Mutation of the Arg191 in FtsZ impairs cytokinetic abscission of *Bacillus subtilis* cells

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Supporting Figures

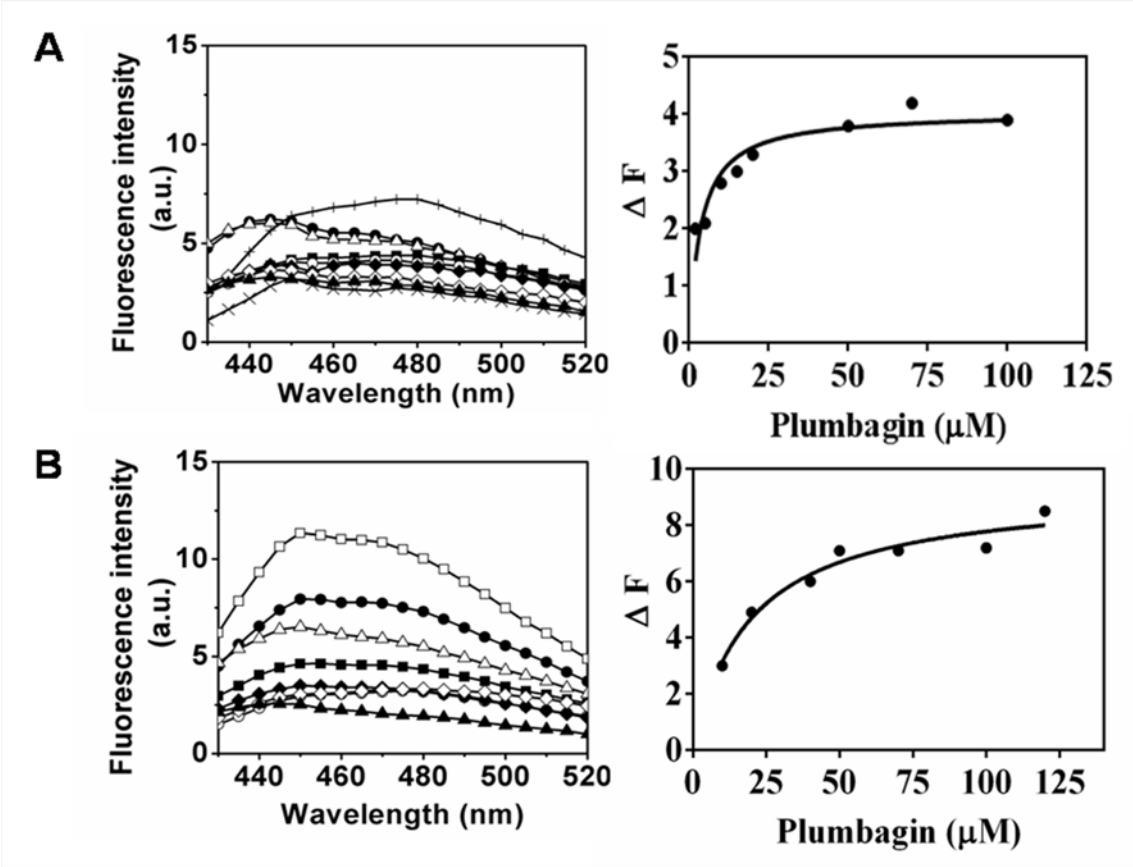

Fig. S1. Effects of plumbagin on the fluorescence intensity of ANS in the presence of WT-FtsZ or with R191A-FtsZ. (A) WT-FtsZ (2 μM) (+) was incubated with 2 (●), 5 (△), 10 (■), 15 (○), 20 (◆), 50 (◇), 70 (▲), and 100 μM (×) plumbagin at 25 ºC for 10 min. (B) R191A-FtsZ (2 μM) (□) was incubated with 10 (●), 20 (△), 40 (■), 50 (○), 70 (◆), 100 (◇) and120 μM (▲) plumbagin at 25 ºC for 10 min. Then, ANS (30 μM) was added in the samples and incubated for 30 min at 25 ºC. The fluorescence spectra were recorded in the range of 430-520 nm using 350 nm as an excitation wavelength. The fluorescence spectra of plumbagin (0-120 μM) was also recorded as a blank. The change in fluorescence at 475 nm was calculated by subtracting blank from the respective data sets. A dissociation constant of the binding interaction of plumbagin with WT-FtsZ and with R191A-FtsZ was determined from the fluorescence change data as described previously[1].

