The Role and Regulation of FtsZ Dynamics in Bacterial Cytokinesis

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Introduction: Cytokinesis in bacteria is carried out by a large group of proteins collectively known as the divisome[1,2] (Fig. 1). The best-studied member of the divisome is FtsZ, a tubulin homolog that forms a ring structure, termed the Z ring, at the division site[3,4] (Fig. 2a). The Z ring recruits other divisome proteins to the division site and constricts as the cell divides[2,5]. One group of divisome proteins is recruited to the Z ring early in the cell cycle to regulate FtsZ[2,6]. Concurrent with cytokinesis, other divisome proteins synthesize a new cell wall, called a septum, between the daughter cells[1]. I will focus my research on the gram-positive, rod-shaped *Bacillus subtilis*, but much of this same division machinery is conserved across most bacteria and in some archaea, plastids, and mitochondria[7].

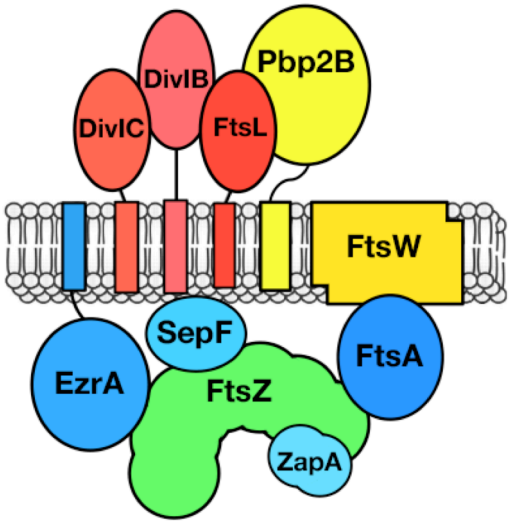

Figure 1: Selected members of the *B. subtilis* divisome. The divisome is a protein complex involved in cytokinesis in bacteria that includes the tubulin homolog FtsZ (green), proteins involved in FtsZ organization (blue), cell wall synthesis proteins (yellow), and structural proteins (red). Adapted from [6].

Although many of the proteins involved in cell division have been identified, the mechanism by which they function together to orchestrate cytokinesis is still unclear. One model suggests that FtsZ filaments in the Z ring change conformation, pulling the membrane inward[3,8]. *In vitro* experiments have shown that FtsZ filaments can generate pulling forces on membranes[9,10], but this has not been demonstrated in live cells. Another model proposes that cell wall synthesis drives cytokinesis, with the septum pushing inward on the membrane as it is made[5]. This model is supported by the fact that the Pbp2B, a divisome-associated cell wall transpeptidase, is essential for division, and by recent work suggesting that Pbp2B-mediated cell wall synthesis is the rate-limiting step in cytokinesis[11,12]. Understanding the molecular mechanism of cytokinesis will require additional study of the behavior of FtsZ in the Z ring, and of the relationship between FtsZ and septal synthesis.

Recent research has suggested that FtsZ filaments, rather than behaving as a static scaffold for other cell division proteins, may be dynamic. FtsZ dynamics have been reported *in vitro* on supported bilayers, where short FtsZ filaments assemble into large motile bundles and highly processive "rotating vortices"[13]. Z rings undergo rapid turnover and dynamic rearrangement *in vivo*[14,15], but previous attempts to image Z ring dynamics in living cells have been limited by either spatial or temporal resolution. Using live cell, high-resolution microscopy techniques, **I will study the role and regulation of FtsZ dynamics in bacterial cytokinesis.** I will ask if FtsZ is dynamic in cells, how FtsZ dynamics are coupled to cell division, and if the cell modulates FtsZ dynamics to regulate cytokinesis.

