Supplementary Material

Laminar flow around corners triggers the formation of biofilm streamers

Roberto Rusconi, Sigolene Lecuyer, Laura Guglielmini, and Howard A. Stone

Supplementary Figures

Figure S1. (a, b) Midplane confocal images acquired after 10 h of constant flow at 0.75 $\mu$l min$^{-1}$ (same experiment of figures 1e and f). (c) Midplane confocal image acquired after 12 h of constant flow at 0.75 $\mu$l min$^{-1}$ (same experiment as figures 1c and d). Scale bars, 100 $\mu$m.
Figure S2. (a, b) Plan view and horizontal perspectives of a 3D software reconstruction obtained from confocal z-scan images acquired at 10X magnification (midplane sections are shown in figures 1c and d). The loss in continuity is partially due to an imperfect reconstruction owing to the oscillatory motion of the streamers.
Figure S3. Green fluorescently labeled lectins, Triticum vulgaris (WGA) and Canavalia ensiformis (ConA), have been used to stain the extracellular matrix in a test with *P. Aeruginosa* strain PA01. The images are taken with a confocal microscope at the middle-height plane of the channel. Scale bar, 50 μm.
Figure S4. Comparison between type IV pili (*pilC*) and flagellar-mediated motility (*flgK*) detective mutant streamers after 6 and 10 h of continuous at 0.75 μl min⁻¹. The initial concentration is equivalent to OD₆₀₀ = 0.5 for both mutant solutions. The images are taken with a phase-contrast microscope and the focus is approximately at half the channel height. Scale bar, 50 μm.
Figure S5. (a) Confocal images of the channel with a lateral hemi-cylindrical bump taken respectively at the bottom, middle, and the top surface after 20 h of continuous flow at 0.25 μl min⁻¹. The height of the channel is 65 μm. The midplane image is a larger view of figure 5f.

(b) Phase-contrast images of other streamers developed around cylindrical bumps after 20 h of flow at 0.75 μl min⁻¹ in a 80 μm-thick channel. White arrows indicated small clusters of cells aligned on a very thin streamer (the focus is approximately in the middle of the channel). Scale bars, 25 μm.
Supplementary Movies

Movie S1. This movie shows a confocal z-scan (from the bottom to the top) of the curved microfluidic channel after 12 h of constant flow (at 0.75 µl min⁻¹) for a solution of GFP-labeled PA14 wild-type. The flow rate during the scanning process is 0.2 µl min⁻¹. Confocal images are taken every µm for the whole thickness of the channel (100 µm). Magnification: 10X. Frame rate: 10 frames s⁻¹.

Movie S2. This movie shows a different part of the curved microfluidic channel used to study the formation of bacterial streamers under the same conditions of movie S1. Magnification: 10X. Frame rate: 10 frames s⁻¹.

Movie S3. This movie shows the temporal development of a streamer around a corner at half the channel height. The time lapse (on a phase-contrast microscope) covers a period of 3 h for a solution of PA14 wild-type after 5 h of constant flow (at 1 µl min⁻¹). Magnification: 40X. Acquisition rate: 1 image min⁻¹. Frame rate: 10 frames s⁻¹.