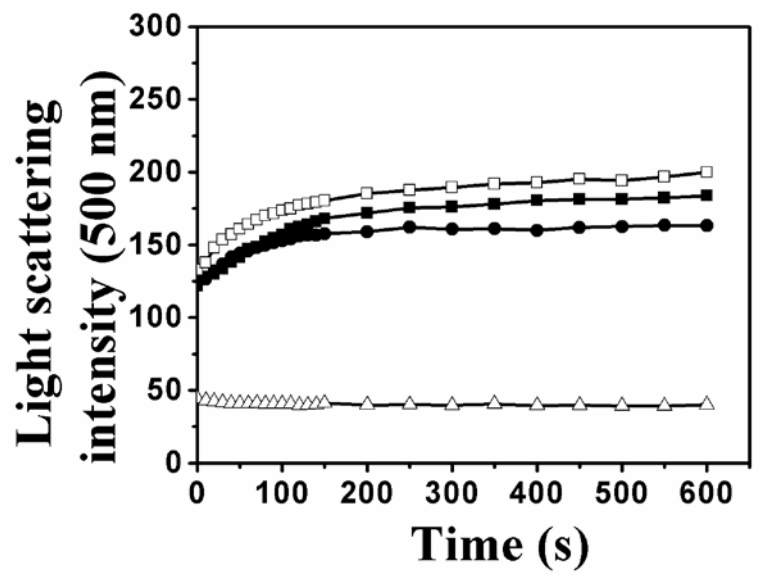

Fig. S2. The effect of plumbagin on the assembly kinetics of R191A-FtsZ. R191A-FtsZ (12 µM) was incubated without (□) or with 20 (■) and 40 µM (●) plumbagin for 15 min on ice and then, the assembly kinetics was monitored by adding 1 mM GTP at 37 ºC. The light scattering intensity of buffer (△) [25 mM PIPES (pH 6.8), 50 mM KCl and 10 mM $MgCl_2$] was also monitored as a blank.