Preliminary Data:

FtsZ moves directionally *in vivo*. We used total internal reflection fluorescence (TIRF) microscopy to image FtsZ dynamics *in vivo*. We created a functional fusion of FtsZ to mNeonGreen, expressed from the native chromosomal locus as the only copy of *ftsZ* in the cell. Time-lapse TIRF microscopy of mNeonGreen-FtsZ demonstrates that the Z ring is highly dynamic, containing bright foci of FtsZ that move processively (Fig. 2b, i). Directional motion is more evident in patches of labeled FtsZ that are visible outside of Z ring, where it is much easier

to resolve individual foci (Figure 2b, ii). Together, these observations demonstrate that FtsZ polymers move processively both inside and outside of the Z ring.

FtsZ dynamics are driven by treadmilling. After observing that FtsZ filaments move processively, we asked whether these dynamics are the result of treadmilling. FtsZ treadmilling has been reported *in vitro*[13], and there is indirect evidence for FtsZ dynamics *in vivo*[16], but this has not been conclusively observed in live cells. To test this, we expressed mNeonGreen-FtsZ at low levels in cells containing unlabeled WT FtsZ to achieve speckle labeling. If the processivity of FtsZ filaments is caused by translocation, then individual molecules within the filaments should show the same processivity. On the other hand, if dynamics are caused by treadmilling, individual molecules within the filament should not move, with apparent motion of the filament caused by preferential addition of subunits at one end and loss from the other end. This is indeed what we observe: single molecules of FtsZ can be seen to localize to a fixed point on the membrane, remain in place for seconds, and then disappear (Fig. 2b, iii). This is consistent with previous reports of non-moving FtsZ single molecules in the Z ring[17].

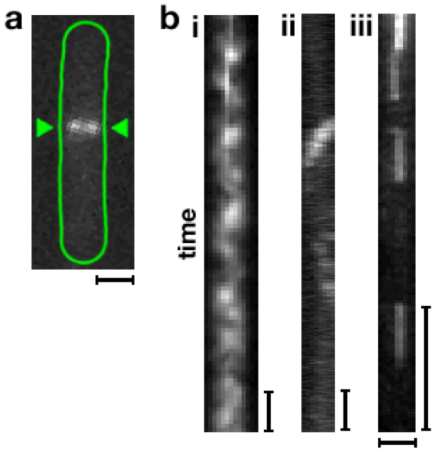

Figure 2: FtsZ treadmilling *in vivo*. a: Epifluorescence image of mNeonGreen-FtsZ. Cell outline indicated in green. Arrows: position of the Z ring. b: Kymographs of: i) FtsZ dynamics in Z rings, ii) FtsZ dynamics outside of Z rings, iii) Single molecule FtsZ, high time resolution. Horizontal axis: position along the short axis of the cell, vertical axis: time. Diagonal lines in the kymograph indicate directional movement. Scale bars: 1 μm (horizontal), 30 s (vertical).

FtsZ dynamics may be involved in cytokinesis. Next, we asked if changes to FtsZ dynamics could have any effect on cytokinesis. To alter FtsZ dynamics, we expressed the FtsZ(D213A) mutant; this mutation is in the conserved T7 synergy loop which is required for GTP hydrolysis[20], and is known in *E. coli* (where it is D212A) to substantially reduce FtsZ GTPase activity[16]. *In vitro*, it has been demonstrated that GTP hydrolysis is not required for FtsZ filament assembly but is necessary for subunit turnover[21], which is needed for treadmilling. When we expressed low levels of FtsZ(D213A) in the presence of WT FtsZ, the rate of FtsZ motion slowed, as expected for decreased treadmilling. Furthermore, expression of this mutant slows the rate of Z ring constriction in a concentration-dependent manner (Fig. 3).

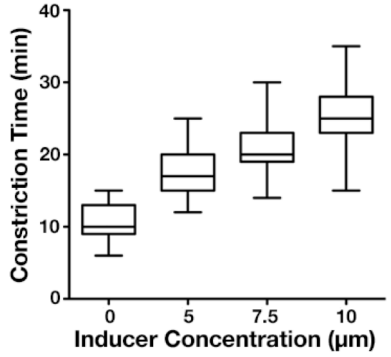

Figure 3: Expression of FtsZ(D213A) mutant slows Z ring constriction. X axis: induction of mutant expression. Y axis: Z ring constriction time for 100 rings. Box: first quartile, median, third quartile; whiskers: minimum and maximum.

