Cell shape-independent FtsZ dynamics in synthetically remodeled bacterial cells

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FtsZ is the main regulator of bacterial cell division. It has been implicated in acting as a scaffolding protein for other division proteins, a force generator during constriction, and more recently, as an active regulator of septal cell wall production. FtsZ assembles into a heterogeneous structure coined the Z-ring due to its resemblance to a ring confined by the midcell geometry. Here, to establish a framework for examining geometrical influences on proper Z-ring assembly and dynamics, we sculpted Escherichia coli cells into unnatural shapes using division- and cell wall-specific inhibitors in a micro-fabrication scheme. This approach allowed us to examine FtsZ behavior in engineered Z-squares and Z-hearts. We use stimulated emission depletion (STED) nanoscopy to show that FtsZ clusters in sculpted cells maintain the same dimensions as their wild-type counterparts. Based on our results, we propose that the underlying membrane geometry is not a deciding factor for FtsZ cluster maintenance and dynamics in vivo.
Most bacterial cells divide by binary fission, whereby one mother cell splits into two identical daughters. Decades of study have led to a detailed understanding of how the cell division machinery, the divisome, carries out this task during the later stages of the cell cycle. At the heart of this process is the eukaryotic tubulin homolog, FtsZ, that, together with its membrane anchors FtsA and ZipA (in E. coli), forms an intermediate structure called the proto-ring (Fig. 1a). Functioning as a recruitment base, the proto-ring components then enlist the remaining essential division proteins to form a mature divisome. As soon as it is fully assembled, the divisome starts to constrict the remaining essential division proteins to form a mature divisome. During the later stages of the cell cycle, the divisome, the cell division machinery, carries out this task how the cell division machinery, the divisome, carries out this task.

Midcell FtsZ-ring assembly is unaffected by increased cell diameter. In rod-shaped model bacteria such as Escherichia coli and Bacillus subtilis, FtsZ is believed to organize into short bundles of filaments, roughly 100 nm in length. The divisome is the eukaryotic tubulin homolog, FtsZ, that, together with its membrane anchors FtsA and ZipA (in E. coli), forms an intermediate structure called the proto-ring (Fig. 1a). Functioning as a recruitment base, the proto-ring components then enlist the remaining essential division proteins to form a mature divisome. As soon as it is fully assembled, the divisome starts to constrict the remaining essential division proteins to form a mature divisome. During the later stages of the cell cycle, the divisome, the cell division machinery, carries out this task how the cell division machinery, the divisome, carries out this task.
maintenance and stability are currently unclear. In vitro data have shown that FtsZ can self-organize into swirling rings on supported bilayers when unconfined by geometrical constraints21, but we were curious as to whether geometrical changes to cell shape would influence Z-ring formation and dynamics, as this would result in a better understanding of FtsZ behavior in live cells. In this study, we systematically examine FtsZ formation, organization and behavior in E. coli cells that are sculpted into complex geometrical shapes in micron sized holes. We show that FtsZ formation and dynamics are independent of cell shape and membrane curvature.

Results
FtsZ structure and dynamics in Z-rings are not sensitive to increased ring size. As a reference for unmodified division rings, we imaged Z-rings in E. coli cells expressing FtsZ-mNeonGreen as the only source of FtsZ25. Under our experimental conditions, this strain produced normal-looking, sharp Z-rings (Supplementary Figure 1) and grew and divided similarly to wild-type (WT) E. coli (MC4100) (Supplementary Figure 2a-e). We then trapped the cells in a vertical position in micron-sized holes that were produced in agarose pads using silica micron pillar arrays14 (Fig. 1b, Supplementary Figure 3), and imaged the cells using super-resolution time-gated STimulated Emission Depletion (gSTED) nanoscopy. In these standing cells, a heterogeneous Z-ring with distinct FtsZ-mNeonGreen clusters was clearly seen traversing the circumference of the cell (Fig. 1c), similar to what has been observed before12,14.

Previous work has shown that FtsZ clusters generally maintain the same length throughout envelope constriction12,14. We wanted to see if this was also true for unnaturally large cells, i.e., would FtsZ clusters maintain the same dimensions in Z-rings of cells with increased diameter at midcell? In order to increase cell diameter, we treated E. coli cells with A22 and cephalaxin (hereafter collectively referred to as “drugs”), in a way similar to what has previously proven successful for cell shape manipulations23. A22 disrupts MreB dynamics and therefore perturbs the characteristic rod-shape of E. coli cells19,24, while cephalaxin blocks cell division by inhibiting the transpeptidase activity of FtsZ25. The net effect of this dual drug treatment is the growth of cells into shapeable blebs that are unable to divide (Supplementary Figure 4a).

