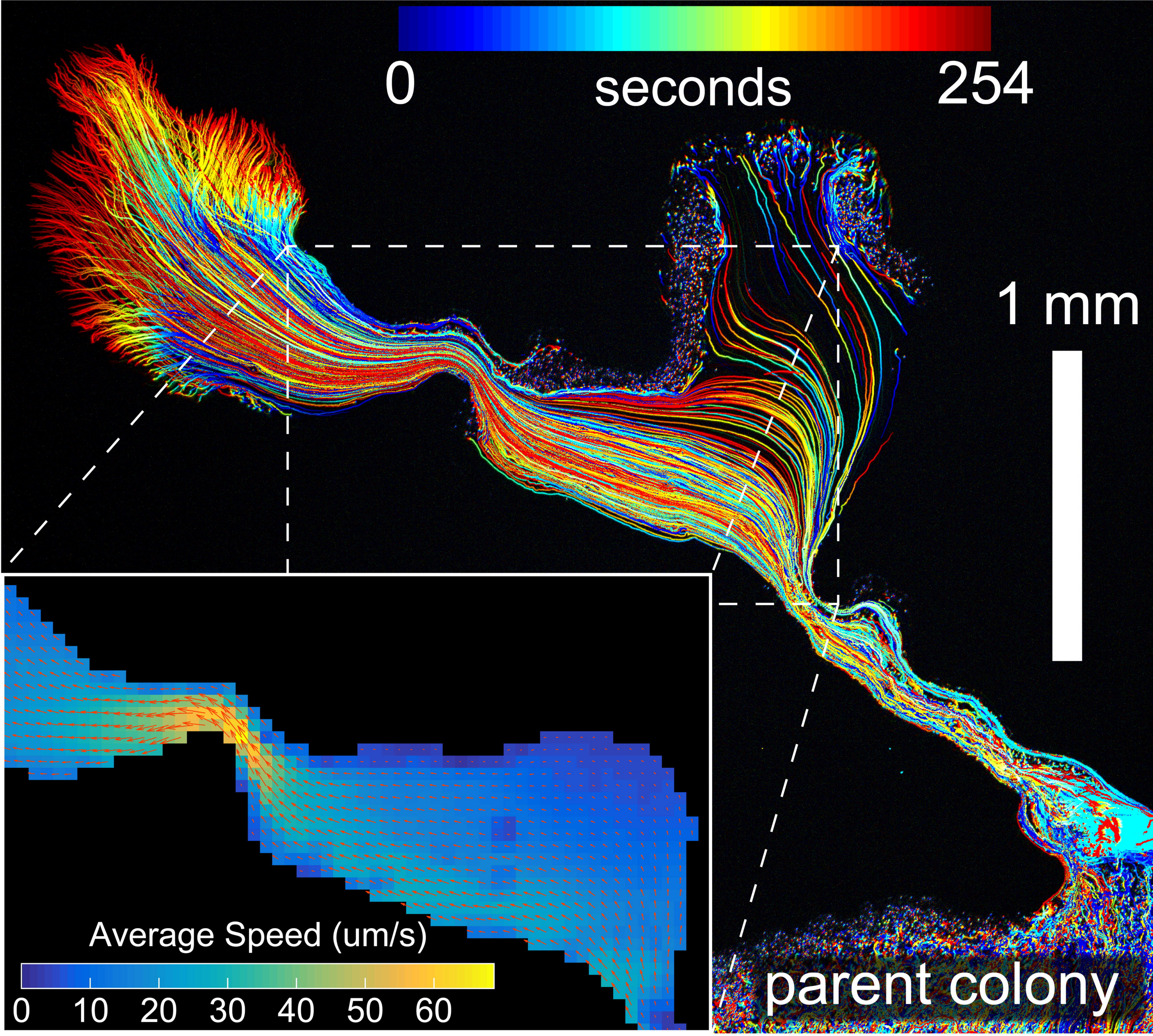
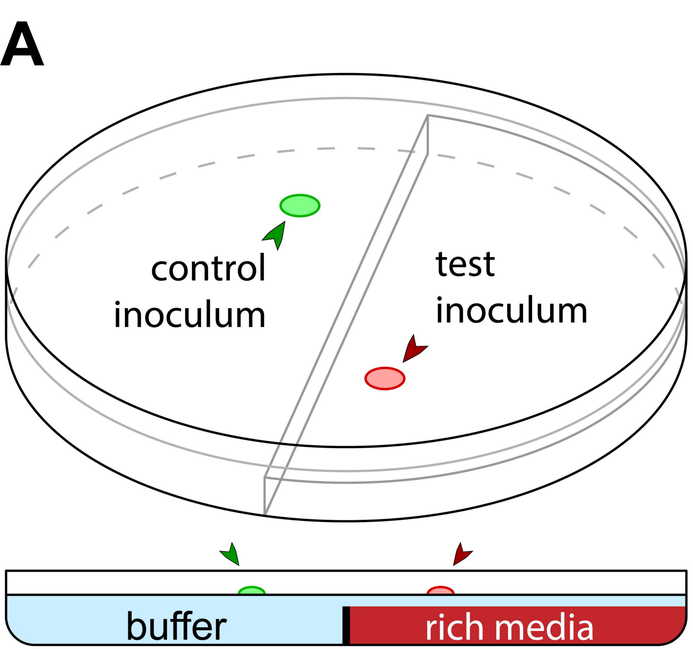