To further probe the connection between FtsZ dynamics and cytokinesis, we treated cells with MciZ. MciZ is a peptide capper of FtsZ filaments that is known to increase the dynamics of FtsZ filaments *in vitro*, likely by increasing the rate of FtsZ subunit turnover in filaments[18,19]. Normally, our cell populations divide asynchronously, but following induction of MciZ, we made a striking observation: almost all of the labeled Z rings in the field of view constricted simultaneously. Labeling with membrane stain showed that these MciZ-induced Z ring constrictions cause cytokinesis: during MciZ-induced Z ring constriction, the membrane also constricts. Together, these experiments suggest that FtsZ dynamics may play a role in the rate and timing of cytokinesis.

Specific Aims: Together, these preliminary results demonstrate that Z rings are dynamic *in vivo*, that these dynamics are caused by FtsZ treadmilling, and that changes in these dynamics can alter the rate of cytokinesis. However, many questions remain about the role of FtsZ dynamics in cell division. Are FtsZ dynamics coupled to processes that are required for cytokinesis, most notably septal synthesis? Do FtsZ treadmilling dynamics change over the course of the cell cycle, particularly during Z ring constriction? Are FtsZ dynamics actively regulated throughout the cell cycle as a means to regulate cytokinesis? To characterize the role and regulation of FtsZ dynamics in cytokinesis, I will: 1) elucidate the coupling between FtsZ dynamics and septal synthesis, 2) measure changes in FtsZ treadmilling behavior throughout the cell cycle, and 3) examine the control of FtsZ dynamics by known FtsZ regulators.

Aim 1: Elucidate the coupling between FtsZ dynamics and septal synthesis. Although our preliminary data suggests that FtsZ dynamics may be involved in cytokinesis, the mechanism for this involvement is unclear. One possibility is that FtsZ dynamics could be coupled to septal synthesis by organizing the activity of Pbp2B, an essential transpeptidase for septal synthesis[11,6]. We have begun testing this hypothesis by studying the dynamics of Pbp2B.

Pbp2B moves directionally at the single molecule level. We first asked whether Pbp2B moved directionally with FtsZ, since they are both in the divisome complex[6]. TIRF images of a mNeonGreen-Pbp2B fusion showed dynamic foci of Pbp2B at the septum, similar to the dynamics seen for FtsZ. We next imaged the behavior of individual Pbp2B molecules. For this experiment, we created a fusion of Pbp2B to HaloTag, a protein tag that binds covalently to chloroalkane ligands *in vivo*[22]. Labeling with a low concentration of an organic dye conjugated to this ligand thus allows for single molecule labeling. Since Pbp2B is thought to be in a complex with FtsZ, one might expect Pbp2B to treadmill along with FtsZ, in which case individual Pbp2B molecules should not move. However, this is not what we observe. The majority of labeled Pbp2B molecules are diffusive on the membrane, but there is a subpopulation of Pbp2B that moves processively (Fig. 4a). These molecules move along the short axis of the cell with an apparent velocity of 36 ± 8 nm/s, much like FtsZ, which has an apparent velocity of 33 ± 8 nm/s (Fig. 4b).

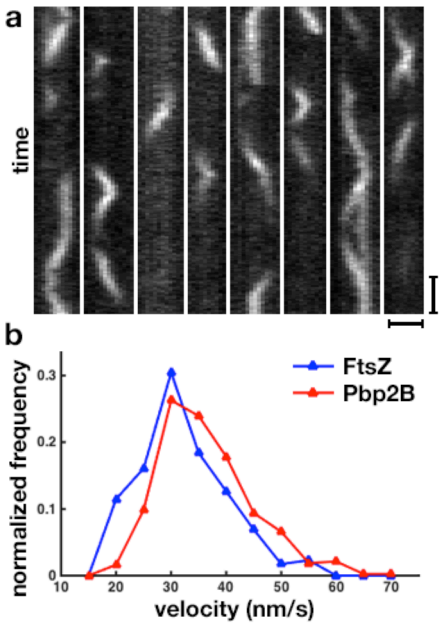

Figure 4: Directional motion of Pbp2B. a: Kymographs of single molecule Pbp2B at the Z ring, made as described in Fig. 2. Scale bar: 1 μm (horizontal), 30 s (vertical). b: Normalized histograms of the velocities of FtsZ (blue, n=174), and Pbp2B (red, n=426).