We hypothesized that as long as cell width remains less than cell length, FtsZ molecules should be directed to midcell by the Min system23 and other FtsZ placements systems26, such that a cell length, FtsZ molecules should be directed to midcell by the

The FtsZ-square. Next, we wanted to know if drug-treated cells placed in deep (5 μm) rectangular volumes would adapt to these shapes and effectively form Z-rectangles or Z-squares instead of Z-rings. Previous work has shown that cells can adapt to rectangular shapes in shallow wells, approximately 1 μm deep23. Here, we produced quadrilateral patterns in agarose pads using silica micron pillar arrays similar to those previously described14, with the exception that the pillars were rectangular and 5.5 ± 0.5 μm in height. Sides of the micron chambers were up to 3.5 μm in length (Supplementary Figure 11), resulting in well volumes up to 80 μm3, roughly 50-fold larger than the volume of a WT cell (assuming a WT cell size of 2 μm in length and 1 μm in width) (Supplementary Figure 12).

Drug-exposed cells expressing FtsZ-mNeonGreen were placed in rectangular micron holes and incubated at room temperature
for 300–420 min (longer incubation times were needed due to increased well size). The cells adapted to their new shapes and formed rectangular cuboids with only one Z-square per cell (Fig. 3a, Supplementary Movie 4). Notably, FtsZ clusters were observed both in the sharp corners and along the sides of the rectangles (Fig. 3b, Supplementary Figure 13). Quantification of the FtsZ-mNeonGreen clusters showed that they had similar dimensions to those in untreated cells, with an average length of 105.4 ± 39.6 nm and radial width of 79.6 ± 18.2 nm (n = 147) (Fig. 3c). This suggests that FtsZ cluster dimensions in vivo are insensitive to membrane curvature (or lack thereof).

To generate a fluorescent FtsZ fusion protein that could be used for both super-resolution STED imaging and examination of FtsZ dynamics when grown in rich media at 37°C, we constructed a plasmid-expressed FtsZ-mCitrine fusion. FtsZ-mCitrine was expressed from an IPTG-inducible, medium copy-number plasmid, pTrc99a, at a level approximately equal to 30% of total cellular FtsZ. Under these conditions, FtsZ-mCitrine formed normal-looking, sharp Z-rings (Supplementary Figures 1 and 2). Cells expressing FtsZ-mCitrine were then exposed to drugs, trapped in rectangular micron-sized holes, and incubated and 2). Cells expressing FtsZ-mCitrine were then exposed to drugs, trapped in rectangular micron-sized holes, and incubated and untreated cells (~25 nm s

**FtsZ dynamics in rectangular-shaped cells.** In order to examine the dynamics of FtsZ in rectangular cells, we performed time-lapse imaging on cells expressing either FtsZ-mCitrine or FtsZ-GFP. Although a few fluorescence spots were abnormally bright and immobile (~1 spot/5 cells, with a maximum of 2 spots in one cell) (Fig. 4b, Supplementary Movie 7, red arrow), the majority of FtsZ clusters were highly dynamic (Fig. 4a, b, Supplementary Movies 5–6). Note that the bright, immobile spots were excluded from treadmilling analyses. Close inspection of time-lapse sequences suggested that FtsZ clusters in rectangular-shaped cells could treadmill continuously around the perimeter of the cells (Fig. 4a), and importantly, even across sharp corners, without an apparent change in speed (Fig. 4b, c, Supplementary Movie 8). The average treadmilling speed of FtsZ-mCitrine clusters in rectangular cells with perimeter lengths up to 13 μm (more than four times the circumference of a WT cell) was 27.6 ± 12.5 nm s−1 (n = 109), which was consistent with the measured treadmilling speed of FtsZ-GFP in rectangular cells (25.3 ± 11.3 nm s−1, n = 122) (Fig. 4d), large cylindrical cells (30 ± 18 nm s−1, Fig. 1m) and untreated cells (~25 nm s−1)13,14.