A
11 min
1 mm
27 min
1 mm
43 min
1 mm
49 min
1 mm
53 min
1 mm
62 min
1 mm
fluidized
jammed
monolayer
bilayer
trilayer
B
0.15 um/s
0.18 um/s
0.24 um/s
0.26 um/s
emergence
of bilayers
coalescence
of bilayers
10 min
200 um

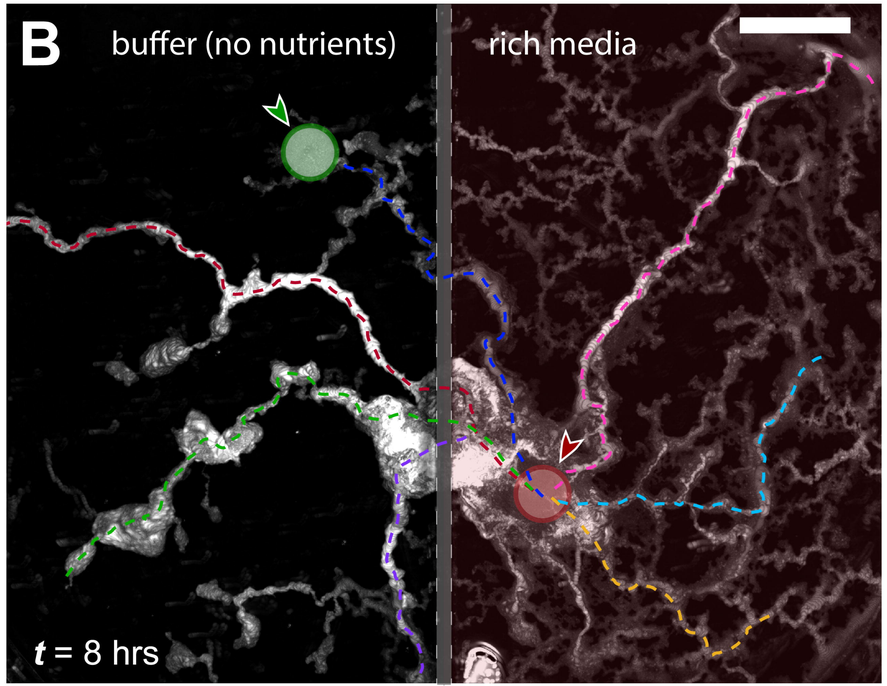

A
dehydration
0 mins
2 mins
4 mins
6 mins
8 mins
10 mins
B
upon dehydration
mean activity (au)
1
0.5
0
0
2
4
6
8
10
time (minutes)
C
rehydration
0 mins
4 mins
8 mins
12 mins
16 mins
20 mins
D
upon rehydration
mean activity (au)
1
0.5
0
0
5
10
15
20
25
time (minutes)