Pbp2B dynamics require FtsZ. To test if Pbp2B dynamics require FtsZ, we constructed a strain containing both mNeonGreen-FtsZ and HaloTag-Pbp2B, enabling us to image FtsZ and single molecules of Pbp2B in the same cells. All directionally moving Pbp2B molecules are localized to Z rings, suggesting that an interaction between Pbp2B and other divisome proteins is required for processive motion of Pbp2B. To further test the link between FtsZ and Pbp2B, we treated cells with PC190723 (PC19), a small molecule inhibitor of cell division[23]. PC19 stabilizes FtsZ filaments and induces their bundling *in vitro*; *in vivo*, PC19 causes FtsZ to partially delocalize from the Z ring into inactive aggregates[23,24]. Following treatment with PC19, Pbp2B molecules no longer move, and instead persist in the same location for seconds.

These experiments demonstrate that the processivity of Pbp2B depends on FtsZ, but it is unclear how, since individual molecules of FtsZ and Pbp2B do not move together. I propose two possible models for how Pbp2B dynamics could depend on FtsZ. In the first model, Pbp2B processivity is driven by its enzymatic activity, as has been shown for transpeptidases involved in cell elongation[25]. The Z ring could be required to activate Pbp2B's enzymatic activity or to constrain Pbp2B motion to one dimension. However, this model does not necessarily predict that the rates of motion of FtsZ and Pbp2B should be the same, or that Pbp2B molecules should entirely stop moving following treatment with PC19, since they are still colocalized with FtsZ. The second model proposes that Pbp2B dynamics are driven by FtsZ treadmilling. In this model, Pbp2B travels together with a treadmilling FtsZ filament.

To determine whether the enzymatic activity of Pbp2B is required for its motion, as proposed by model 1, I will treat cells with penicillin G, an antibiotic that specifically inhibits the activity of transpeptidases involved in cell wall synthesis[26]. We have shown that treatment with penicillin, along with other antibiotics that inhibit other aspects of cell wall synthesis, does not alter FtsZ dynamics. Thus, if penicillin treatment abolishes Pbp2B dynamics, it is clear that these dynamics require enzymatic activity, whereas if Pbp2B continues to move, it is unlikely that enzymatic activity is required for processivity. To confirm this result, I will create an enzymatically inactive mutant of Pbp2B by amino acid substitution of its catalytic serine, as has been done with other PBPs[27]. I will use speckle labeling to image the single molecule dynamics of this mutant, and confirm that they are consistent with the dynamics of WT Pbp2B after penicillin G treatment.

To determine whether Pbp2B processivity requires FtsZ treadmilling (model 2), I will express the FtsZ(D213A) mutant, which decreases FtsZ dynamics. I will image single Pbp2B molecules under these conditions and measure their rates of motion. If Pbp2B dynamics depend on FtsZ treadmilling, Pbp2B will slow down when the mutant FtsZ is expressed, matching the slower rate of FtsZ treadmilling. I will image FtsZ dynamics and Pbp2B dynamics simultaneously in these cells, allowing me to compare their rates directly. Model 2 makes the strong prediction that the rates of FtsZ treadmilling and Pbp2B motion should always match, so testing this prediction with the FtsZ mutant is a clear way to verify or invalidate this model.

Aim 2: Measure changes in FtsZ treadmilling behavior throughout the cell cycle. One longstanding question about cytokinesis in bacteria is the molecular mechanism underlying Z ring constriction. While our preliminary results demonstrate that experimentally induced changes in FtsZ dynamics can change cytokinesis, it is unclear whether a similar process occurs during normal cell division. In particular, given the striking observation that an increase in FtsZ dynamics (induced by MciZ) can trigger cytokinesis, it is interesting to ask whether normal cytokinesis is accompanied by a similar increase in dynamics.

