

1 **Treadmilling FtsZ polymers drive the directional movement of sPG-**
2 **synthesis enzymes *via* a Brownian ratchet mechanism**

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13

14 **Abstract**

15

16 FtsZ, a highly conserved bacterial tubulin GTPase homolog, is a central component of
17 the cell division machinery in nearly all walled bacteria. FtsZ polymerizes at the future
18 division site and recruits > 30 proteins that assemble into a macromolecular complex
19 termed divisome. Many of these divisome proteins are involved in septal cell wall
20 peptidoglycan (sPG) synthesis. Recent studies found that FtsZ polymers undergo GTP
21 hydrolysis-coupled treadmilling dynamics along the septum circumference of dividing
22 cells, which drives processive movements of sPG synthesis enzymes. The mechanism
23 of FtsZ treadmilling-driven directional transport of sPG enzymes and its precise role in
24 bacterial cell division are unknown. Combining theoretical modeling and experimental
25 testing, we show that FtsZ treadmilling drives the directional movement of sPG-
26 synthesis enzymes *via* a Brownian ratchet mechanism, where the shrinking end of FtsZ
27 polymers introduces an asymmetry to rectify diffusions of single enzyme molecules into
28 persistent end-tracking movement. Furthermore, we show that processivity of this
29 directional movement hinges on the balance between the enzyme's diffusion and FtsZ's
30 treadmilling speed, which provides a mechanism to control the level of available
31 enzymes for active sPG synthesis, explaining the distinct roles of FtsZ treadmilling in
32 modulating cell wall constriction rate observed in different bacterial species.

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36 During cell wall constriction in most gram negative bacteria, new septal
37 peptidoglycan (sPG) synthesis and old cell wall degradation occur simultaneously¹. A
38 large number of cell wall enzymes and their regulators involved in this process has been
39 identified. However, it remains unclear how these proteins are orchestrated in time and
40 space to achieve successful cytokinesis and at the same time maintain the structural
41 integrity of the septal cell wall^{2,3}. Perturbations of PG remodeling at septum compromise
42 cell division and often lead to cell lysis⁴.

43

44 Recent studies have indicated that FtsZ, an essential component of the bacterial
45 cell division machinery, may play a central part in regulating the spatiotemporal
46 coordination of sPG synthesis enzymes. FtsZ is a highly conserved bacterial tubulin
47 homologue and GTPase⁵⁻⁷. In *E. coli* during cell division, FtsZ polymerizes at the
48 cytoplasmic face of the inner membrane to form a ring-like structure (Z-ring) at mid-cell⁸⁻
49 ¹⁰. The Z-ring then locally recruits an ensemble of more than 30 proteins, many of which
50 are sPG-remodeling enzymes^{1,11}, to initiate septal cell wall constriction. New studies
51 employing super-resolution and single-molecule imaging *in vitro* and *in vivo* have
52 demonstrated that the FtsZ polymers exhibit GTP hydrolysis-driven treadmilling
53 dynamics, which are the continuous polymerization at one end and depolymerization at
54 the other end, with individual FtsZ monomers remaining stationary in the middle^{12,13}.
55 Most interestingly, it was found that FtsZ's treadmilling dynamics drives processive
56 movements of the essential sPG transpeptidase (TPase, FtsI in *E. coli* and PBP2B in *B.*
57 *subtilis*)^{12,13} and glycosyltransferase FtsW¹⁴. Consequently, it was proposed that the

58 FtsZ's treadmilling dynamics spatially and temporally distribute sPG synthesis enzymes
59 along the septum plane to ensure smooth septum morphogenesis¹³. However, it is
60 unknown how FtsZ's treadmilling dynamics with stationary monomers in the cytoplasm
61 are transduced into the periplasm to drive the persistent and directional movement of
62 cell wall synthesis enzymes. The role of FtsZ's treadmilling dynamics in modulating sPG
63 synthesis activity also remains elusive, as it was shown that the cell wall constriction
64 rate is dependent on FtsZ's treadmilling speed in *B. subtilis*¹² but not in *E. coli*¹³, *S.*
65 *aureus* or *S. pneumoniae*^{15,16}.