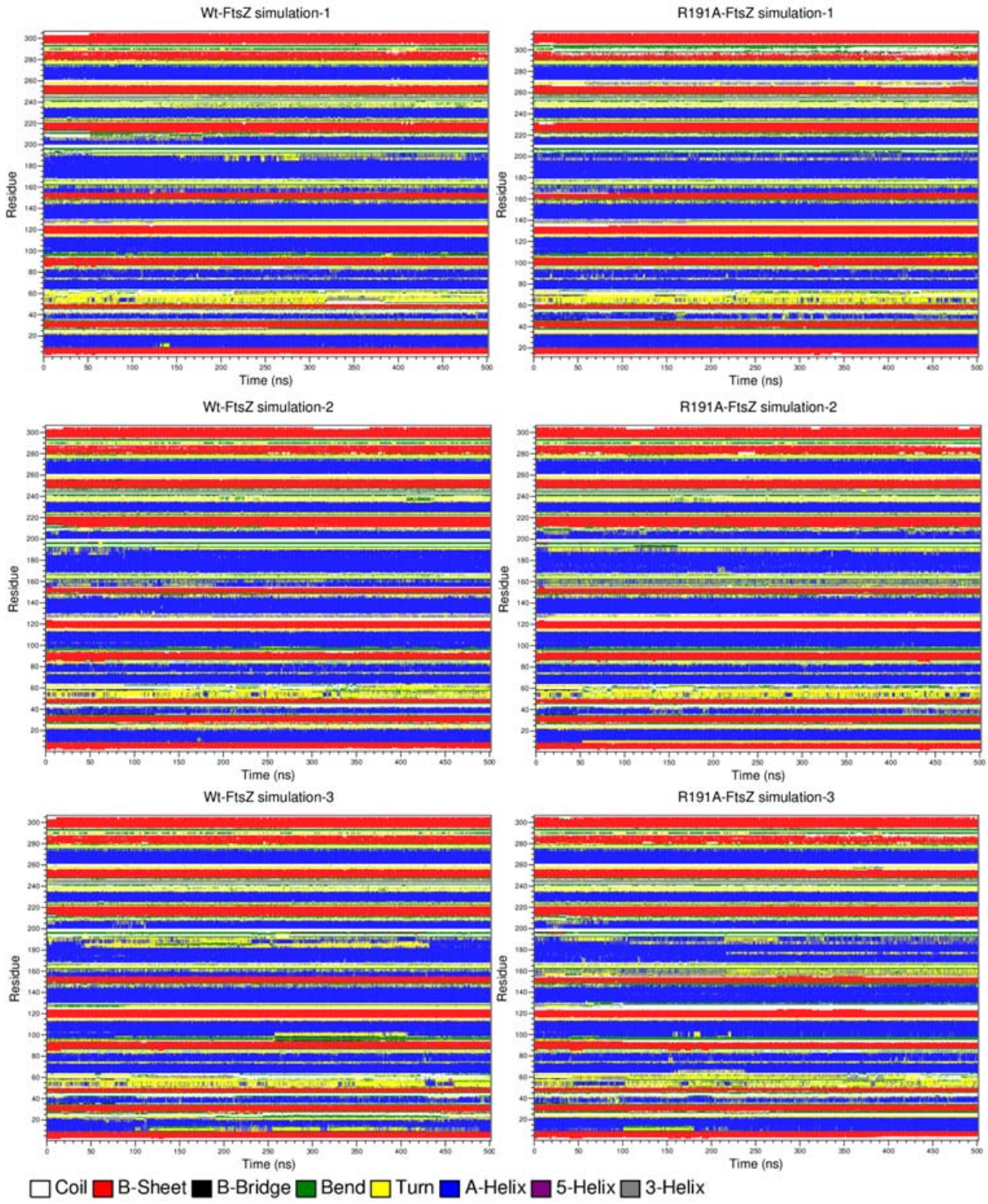

Fig. S3: Secondary structures of WT-FtsZ and R191A-FtsZ. Molecular dynamics simulations of WT-FtsZ and R191A-FtsZ generated 20,000 structures in one trajectory. For each structure, secondary structure was calculated using DSSP tool from GROMACS package. On the y-axis secondary structure of each residue is shown and x-axis shows how the secondary structure of each residue evolved during the course of the simulation. H5-helix: residues 179-203 and T7-loop: residues 204 to 210.