Thus far, we have used a native mNeonGreen-FtsZ fusion to image Z ring dynamics. This construct has allowed us to detect FtsZ dynamics in moving foci of FtsZ, and in Z rings early in the cell cycle (Fig. 2). As the cell cycle proceeds, however, the Z ring condenses and constricts, becoming too small and densely labeled to resolve FtsZ motion. To resolve treadmilling dynamics of FtsZ filaments throughout the cell cycle, I will use speckle labeling of FtsZ to measure the residence time of FtsZ molecules in the Z ring. In our preliminary data, we observe FtsZ molecules that appear in one spot and persist for seconds before disappearing; I will measure the duration of these events across the cell cycle. I will use the localization of mNeonGreen-Pbp2B to resolve the cell cycle into three stages: 1) initial assembly of the Z ring

(Pbp2B will be delocalized from the Z ring), 2) recruitment of the divisome (Pbp2B will localize to the division site), and 3) cytokinesis (Pbp2B will appear to constrict along with the Z ring)[6].

This experiment relies on the claim that the immobile FtsZ molecules seen in speckle labeling are stationary molecules in treadmilling filaments, as we and others have thought[17]. To confirm this, I will express FtsZ(D213A) and measure residence time. Because FtsZ dynamics are known to depend on GTP hydrolysis[21], I expect residence time to increase under these conditions. I will also need a control to confirm that my measurements are not artifacted by photobleaching. The effects of photobleaching are strongly dependent on laser power and on the total amount of illumination during the experiment[28,29], so I will repeat my experiments at a range of laser powers and illumination times, and verify that the residence time distribution does not significantly change in the imaging regime I am using.

Aim 3: Examine the control of FtsZ dynamics by known FtsZ regulators. We have shown that changes in FtsZ dynamics can alter cytokinesis rate; it is therefore interesting to ask whether the cell actively regulates FtsZ dynamics. In the simplest case, such regulation could be necessary to maintain an appropriately dynamic Z ring. Alternately, the cell could actively change FtsZ dynamics based on cell cycle state, as discussed in Aim 2. Because FtsZ treadmills, the FtsZ dynamics we observe are interrelated with FtsZ GTPase activity, subunit turnover, filament length, and bundling. Each of these is thought to be modulated by FtsZ regulators, so known FtsZ regulators are likely to have impacts on FtsZ dynamics[2,14].

Multiple divisome-associated proteins are involved in FtsZ regulation, including SepF, EzrA, and ZapA[2,14]. ZapA promotes FtsZ filament assembly and stability[30], SepF is thought to be required for normal Z ring structure and septum morphology[31], and EzrA was originally characterized as a negative regulator of FtsZ but may have other functions[14,32]. None of these proteins is essential, but pairs of deletions of these genes (*zapA* and *ezrA*, *sepF* and *ezrA*) are synthetically lethal, illustrating the importance of these FtsZ regulators in cell division[30,31].

I propose to study the roles of these proteins in regulating FtsZ dynamics and cytokinesis. I will express each protein under the control of an inducible promoter, and measure the effect of protein level on FtsZ dynamics. Initially, I can measure changes in FtsZ dynamics using the mNeonGreen-FtsZ total labeling construct, as in our preliminary data (Fig. 2). If I successfully establish single molecule residence time measurements in Aim 2, I can use speckle labeling to detect the effects of SepF, ZapA, and EzrA on FtsZ dynamics across the cell cycle.

I will also measure the effects of these regulators on cytokinesis. Using mNeonGreen-FtsZ, I will measure the rate of Z ring constriction at various expression levels of each protein. Null mutants of *sepF* and *ezrA* both give rise to elongated cells, so I expect that both of these regulators will have some effect on cell division[31,32]. *zapA* null cells have normal morphology, so it will be interesting to see whether FtsZ dynamics are affected by *zapA* deletion and, if so, how cells lacking ZapA are still able to divide normally[30]. Together, these experiments will illustrate how cells may regulate FtsZ dynamics throughout the cell cycle, and whether this regulation is necessary for cytokinesis.