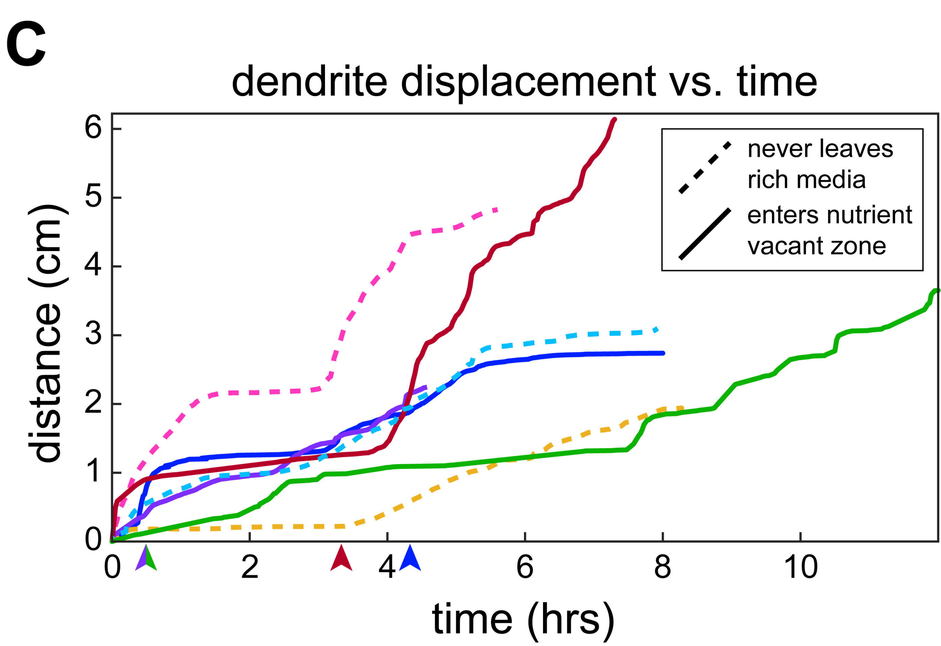

A
voids
Y (mm)
X (mm)
Height (um)
B
Profile Height (um)
Position (mm)
C
Y (mm)
X (mm)
Height (um)
D
Profile Height (um)
Position (mm)


A
100 um
$t_1$
$t_2$
$t_3$
$t_4$
B
×10^4
10
9
8
7
Island Area (um^2)
frame movement
$t_1$
$t_2$
$t_3$
$t_4$
0
20
40
60
80
100
120
Time (s)


0
seconds
254
1 mm
parent colony
Average Speed (um/s)
0
10
20
30
40
50
60


A
control
inoculum
test
inoculum
buffer
rich media


B
buffer (no nutrients)
rich media
t = 8 hrs


C
dendrite displacement vs. time
never leaves
rich media
enters nutrient
vacant zone
distance (cm)
time (hrs)
0
1
2
3
4
5
6
0
2
4
6
8
10