66
67 In this work, we combined agent-based theoretical modeling with single-molecule
68 imaging-based experimental testing to address the mechanism involved in FtsZ
69 treadmilling-dependent processive movement of sPG enzymes and the associated role
70 in bacterial cell division. We found that a Brownian ratchet mechanism underlies the
71 persistent and directional movement of single sPG synthesis enzyme molecules (using
72 FtsI as the model) driven by FtsZ's treadmilling dynamics. Most importantly, the
73 processivity of the Brownian ratchet is modulated by the balance between FtsI's random
74 diffusion and FtsZ's treadmilling speed. This finding indicates that different bacterial
75 species could harness the same FtsZ treadmilling machinery – but modulate diffusions
76 of sPG synthesis enzymes – to achieve distinct processivities of sPG enzymes and
77 control their availability for sPG synthesis. As such, FtsZ's treadmilling dynamics impact
78 the rate of cell wall constriction differentially in different species. Given the lack of linear
79 stepping motors in the prokaryotic world, our work suggests a general framework on
80 how polymer dynamics coupled with Brownian-ratcheting could underlie directional

81 transport of cargos and be shaped by evolution to meet the needs of different cellular
82 milieus.

83

84 **Results**

85 **Model description**

86

87 Our model is based on the concept of a Brownian Ratchet, where FtsZ's treadmilling
88 events introduce an asymmetry to bias the random diffusion of FtsI molecules in the
89 periplasm, upon which FtsI persistently follows the shrinking end of a treadmilling FtsZ
90 filament (Fig. 1). The quantitative details of the model are rooted in the physical and
91 chemical properties of key components of the system, which can be well characterized
92 by experiments.

93

94 As shown in Fig. 1, the model describes the movement of a FtsI molecule at the septum
95 as a quasi-1D problem. The model assumes that FtsI, the essential TPase with a single
96 transmembrane domain and a cytoplasmic tail, can freely diffuse along the inner
97 membrane at the septum or interact indirectly with the treadmilling FtsZ filament
98 underneath the inner membrane (Fig. 1A). The dynamics of a single FtsI molecule at
99 septum is thus determined by three parameters, the constant of FtsI's free diffusion (D),
100 the treadmilling speed (V_Z) of FtsZ filaments, and the attraction force determined by the
101 binding potential (U) between FtsI and FtsZ (Fig. 1B and C). The diffusion constant of
102 FtsI in *E. coli* and PBP2B in *B. subtilis* were measured to be in the range of $\sim 10^{-3}$ – 10^{-1}
103 $\mu\text{m}^2/\text{s}$ using single-molecule tracking (SMT)^{17,18}; the average treadmilling speed of FtsZ

104 was at $\sim 20 - 40$ nm/s *in vivo* but can be a few-fold faster *in vitro*, therefore we set a
105 large range of $10 - 100$ nm/s^{19,20}. FtsI interacts with FtsZ at septum indirectly through a
106 relay of protein-protein interactions that include FtsN, FtsA, and/or FtsEX^{1,21}. For
107 simplicity we omit the details of the protein-protein interaction relay but refer to it as the
108 interaction between FtsI and FtsZ. The indirect interaction between a FtsZ monomer
109 and a nearby FtsI molecule constitute an attractive force for each other and can be
110 described as a short-ranged harmonic binding potential (Fig. 1C). We assumed the
111 potential range at ± 2.5 nm, commensurate with the size of a FtsZ monomer (~ 5 nm)²²⁻
112 ²⁵, and the potential's magnitude at $\sim 10 k_B T$, corresponding to a K_d in the μM range,
113 which is typical for protein-protein interactions in the bacterial divisome system²⁶⁻³⁰.