Discussion: In this proposal, FtsZ dynamics form the basis for new studies on the mechanism and regulation of cytokinesis. Measurements of FtsZ dynamics across the cell cycle will yield new insights into the behavior of FtsZ during cytokinesis, the control of this behavior by FtsZ regulators, and the relationship between FtsZ and septal synthesis. Together, these experiments represent a new look at longstanding questions in bacterial cell division.

References:

1. Errington, J., Daniel, R. A., & Scheffers, D. J. (2003). Cytokinesis in Bacteria. *Microbiology and Molecular Biology Reviews*, *67*(1), 52–65.
2. Adams, D. W., & Errington, J. (2009). Bacterial cell division: assembly, maintenance and disassembly of the Z ring. *Nature Publishing Group*, *7*(9), 642–653.
3. Erickson, H. P., Anderson, D. E., & Osawa, M. (2010). FtsZ in bacterial cytokinesis: cytoskeleton and force generator all in one. *Microbiology and Molecular Biology Reviews : MMBR*, *74*(4), 504–528.
4. Bi, E. F., & Lutkenhaus, J. (1991). FtsZ ring structure associated with division in Escherichia coli. *Nature*, *354*(6349), 161–164.
5. Meier, E. L., & Goley, E. D. (2014). Form and function of the bacterial cytokinetic ring. *Current Opinion in Cell Biology*, *26*, 19–27.
6. Gamba, P., Veening, J.-W., Saunders, N. J., Hamoen, L. W., & Daniel, R. A. (2009). Two-step assembly dynamics of the Bacillus subtilis divisome. *Journal of Bacteriology*, *191*(13), 4186–4194.
7. Margolin, W. (2005). FtsZ and the division of prokaryotic cells and organelles. *Nature Reviews Molecular Cell Biology*, *6*(11), 862–871.
8. Erickson, H. P. (2009). Modeling the physics of FtsZ assembly and force generation. *Proceedings of the National Academy of Sciences*, *106*(23), 9238–9243.
9. Osawa, M., Anderson, D. E., & Erickson, H. P. (2008). Reconstitution of contractile FtsZ rings in liposomes. *Science*, *320*(5877), 792–794.
10. Osawa, M., & Erickson, H. P. (2011). Inside-out Z rings - constriction with and without GTP hydrolysis. *Molecular Microbiology*, *81*(2), 571–579.
11. Daniel, R. A., Harry, E. J., & Errington, J. (2000). Role of penicillin-binding protein PBP 2B in assembly and functioning of the division machinery of Bacillus subtilis. *Molecular Microbiology*, *35*(2), 299–311.
12. Coltharp, C., Buss, J., Plumer, T. M., & Xiao, J. (2016). Defining the rate-limiting processes of bacterial cytokinesis. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(8), E1044–53.
13. Loose, M., & Mitchison, T. J. (2014). The bacterial cell division proteins FtsA and FtsZ self-organize into dynamic cytoskeletal patterns. *Nature Cell Biology*, *16*(1), 38–46.
14. Anderson, D. E., Gueiros-Filho, F. J., & Erickson, H. P. (2004). Assembly Dynamics of FtsZ Rings in Bacillus subtilis and Escherichia coli and Effects of FtsZ-Regulating Proteins. *Journal of Bacteriology*, *186*(17), 5775–5781.
15. Strauss, M. P., Liew, A. T. F., Turnbull, L., Whitchurch, C. B., Monahan, L. G., & Harry, E. J. (2012). 3D-SIM super resolution microscopy reveals a bead-like arrangement for FtsZ and the division machinery: implications for triggering cytokinesis. *PLoS Biology*, *10*(9), e1001389.
16. Redick, S. D., Stricker, J., Briscoe, G., & Erickson, H. P. (2005). Mutants of FtsZ targeting the protofilament interface: effects on cell division and GTPase activity. *Journal of Bacteriology*, *187*(8), 2727–2736.
17. Niu, L., & Yu, J. (2008). Investigating intracellular dynamics of FtsZ cytoskeleton with photoactivation single-molecule tracking. *Biophysical Journal*, *95*(4), 2009–2016.
18. Handler, A. A., Lim, J. E., & Losick, R. (2008). Peptide inhibitor of cytokinesis during sporulation in Bacillus subtilis. *Molecular Microbiology*, *68*(3), 588–599.
19. Bisson-Filho, A. W., Discola, K. F., Castellen, P., Blasios, V., Martins, A., Sforça, M. L., et al. (2015). FtsZ filament capping by MciZ, a developmental regulator of bacterial division. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(17), E2130–8.
20. Scheffers, D.-J., de Wit, J. G., den Blaauwen, T., & Driessen, A. J. M. (2002). GTP hydrolysis of cell division protein FtsZ: evidence that the active site is formed by the association of monomers. *Biochemistry*, *41*(2), 521–529.
21. Mukherjee, A., & Lutkenhaus, J. (1998). Dynamic assembly of FtsZ regulated by GTP hydrolysis. *The EMBO Journal*, *17*(2), 462–469.
22. Los, G. V., Encell, L. P., McDougall, M. G., Hartzell, D. D., Karassina, N., Zimprich, C., et al. (2008). HaloTag: a novel protein labeling technology for cell imaging and protein analysis. *ACS Chemical Biology*, *3*(6), 373–382.