To determine whether the dynamics of FtsZ subunit exchange are affected by changes to circumferential length and shape, we collected FRAP measurements on FtsZ bundles in rectangular-shaped cells (Fig. 4e, Supplementary Movie 9). The recovery times of half-bleached rectangles of varying sizes matched those of rings, with mean t1/2 recovery times of 9.85 ± 2.58 s (n = 24) and 9.15 ± 2.55 s (n = 22) for FtsZ-mCitrine and FtsZ-GFP, respectively (Fig. 4f). This suggests that subunit exchange from the cytoplasmic FtsZ pool is independent of circumference length and membrane curvature. The data thus far indicate that the maintenance and dynamics of FtsZ clusters are preserved in both large Z-rings and Z-rectangles of varying size.
heart-shaped (Supplementary Figure 15). Heart shapes were chosen because they would sculpt cells in such a way that highly curved, straight, and angled membrane segments would be present within a single cell. Drug-treated E. coli cells expressing cytoplasmic GFP, FtsZ-mNeonGreen or FtsZ-mCitrine were sculpted into hearts as described above (Fig. 5a). Perhaps not surprisingly, quantification of 155 individual FtsZ clusters from the heart-shaped cells revealed dimensions similar to those in round and rectangular cells (129 ± 44 nm long and 84 ± 9 nm wide) (Fig. 5b). We also found that the average treadmilling speed of FtsZ-mCitrine in heart-shaped cells (22 ± 10 nm s⁻¹, n = 44) was essentially the same as that in untreated cells (Fig. 5c, Supplementary Movie 10).

For about one-third of the heart-shaped cells, we noticed bright spots of internalized FtsZ-FP signal that accumulated close to the cell center (Fig. 5c, green arrowhead). Although we couldn’t distinguish whether these were true FtsZ clusters or aggregated protein, cytoplasmic clustering of FtsZ in WT cells have previously been reported. Furthermore, although most hearts had FtsZ-FP signal spanning the full perimeter of the cell, approximately 20% were only half-full (Fig. 5d, lower left). We do not fully understand the underlying reason for this, however it is unlikely due to image focus or cell tilt issues, as every cell was scanned in the z-direction prior to imaging. Nevertheless, when we subjected the heart-shaped cells to FRAP, fluorescence recovery rates were equal for both full and half-full hearts (Fig. 5d), with mean t₁/₂ recovery times of 7.1 ± 1.1 s (n = 24) and 6.9 ± 0.9 s (n = 9), respectively (Fig. 5e).

FtsZ-rings form in complex cell shapes. To explore if cell geometry plays a role in Z-ring formation, we set out to remodel cells into other complex shapes. Even though highly complex-shaped bacteria occur in nature, such as star-shaped bacteria, we wanted to test whether rod-shaped E. coli cells would allow themselves to be drastically remodeled. Using micron pillars of various shapes, we produced holes in agarose pads such that drug-exposed cells could be sculpted into complex shapes, such as pentagons, half-moons, stars, triangles and crosses (Fig. 6a, middle row, Supplementary Figure 15). The cells deformed remarkably well to these shapes, forming sharp boundary angles < 70° (Fig. 6a, star). After we confirmed that cells could adapt to these complex shapes, we placed cells expressing FtsZ-mCitrine into the micron holes, allowed for reshaping to occur, and then imaged the cells using STED nanoscopy. Cells of all tested shapes produced FtsZ-shapes at midcell (Fig. 6a, top row). Following analysis of the lengths and widths of the FtsZ clusters revealed little difference in dimensions between the different shapes, suggesting a minimal role of cell shape in determining FtsZ cluster dimensions in vivo (Fig. 6b).