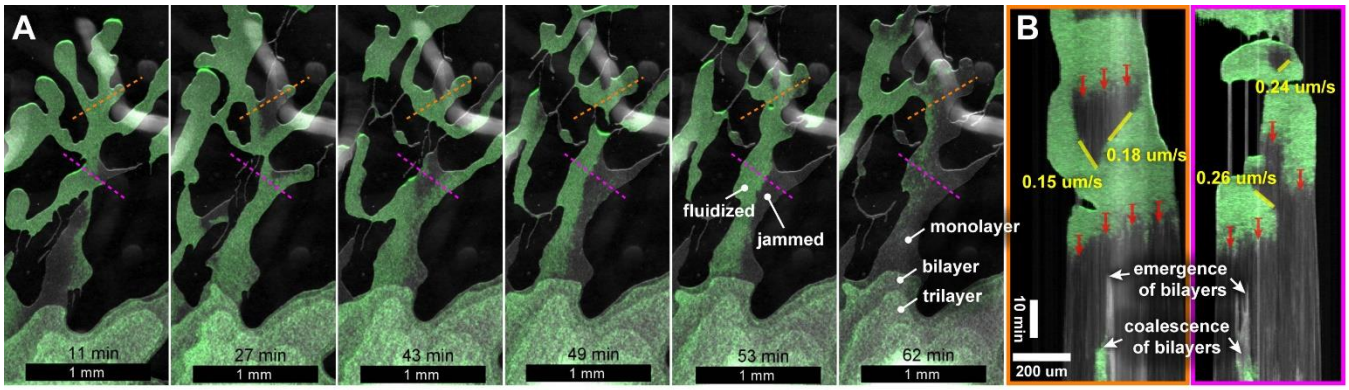



**Figure 1. Isogenic cells exist in motile and immotile phases within the same colony. (A)** Time-lapse imaging of wild-type *B. subtilis* spreading dendritically on a nutrient-rich soft-agarose surface. We applied a computational image filter that uses local intensity fluctuations over time to report on the degree of movement. Green regions are high motile activity and stationary regions are left gray. Transient motile regions and fluctuating boundaries between motile and stationary regions emerged and dissipated on timescales shorter than (potential) phenotypic changes (see Movie S1). **(B)** Kymographs along the colored dashed lines in (A) (time is downward). Transitions from large-scale motility (green) to immobilized (jammed) states are denoted by the red arrows. Regions could transition from jammed back to fluidized if they came in contact with a fluidized region (see boundary movements in yellow). At later times, once the entire population had become immotile, cells grew 'upward' into bilayers that frequently coalesced. See Movie S6.