23. Haydon, D. J., Stokes, N. R., Ure, R., Galbraith, G., Bennett, J. M., Brown, D. R., et al. (2008). An inhibitor of FtsZ with potent and selective anti-staphylococcal activity. *Science*, *321*(5896), 1673–1675.
24. Andreu, J. M., Schaffner-Barbero, C., Huecas, S., Alonso, D., Lopez-Rodriguez, M. L., Ruiz-Avila, L. B., et al. (2010). The antibacterial cell division inhibitor PC190723 is an FtsZ polymer-stabilizing agent that induces filament assembly and condensation. *The Journal of Biological Chemistry*, *285*(19), 14239–14246.
25. Garner, E. C., Bernard, R., Wang, W., Zhuang, X., Rudner, D. Z., & Mitchison, T. (2011). Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in B. subtilis. *Science*, *333*(6039), 222–225.
26. Ghuysen, J. M. (1991). Serine beta-lactamases and penicillin-binding proteins. *Annual Review of Microbiology*, *45*(1), 37–67.
27. Broome-Smith, J. K., Hedge, P. J., & Spratt, B. G. (1985). Production of thiol-penicillin-binding protein 3 of Escherichia coli using a two primer method of site-directed mutagenesis. *The EMBO Journal*, *4*(1), 231–235.
28. Patterson, G. H., & Piston, D. W. (2000). Photobleaching in two-photon excitation microscopy. *Biophysj*, *78*(4), 2159–2162.
29. Kim, S. Y., Gitai, Z., Kinkhabwala, A., Shapiro, L., & Moerner, W. E. (2006). Single molecules of the bacterial actin MreB undergo directed treadmilling motion in Caulobacter crescentus. *Proceedings of the National Academy of Sciences*, *103*(29), 10929–10934.
30. Gueiros-Filho, F. J., & Losick, R. (2002). A widely conserved bacterial cell division protein that promotes assembly of the tubulin-like protein FtsZ. *Genes & Development*, *16*(19), 2544–2556.
31. Hamoen, L. W., Meile, J.-C., de Jong, W., Noirot, P., & Errington, J. (2006). SepF, a novel FtsZ-interacting protein required for a late step in cell division. *Molecular Microbiology*, *59*(3), 989–999.
32. Levin, P. A., Kurtser, I. G., & Grossman, A. D. (1999). Identification and characterization of a negative regulator of FtsZ ring formation in Bacillus subtilis. *Proceedings of the National Academy of Sciences*, *96*(17), 9642–9647.