Discussion
Cells, both bacterial and eukaryotic, have the ability to adapt to their local environments, reverting to their original shapes after stress and dividing with striking midcell accuracy even when remodeled into irregular cell shapes. In bacteria, the tubulin homolog FtsZ assembles into a ring-like structure at midcell and is responsible for overall maintenance of the cell division machinery. The general dynamics and organization of the FtsZ-ring have been shown to be quite similar across many bacterial species. Common to these species is confinement of the FtsZ-ring to a circular geometry at midcell. Strikingly, when purified FtsZ is placed on supported lipid bilayers, it assembles into a dynamic, swirling ring-like assembly with a diameter resembling that of wild-type E. coli cells (approximately 1 μm). This phenomenon is observed when FtsZ...
Fig. 4 FtsZ dynamics in rectangular-shaped cells. The dynamics of FtsZ in rectangular shapes were assessed by time-lapse imaging and FRAP measurements on E. coli cells expressing FtsZ-mCitrine (FtsZ-mCit) or FtsZ-GFP. a–c Snapshot images from time-lapse series of FtsZ-mCitrine or FtsZ-GFP in rectangular shaped cells. Corresponding kymographs are shown next to each image. a Kymographs were taken around the entire perimeter (starting at the upper left corner, moving counter-clockwise, indicated by the yellow arrowheads). b Kymographs were taken along the white line (left kymograph), or over the bright spot indicated by the red arrow (right kymograph). FtsZ can clearly be seen treadmilling continuously across the sharp corner (indicated by the red arrowhead). The red arrows and arrowheads in the images correspond to the arrows and arrowheads on the kymographs. The black dashed line indicates the upper left corner of the cell. b The red arrowhead). The red arrows and arrowheads in the images correspond to the arrows and arrowheads on the kymographs. The black dashed line in b indicates the upper left corner of the cell. c Kymograph taken between the yellow arrowheads (top to bottom is left to right in the kymograph). d Average treadmilling speed of FtsZ-mCitrine and FtsZ-GFP in rectangles was 27.6 ± 12.5 nm s⁻¹ (n = 97) and 25.3 ± 11.3 nm s⁻¹ (n = 122), respectively. e Typical FRAP measurement of FtsZ-GFP in a rectangular E. coli cell. Half of the rectangle was bleached. f Average recovery times for FtsZ-mCitrine (dark, n₀ = 24) and FtsZ-GFP (light, n₀ = 22) in FtsZ-rectangles of various perimeter lengths. FRAP recovery times for rectangular cells with different perimeters: FtsZ-mCitrine t₁/₂ recovery times: 9 ± 2.9 s (Circ. 8 ± 1 μm, n = 6), 10 ± 3.2 s (Circ. 10 ± 1 μm, n = 7), 10.4 ± 2.3 s (Circ. 12 ± 1 μm, n = 7), 9.9 ± 1.9 s (Circ. 14 ± 1 μm, n = 4). FtsZ-GFP t₁/₂ recovery times: 8.1 ± 1.9 s (Circ. 8 ± 1 μm, n = 5), 8.8 ± 2.6 s (Circ. 10 ± 1 μm, n = 5), 10.1 ± 2.5 s (Circ. 12 ± 1 μm, n = 8), 9.7 ± 3.2 s (Circ. 14 ± 1 μm, n = 4). Circ. = Cell circumference. Values represent mean ± S.D. Dots represent individual data points, bars represent mean with error bars representing S.D. Scale bars = 1 μm.

is anchored to the lipid bilayer, either by FtsA or a membrane targeting sequence, hinting at an intrinsically preferred FtsZ-ring curvature.²¹,⁴³

In this study, we characterized FtsZ midcell accumulation and dynamics in cell shape-determining environments by ‘looking through the Z-ring’ along the long-axis of cells. We observed normal-looking FtsZ-rings in cells with diameters three times the size found in WT cells. However, this might not be surprising, as the total intensity fluorescence increased in large cells (Supplementary Figure 7) and considering only ~30% of the pool of FtsZ molecules are in the ring of WT cells at any given point in time.²⁷ Quantification of FtsZ cluster dimensions revealed little variation between different cell shapes, such as squares, pentagons, triangles and stars (on average 123 ± 80 nm, length × width, respectively, and summarized in Table 1), suggesting that local membrane geometry has minimal influence on FtsZ cluster dimensions. Compared to untreated cells, rectangular and heart-shaped cells with perimeter lengths more than four times that of a WT cell exhibited similar FtsZ cluster dynamics, as FtsZ-FP clusters treadmilled continuously at the same average velocity throughout the perimeter of the shaped cells (including over sharp corners and regions with severe angles), and FtsZ subunit exchange occurred at similar rates, independent of cell shape and size (Table 2).