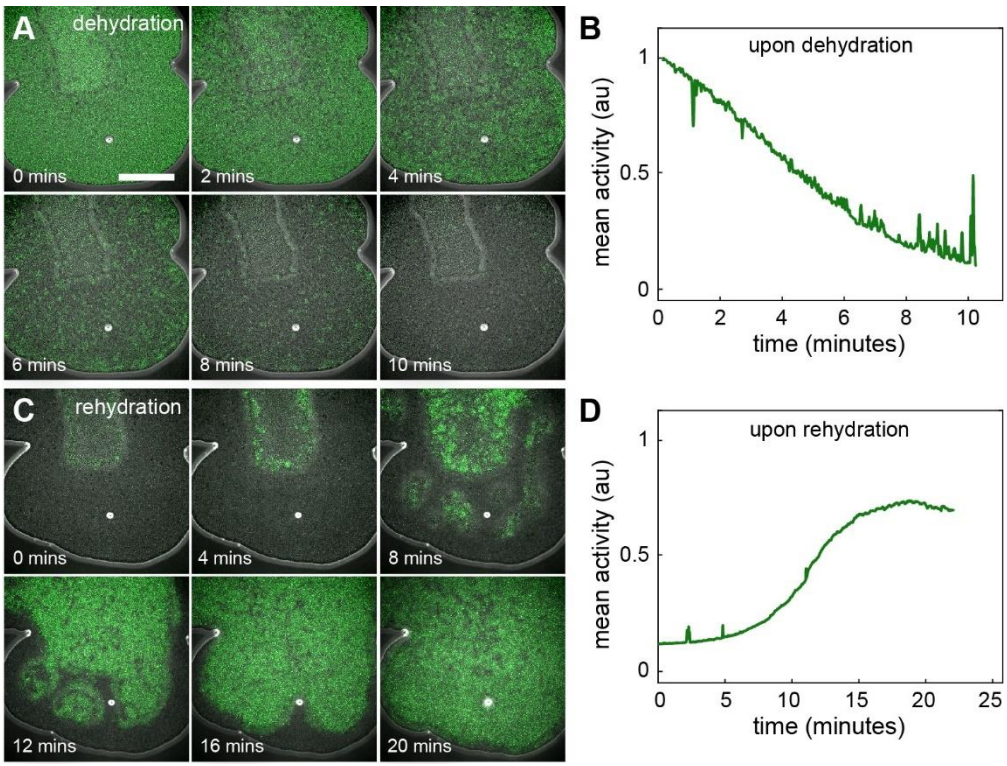



**Figure 2. Water availability controls a jamming-like transition**. **(A)** Time-lapse imaging of the tip of a wild- type *B. subtilis* dendrite on a nutrient-rich low-agarose surface. At $t$ = 0 the plate was unsealed and its surface was exposed to air. Subsequent evaporation reduced water availability at the surface, causing both collective motion (green color) and the computational measure of mean motile activity **(B)** to drop as the cellular group entered the jammed state. Across the entire dendrite tip collective motility halted. **(C - D)** The same plate was then immediately resealed and imaged over 20 minutes. The punctuated drop in evaporation rate allowed cells to retain more water from the gel. Fluidized domains of motile cells subsequently emerged and coalesced until the entire dendrite was, once again, collectively motile. In both sets of images, the rectangular structure toward the top is a cellular bilayer (see Fig. 3). Noise in the activity measurement (B) was (in part) due to manual refocusing required by the retraction of the surface as water evaporated. Scale bar is 200 µm. See Movies S7A and S7B.