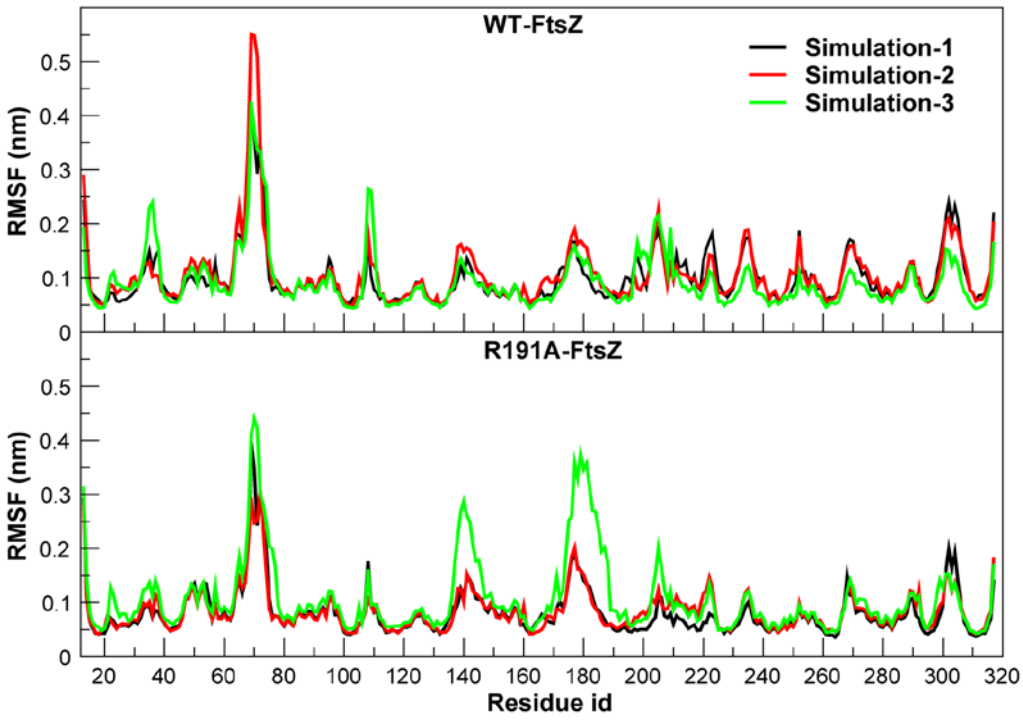

Fig. S4: Root mean square fluctuation analysis of WT-FtsZ and R191A-FtsZ simulations. Root mean square fluctuation analysis was performed to identify regions of structural change**.** Three simulations each for the WT-FtsZ and R191A-FtsZ that were performed are shown in black, red, and green.