In summary, our results from different shaped cells show that Z-ring formation and dynamics are not limited to cells of a certain shape or size. This agrees with previous findings, which show that internal cellular structures are maintained in cells that have been reshaped into unnatural forms.²³ Our observation that FtsZ clusters conform to the geometric shape of the membrane at midcell suggests that FtsZ-ring formation is not affected by changes in membrane curvature. Indeed, cell shape and size are important for proper cellular functions, however, with the many naturally-occurring shape variations of bacteria, it is...
perhaps not surprising that FtsZ can adapt to changing environments without compromising its own ability to maintain fundamental functionality. Although our data do not explicitly show that sculpted cells can divide (since downstream division proteins were inhibited), the fact that the dynamic properties of FtsZ were conserved in these cells shows that the Z-ring can be decoupled from the constriction process. Presently, we have shown in vivo that E. coli FtsZ-ring formation and dynamics are conserved, irrespective of cell shape and size.

Methods

Bacterial growth. All experiments were performed in E. coli strain MC4100, unless otherwise stated. Pre-cultures were grown overnight in 20 ml of rich media (LB) at 37 °C or M9 minimal media supplemented with 1 μg ml⁻¹ thiamine, 0.2% (w/v) glucose and 0.1% (w/v) casamino acids. The following morning, cultures were back-diluted 1:50 in either LB or M9 (with supplements) and antibiotics (ampicillin 25 μg ml⁻¹) when needed, and incubated at 30 or 37 °C.

Fluorescent protein production. Chromosomally-encoded FtsZ-mNeonGreen was integrated at the native ftsZ locus and did not require any inducer.

Fig. 5 FtsZ cluster dimensions and dynamics in heart-shaped cells. FtsZ behavior in E. coli cells sculpted into heart shapes. a Upper left, Cartoon representation of a WT E. coli cell and a heart shape (both colored red for visualization), highlighting the large and complex structural changes of a cell-to-heart transition, approximately to scale. Upper right, Drug-treated cell expressing cytoplasmic GFP, shaped as a heart. Lower, STED image of an FtsZ-heart (FtsZ-mCitrine) in a drug-treated E. coli cell. b Lengths and widths of 155 individual FtsZ-mNeonGreen fluorescence clusters in cells shaped as hearts. Average length = 129 ± 44 nm and width = 84 ± 9 nm. Boxes represent S.D., with red lines indicating mean. Whiskers on the box plots encompass 95.5% of the distribution. c Upper row, SIM image from a time-lapse series (epi-fluorescence) of a heart-shaped cell expressing FtsZ-mCitrine. Green arrowhead indicates internal FtsZ clustering. Corresponding kymograph is shown adjacent to the image, and was generated starting at the yellow arrowhead in the SIM image, moving counter-clockwise for the indicated length. The yellow arrow points to an FtsZ trajectory. Lower, average treadmilling speed of FtsZ-indicated internal FtsZ clustering. Corresponding kymograph is shown adjacent to the image, and was generated starting at the yellow arrowhead in the SIM image, moving counter-clockwise for the indicated length. The yellow arrow points to an FtsZ trajectory. Lower, average treadmilling speed of FtsZ-indicated internal FtsZ clustering. Corresponding kymograph is shown adjacent to the image, and was generated starting at the yellow arrowhead in the SIM image, moving counter-clockwise for the indicated length. The yellow arrow points to an FtsZ trajectory. Lower, average treadmilling speed of FtsZ-indicated internal FtsZ clustering.

Fig. 6 FtsZ bundle dimensions in complex shapes. a Cells expressing cytosolic GFP or FtsZ-mCitrine were remodeled into various shapes. Top row, schematic representation of the cell shapes (colored green for visualization). Middle row, representative cells expressing cytosolic GFP, and sculpted in the corresponding shapes. Bottom row, an FtsZ-pentagon, FtsZ-half-moon, FtsZ-star, FtsZ-triangle and FtsZ-cross in sculpted cells. Scale bars = 1 μm. Dots represent individual data points, bars represent mean with error bars representing S.D.
Chromosomally-encoded FtsZ-GFP (strain B8001), GFP-CYTO (strain B8008) and ZipA-GFP were induced with 2.5 μM, 5 μM and 50 μM IPTG, respectively. The plasmid pTrc99a (ftsZ-mCitrine) was constructed using Gibson assembly to generate an IPTG-inducible ftsZ-mCitrine fusion expressed from pTrc99a. PCR was performed using Q5 High-Fidelity DNA polymerase (New England Biolabs). A DNA fragment containing ftsZ was amplified from E. coli MC4100 genomic DNA using primers FtsZ(F) (5′-caattcagaaacgaacagcttggtcga-3′) and FtsZ(R) (5′-gcggcttcggatcagttggtg-3′) and mCitrine was amplified from mCitrine-N1 plasmid DNA using primers mCitrine(F) (5′-ccgccaaaacagccaagcttttacttgtacagctcgtccatgc-3′) and mCitrine(R) (5′-ccgcagcaacaggttcagttggtg-3′) and pTrc99a(R) (5′-aaccgcttcggatcagttggtg-3′) and pTrc99a(R) (5′-aaccgcttcggatcagttggtg-3′) and pTrc99a(R)

