

1 **Supplementary Information:**

2 **Probing bacterial cell wall growth by tracing wall-anchored protein complexes**

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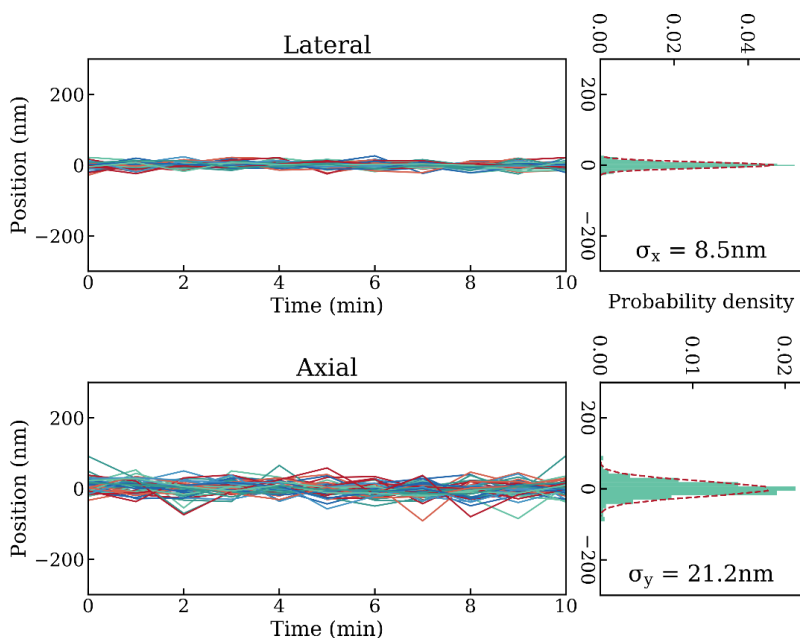
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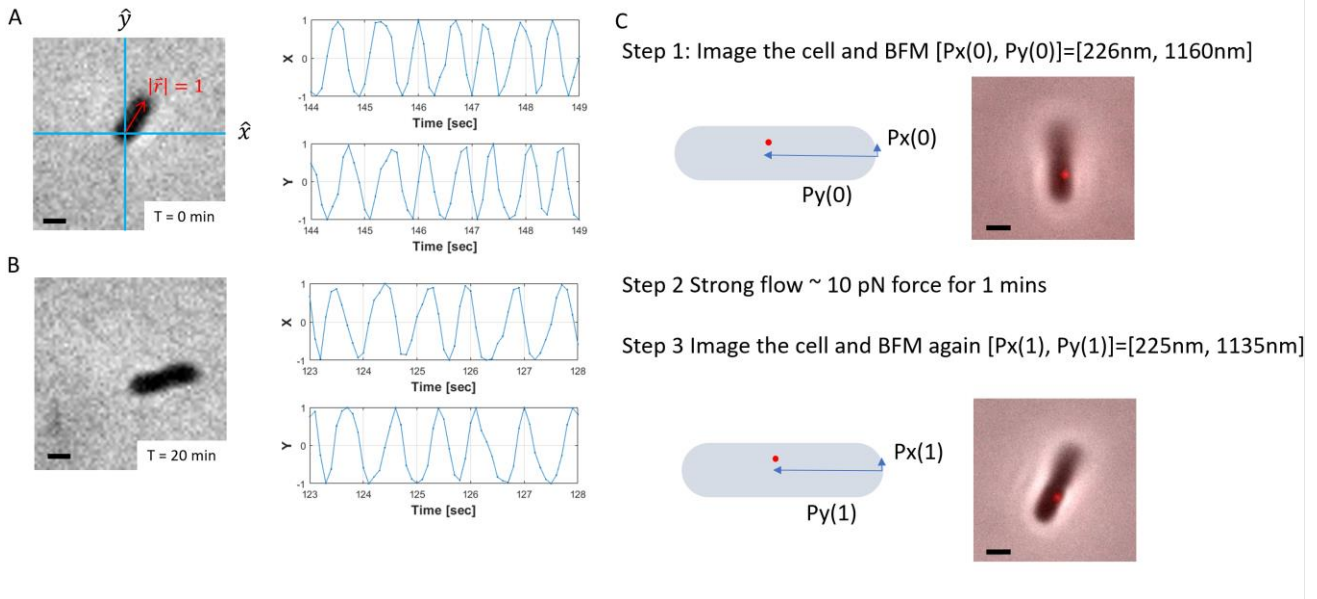
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11 work