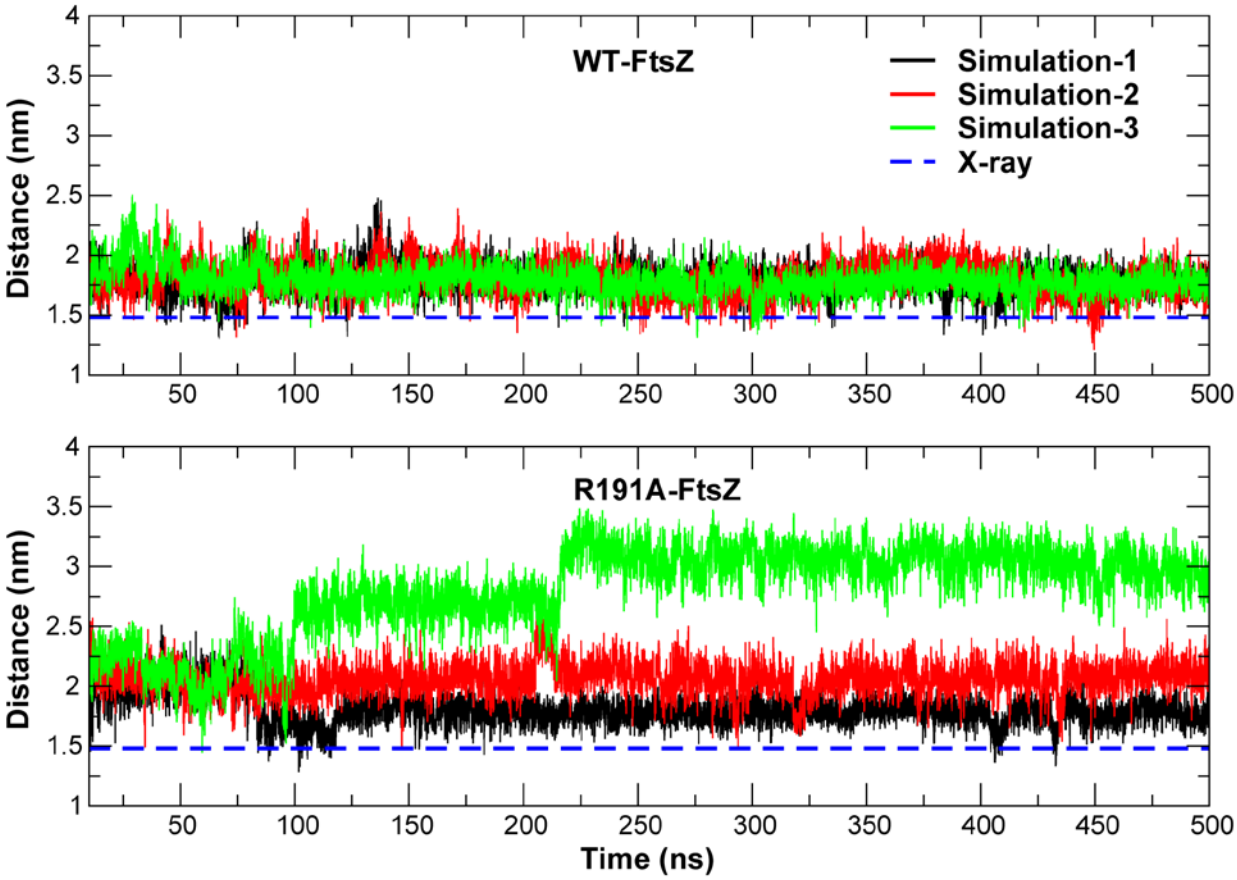

Fig. S5: Distance plot between the residue F138 and N176 C-alpha atoms in WT-FtsZ and R191A-FtsZ simulations**.**

Table S1. The role of different domains of FtsZ.

Domian or residues of FtsZ	**Function**
1. N-terminal domain and C-terminal domain of *T. maritima* FtsZ	1. Both domains can fold independently into functional tertiary structure.[2]
2. Poorly conserved last 6 residues (NRNKRG) of *B. subtilis* FtsZ	2. These residues are essentially required to promote the high degree of lateral interactions between FtsZ polymers. The change in this region of FtsZ produces significant defect in cell division *in vivo*.[3]
3. a) N1-FtsZ (1−178 residues) and N2-FtsZ (1−204 residues) of *B. subtilis* FtsZ b) C1-FtsZ (205−366 residues), C2-FtsZ (176-366 residues) and C3-FtsZ (176-382 residues) of *B. subtilis* FtsZ	3. a) Both N-domains have ability to polymerize and form filamentous polymers independently.[4] b) These C-domains cannot form polymers and also inhibited the Polymerization of FL-FtsZ.[4]
4) Mutations of Asn207, Asp209, and Asp212 in the T7 loop of EcFtsZ	4) Severely affected GTP hydrolysis.[5]
5) H7 helix of *Methanococcus jannaschi* FtsZ	5) H7-helix maintains communication between N-and C-terminal domain, and bending of it can regulate assembly/disassembly of FtsZ.[6]

Table S2. Comparison of root mean square deviation of backbone atoms of entire protein and only helices H4 and H7.

	Simulation-1		Simulation-2		Simulation-3	
	Entire protein (nm)	H5 & H7(nm)	Entire protein(nm)	H5 & H7(nm)	Entire protein(nm)	H5 & H7(nm)
WT-FtsZ	0.16 ± 0.02	0.19 ± 0.02	0.19 ± 0.04	0.2 ± 0.03	0.17 ± 0.02	0.18 ± 0.03
R191A-FtsZ	0.17 ± 0.02	0.2 ± 0.03	0.17 ± 0.02	0.19 ± 0.02	0.19 ± 0.02	0.27 ± 0.02

Average ± Standard deviation

Movie S1: The MD simulation of R191A-FtsZ and WT-FtsZ.

Movie is attached as FtsZ_HelixTilt.vlc file.

References:

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