**Western blot analysis**. Cell extracts from a volume corresponding to 0.1 OD600 units were collected for each strain to be analyzed. The extracts were suspended in loading buffer and resolved by SDS-PAGE gel electrophoresis. Proteins were transferred to nitrocellulose membranes using a semi-dry Transfer-Blot apparatus and resolved by SDS-PAGE gel electrophoresis. Proteins were transferred to nitrocellulose membranes using a semi-dry Transfer-Blot apparatus (Bio-Rad). The membranes were blocked with 5% (w/v) milk and probed with anti FtsZ (Agrisera, Sweden) and detected using standard methods.

**Nanofabrication of micro arrays**. Micron pillars were engineered using two different, but related, approaches. The first approach was used for round and square/ rectangular micron pillars, and was adapted from the refs.14,15. Using a multi-step lithography was used. For this, the micron-scale structures were fabricated on a Si surface using UV lithography, to work as a mask for etching. The photolithographic pattern of e-beam resist was created on a Si surface using e-beam lithography. A DNA fragment containing ftsZ was amplified from E. coli MC4100 genomic DNA using primers FtsZ(F) (5′-caattcagaaacgaacagcttggtcga-3′) and FtsZ(R) (5′-gcggcttcggatcagttggtg-3′) and mCitrine was amplified from mCitrine-N1 plasmid DNA using primers mCitrine(F) (5′-ccgccaaaacagccaagcttttacttgtacagctcgtccatgc-3′) and mCitrine(R) (5′-ccgcagcaacaggttcagttggtg-3′) and pTrc99a(R) (5′-aaccgcttcggatcagttggtg-3′) and pTrc99a(R) (5′-aaccgcttcggatcagttggtg-3′) and pTrc99a(R)

**Table 1 Summary of FtsZ cluster dimensions at midcell in various cell shapes**

| Cell shape | FP | Drugs | Length (nm) | Width (nm) |
|------------|----|-------|-------------|-----------|
| Circle (WT) | mNG | – | 123 ± 44 | 80 ± 2 |
| Circle (large) | mNG | + | 132 ± 48 | 88 ± 9 |
| Square | mNG | + | 105 ± 40 | 80 ± 18 |
| Square | mCitrine | + | 118 ± 41 | 86 ± 22 |
| Heart | mNG | + | 129 ± 44 | 84 ± 9 |
| Pentagon | mNG | + | 131 ± 52 | 74 ± 9 |
| Half-moon | mNG | + | 129 ± 45 | 80 ± 10 |
| Star | mNG | + | 140 ± 67 | 76 ± 16 |
| Triangle | mNG | + | 110 ± 35 | 78 ± 11 |
| Cross | mNG | + | 119 ± 21 | 71 ± 18 |

In all cell shapes, the average measured cluster lengths were within 17% of WT, while average widths were within 13%. Numbers represent mean ± S.D. Note that values have been rounded to the nearest integer.

FP fluorescent protein, mNG mNeonGreen, mCitrine mCitrine
*Drugs: A22 [16 μM] and Cephalxin [20 μM]

**Table 2 Summary of FtsZ dynamics in various cell shapes**

| Cell shape | FP | Drugs | Treadmilling speed (nm s⁻¹) | τ₁/₂ recovery (s) |
|------------|----|-------|---------------------------|------------------|
| Circle (WT) | GFP | – | 26 ± 15 | 8 ± 2 |
| Circle (large) | GFP | + | 30 ± 18 | 8 ± 2 |
| Square | mCitrine | + | 28 ± 13 | 10 ± 3 |
| Square | GFP | + | 25 ± 11 | 9 ± 3 |
| Heart | mCitrine | + | 23 ± 10 | 7 ± 1 |

Numbers represent mean ± S.D. Note that values have been rounded to the nearest integer.