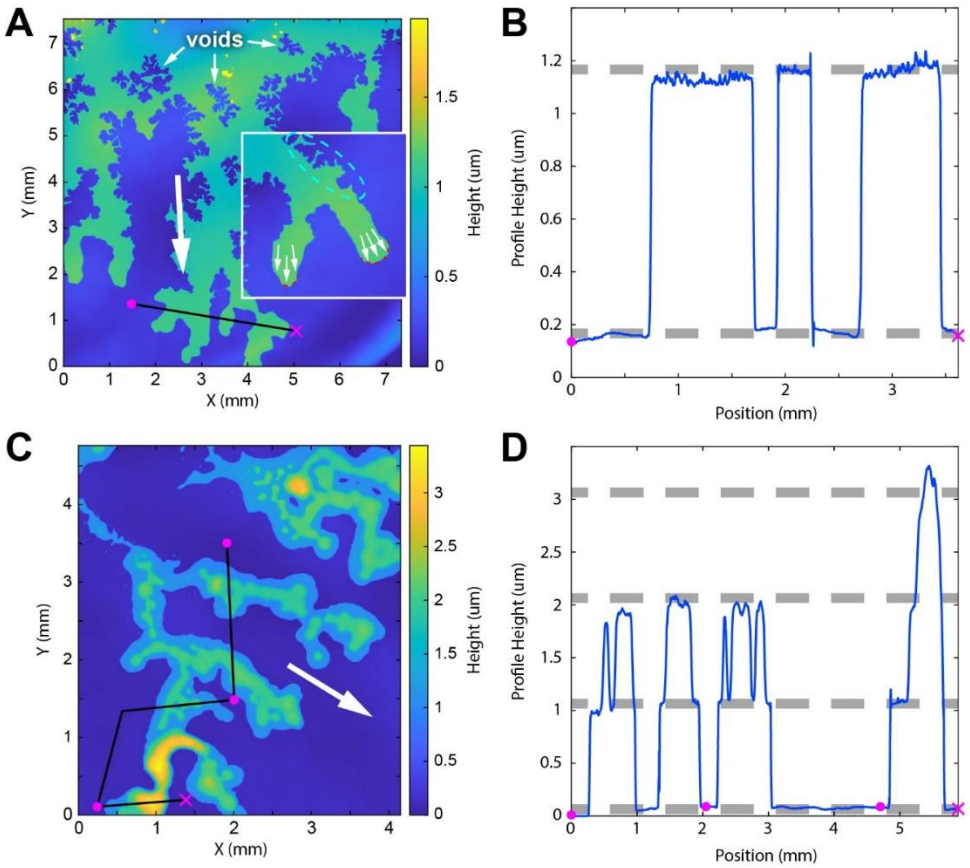



**Figure 3. Dendrites expand in discrete layers.** The left-hand column shows height images of wild-type *B. subtilis* dendrites on soft agarose taken using an interferometric profilometer (see Methods); the image backgrounds have been computationally flattened. **(A)** Advancing dendrites flowed downward (white arrow) and the fronts of those dendrites exhibited overall positive curvature (red dashed lines, inset) indicating outward pressure. Behind the advancing front, boundaries between agarose and cells exhibited a 'lacunar' structure (e.g. inset, cyan dashed ellipse), where the mode of the curvature distribution was negative (SI Fig. 3). Voids appeared behind the front, consistent with negative pressure in those regions overcoming meniscal forces. Both voids and lacunar structure are consistent with viscous fingering and cells de-wetting and being pulled to the front. **(B)** A one-dimensional profile of the surface height along the black line in (A). Here the advancing front is a densely packed cellular monolayer, whose height is one cell thickness as shown by the horizontal grey dashed lines (1 µm apart). **(C)** Same imaging modality as in (A), now after dendrites have jammed on the surface and then grown for ~ 1 hr. The original flow direction is indicated by the white arrow. **(D)** A one dimensional profile of the surface height along the black line in (C). Within contiguous cellular regions, bacterial film height is discretized into layers one cell thick; here the profile shows transitions from monolayer, to bilayer, to trilayer. Discretization ceased above four layers, when presumably cells move in any orientation, rotating about their long axis within the entire vertical structure (see also Movies S6, S8 and S9).

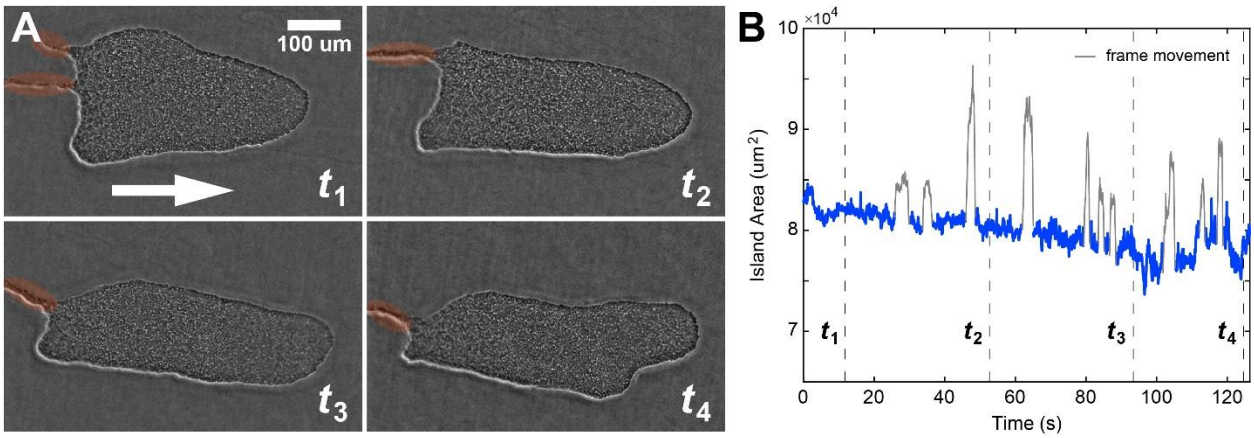
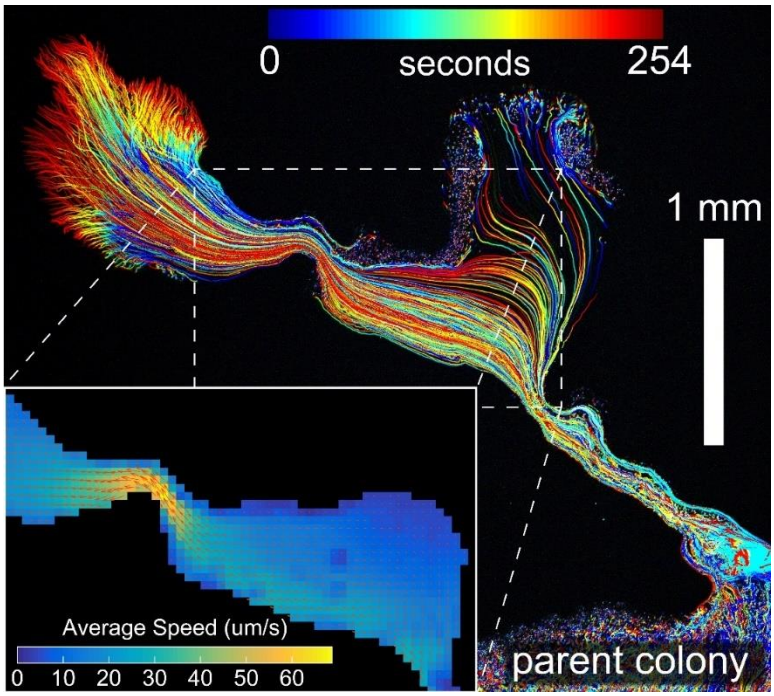