12 **Supplementary Figures**



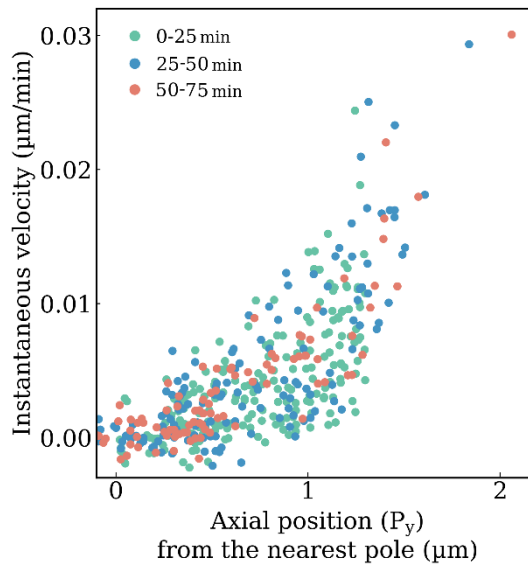
14 Supplementary Fig.1. Stability of the BFM anchoring position in live non-growing cells. BFM  
15 positional traces over 10 min in the buffer solutions (n = 48). The standard deviation of the lateral  
16 and axial coordinates was 8.5 nm and 21.2 nm, respectively.



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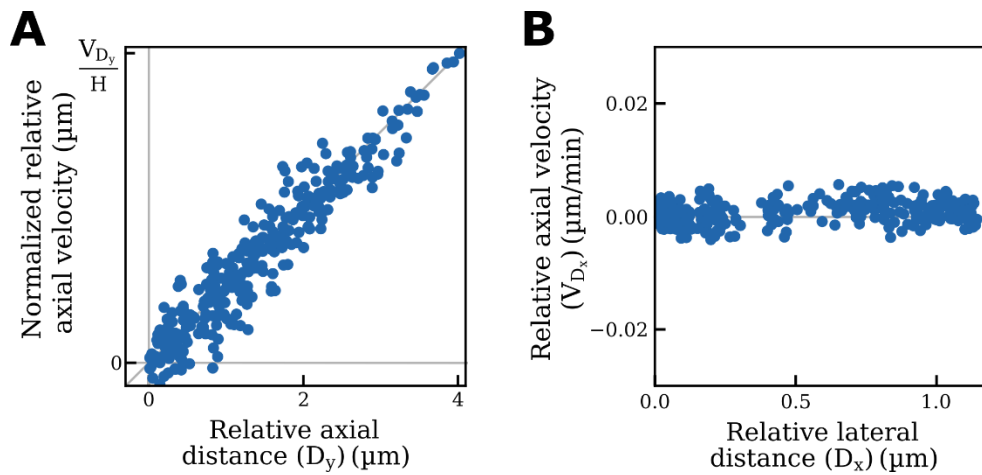
18 Supplementary Fig. 2. Additional experimental results to support that BFMs are firmly anchored to  
 19 the cell wall during cell elongation. (A) A tethered cell experiment showing stable rotation of *E. coli*  
 20 cell body in growth medium. (Scale bar, 1  $\mu\text{m}$ ) (B) The same cell as in (A) showing stable rotation of  
 21 cell body after 20 min in growth medium while the cell elongates. (Scale bar, 1  $\mu\text{m}$ ) (C) A tethered  
 22 cell rotated by a BFM and the hook of the BFM was fluorescently labelled. The coordinate of the BFM  
 23 from the cell pole was [226 nm, 1160 nm]. After a strong hydrodynamic flow, the BFM position from  
 24 the cell pole was [225 nm, 1135 nm]. (Scale bar, 1  $\mu\text{m}$ ).

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27 Supplementary Fig. 3 The relationship between instantaneous velocity of BFM movement during cell  
 28 growth. The measurement time interval is 25 min. Green dots: 0-25 min; blue dots: 25-50 min; red  
 29 dots: 50-75 min.



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31 Supplementary Fig. 4. (A) Normalized BFM relative axial velocity vs. relative axial distance has a  
 32 linear relationship,  $n = 63$  (number of datasets). (B) All BFM lateral velocities from 62 datasets  
 33 showed zero growth rate.

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Strain	MTB9	<i>J. Bacteriol.</i> <b>194</b> , 3495–3501 (2012)	SiteC_flgE
Plasmid	pWR20	<i>PLoS One.</i> <b>7</b> , 1–10 (2012) <i>Biophys. J.</i> <b>117</b> (2019).	Reference for plasmid pWR20
Primer	FlgE_seq	5' GGTGTGATCCGCGGCAACAG 3' 5' CCTGTTGATTCAGTGTCTGG 3'	FlgE primers dedicated for sequencing
Primer	pWR20 backbone	5' AAAGCGGCCGCGGTGATTGATTGAGCAAG 3' 5' AAACCTAGGATGTATATCTCCTTAAGTAGGT 3'	

37 Supplementary Table 1. Strain, plasmid and primers information