

**Movie 1.** Suicidal chemotaxis towards antibiotics. Surface-attached *P. aeruginosa* cells move via twitching motility towards the antibiotic ciprofloxacin ( $C_{MAX} = 10X$  MIC), after an initial period of random motility. A static gradient of ciprofloxacin is generated using a dual-inlet microfluidic device with nutrients (tryptone broth) flowing through one inlet and nutrients supplemented with ciprofloxacin through the other. The panel on the right-hand side shows a magnified view of the region marked with the dashed white box in the first frame.

**Movie 2.** Chemotaxis towards ciprofloxacin is also observed in other strains of *P. aeruginosa*. The left-hand panel shows our focal PAO1 strain, the middle panel shows PA14 and the right-hand panel shows PAK. In all cases, cells were exposed to a static gradient of ciprofloxacin ( $C_{MAX} = 10X$  MIC), with nutrients (tryptone broth) flowing through the top-half of the channel and nutrients supplemented with ciprofloxacin flowing through the bottom-half of the channel. Both halves of the channel were also supplemented with 10% cell-free supernatant collected from liquid cultures of each respective strain as this was found to promote chemotaxis towards antibiotics in experiments with PAO1.

**Movie 3.** A reversal-null  $\Delta pilG$  mutant does not chemotax towards antibiotics. Cells were exposed to a static gradient of ciprofloxacin ( $C_{MAX} = 10X$  MIC), with nutrients (tryptone broth) flowing through the top-half of the channel and nutrients supplemented with ciprofloxacin flowing through the bottom-half of the channel. The right-hand panel shows  $\Delta pilG$  mutant cells, which rarely reverse direction and show significantly reduced movement bias towards ciprofloxacin (Fig. S10). In contrast, the left-hand panel shows complemented mutant cells ( $\Delta pilG_{comp}$ ), whose ability to undergo chemotaxis towards ciprofloxacin is restored. Note that  $\Delta pilG$  cells exhibit reduced motility compared to WT cells in these assays and so we only analyse cells that show appreciable movement from their initial position (Methods).

**Movie 4.** The effects of cell-free supernatant on chemotaxis with and without antibiotics. Left-hand side shows chemotaxis towards antibiotics, where surface-attached *P. aeruginosa* cells move via twitching motility towards ciprofloxacin ( $C_{MAX} = 10X$  MIC; see also Movie 1). Middle panel shows cells in a gradient of cell-free supernatant ( $C_{MAX} = 100\%$  cell free supernatant) extracted from cells grown in tryptone broth within microfluidic devices ('microfluidic cell-free sup'). Cells are repelled by the cell-free supernatant. Right-hand side shows cells in a combined gradient of cell-free supernatant and ciprofloxacin. Cells move towards the ciprofloxacin despite the repulsion of cell-free supernatant (shown in the middle panel).

**Movie 5.** Cells release bacteriocins (pyocins) during chemotaxis towards antibiotics (ciprofloxacin,  $C_{MAX} = 10X$  MIC). Cells turn green when they make bacteriocins as they have a mNeonGreen fluorescent transcriptional reporter for Pyocin R2. Pyocins are released via programmed cell lysis, which can be seen in the movie where it is followed by a transient burst of red colour. This colour is caused by the presence of the dye propidium iodide which binds to the DNA released when cells lyse.