FP fluorescent protein, mCitrine mCitrine
*Drugs: A22 [16 μM] and Cephalxin [20 μM]

**Microscopy**. Gated STED (gSTED) images were acquired on a Leica TCS SP8 STED 3× system, using a HC PL Apo 100× oil immersion objective with NA 1.40. Fluorophores were excited using a white excitation laser operated at 488 nm for mNeonGreen and 509 nm for mCitrine. A STED depletion laser line was operated at 592 nm, using a detection time-delay of 0.8–1.6 ns for both fluorophores. The total depletion laser intensity was in the order of 20–40 MW cm⁻² for all STED imaging. The final pixel size was 13 nm and scanning speed was either 400 or 600 Hz. The pinhole size was set to 0.9 AU.

Epi-fluorescence and confocal images were acquired on either a Zeiss LSM780 or Zeiss ELYRA PS1 (both equipped with a 100× 1.46 NA plan Apo oil immersion objective) with acquisition times between 0.3 and 2 s. Time-lapse series for generating kymographs were recorded at 2 s intervals for a time period of at least 118 s.

SIM images were acquired using a Zeiss ELYRA PS1 equipped with a pcO.edge sCMOS camera. The final pixel size in SIM images was 24 nm. Individual images were acquired using an acquisition time of 200 ms per image (a total of 15 images were acquired per SIM image reconstruction) and subsequently reconstructed from the raw data using ZEN2012 software. Time-lapse movies (containing at least 14 frames) were recorded without time delays between image stacks.

Confocal Z-stacks (focal plane z ± 3.5 μm) were acquired on a Leica TCS SP8 STED 3× system (operated in confocal mode) using predetermined optimal system settings (Leica, LAS X), with 0.22 μm steps (resulting in 30–32 images per stack), and pinhole size 1 AU. All imaging was performed at RT (~23–24°C). In order to...
confirm that the cells were immobile, and that no visible cell movements occurred in the wells, each cell was initially monitored using brightfield and epifluorescence illumination. In this way, we could eliminate the contribution of motion blurring to any observed movements captured during image acquisition.

**FRAP measurements.** Confocal FRAP measurements were performed on a Zeiss LSM780 system using a 100 × 1.4 NA plan apo oil immersion objective and pinhole size 60 μm3. Bleaching was performed for 0.5–0.7 s using 100% laser power applied over the region of interest. Data were collected in time intervals of 1–2 s until steady state was reached. Following background correction, and to account for overall successively bleaching, the fluorescence intensity (F) of the bleached region (half a cell) were normalized to the average ring fluorescence of an unbleached area of the same size, for each time point (t): \( F_{\text{RING}}(t) = \frac{F_{\text{RING}}(t) - F_{\text{RING}}(0)}{F_{\text{RING}}(0)} \). All data were exported to OriginPro and data points were fitted to the single exponential function \( F(t) = F_0 - (F_0 - F_{\text{RING}}(0))e^{-kt} \), where \( F(t) \) is the fluorescence intensity at time t, \( F_{\text{RING}}(0) \) the fluorescence intensity at maximum recovery, \( F_{\text{RING}}(t) \) the fluorescence recovery, and \( k \) is a free parameter. The recovery half-time was then extracted from \( t_{1/2} = \ln \frac{2}{k} \). Importantly, all cells were scanned from top to bottom in order to find the division plane (in which the rings reside).

**Image analysis.** Image analysis was performed using Fiji. When necessary, images were background-corrected using a rolling ball with radius 36. Image stacks were motion-corrected using the plug-in StackReg. Kymographs were generated from motion-corrected image stacks, from which line scans were deconvolved using Huygens Professional deconvolution software (SVI, the Netherlands). FtsZ-ring diameters were extracted from the self-organize into dynamic cytoskeletal patterns. The diffusion coefficient of the FtsZ protein surface through site-directed insertions: discovery of fully functional FtsZ-fluorescent proteins. J. Bacteriol. 199, e00553–16 (2017).

**Supplementary Information**

obtained using line scans (line size 4) over at least

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**Author contributions**

B.S. conceived the study and performed the experiments. A.B. and B.S. designed and engineered the micron pillar arrays. H.C. contributed reagents. B.S. and U.S. analyzed the data. B.S. wrote the manuscript with input from all authors. All authors approved the final version of the manuscript.

**Additional information**

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