**Figure 4. Groups can move independently without growth. (A)** Four snapshots of an independent monolayer 'island' of wild-type *B. subtilis* cells moving to the right (white arrow) on soft agarose. Over the course of imaging, the group moved at an average speed of ~ 6 µm/s and the total amount of movement was ~ 700 µm, necessitating movement of the viewing frame (grey data in B). Common to our observations, such islands leave a trail of cells immobilized on the surface behind them (orange highlighted regions). **(B)** The area of the island decreased over time (time labels correspond between A and B). When combined with our data showing movement in monolayers, these data demonstrate that movement does not require proliferation. See Movie S13.

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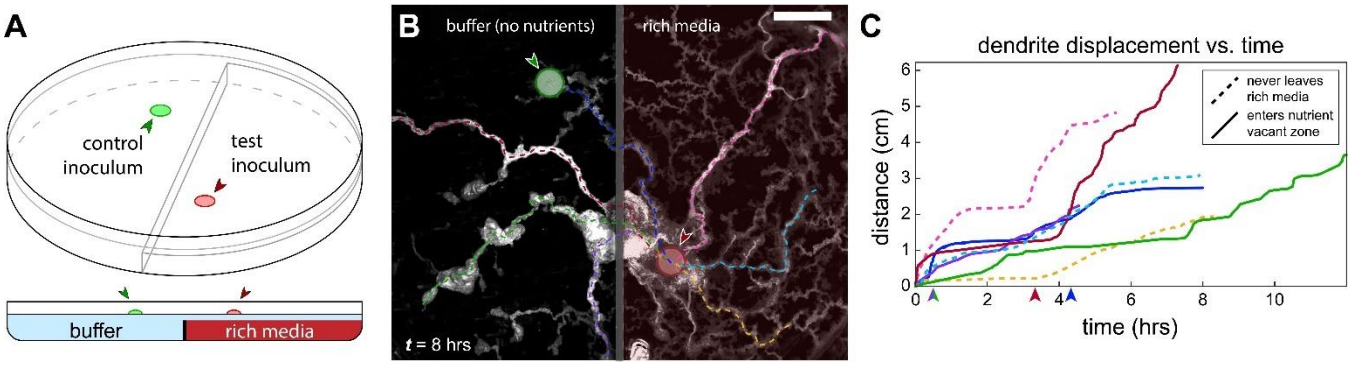


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