114

115 To numerically compute the model, we describe the dynamics of FtsI by a Langevin-
116 type equation (equation (1)) where the viscous drag force on the molecule is in balance
117 with a driving force f and a force from thermal noise ξ .

118
$$\lambda \frac{dx(t)}{dt} = f(x(t)) + \xi(t) \quad \text{Equation (1)}$$

119

120 Here, $x(t)$ represents the location of a FtsI molecule at time t along the 1D septum. λ is
121 the effective viscous drag coefficient for FtsI's movement with $\lambda = k_B T/D$, where D is the
122 diffusion constant of the free FtsI on the inner membrane when it is not interacting with
123 FtsZ. $f(x(t))$ is the attractive force exerted upon the FtsI molecule by the FtsZ binding
124 potential, $U(x, t)$ (Fig. 1C). Specifically, $f(x(t)) = -\partial U(x, t)/\partial x$ at time t . The last term $\xi(t)$

125 reflects the random diffusive motion of the FtsI on the inner membrane with $\langle \xi(t) \cdot \xi(t') \rangle =$
126 $2D \cdot \Delta t \delta(t-t')$, where Δt is the unitary time step in simulation.

127

128 Next, we simulate the shrinking of a FtsZ filament according to equation (2), which
129 describes how the position of the shrinking end of a treadmilling FtsZ filament at time t ,
130 $x_Z(t)$, is related to the shrinking rate, V_Z ,

131
$$\frac{dx_Z(t)}{dt} = V_Z \quad \text{Equation (2)}$$

132 Each time when a FtsZ subunit falls off from the shrinking end of the filament, the
133 associated binding potential vanishes with it.

134

135 To discern principal interactions, the model only considers one FtsI molecule and one
136 FtsZ filament in a self-contained septal section. Hereby, we do not consider complicated
137 cases, in which the movement of one FtsI molecule may be interfered by different FtsZ
138 filaments undergoing treadmilling in the same or opposite directions. Further, we do not
139 consider FtsI molecules that are bound to the middle of FtsZ filaments, as these FtsI
140 molecules will either diffuse away, or eventually start end-tracking FtsZ filaments when
141 the shrinking end of the treadmilling FtsZ filament approaches. These complicated
142 scenarios can be decomposed into the elementary process elucidated by the model.

143

144 Assuming that the FtsZ filament treadmills from left to right with a steady-state length of
145 ~ 200 nm, the model implements a hard-wall boundary condition on the FtsI molecule at
146 the left edge of the system, while keeping the right edge as an open boundary so that
147 the right-ward FtsZ treadmilling is not limited. We also note that the model results

148 presented below reflect the nominal case, whose essence remains robust against the
149 variations of the model parameters within the physical range constrained by existing
150 experimental data.

151

152 **A Brownian-ratchet mechanism can couple FtsI's directional movement to FtsZ's** 153 **treadmilling dynamics**

154

155 As we described above, Brownian-ratcheting hinges on the diffusion of FtsI, its
156 interaction with FtsZ, and FtsZ's treadmilling speed. To examine how the movement of
157 FtsI depends on FtsI's diffusion and the binding potential between FtsI and FtsZ, we
158 kept FtsZ's treadmilling speed constant at an experimentally measured 25 nm/s and
159 carried out a phase diagram study using stochastic simulations. We chose a parameter
160 range of 0.0001 to 0.1 $\mu\text{m}^2/\text{s}$ for FtsI's diffusion based on commonly measured diffusion
161 constants for bacterial inner membrane proteins^{17,18}. The upper limit of the binding
162 potential was set $\sim 20 k_{\text{B}}T$, which corresponded to a dissociation constant K_{d} in the nM-
163 range.

164

165 We considered an initial condition in which both the shrinking end of a FtsZ filament and
166 a FtsI molecule were at the left boundary of the septal section. To be commensurate
167 with our experimental analysis, we counted a FtsI trajectory as being moving
168 directionally if it tracked the shrinking end of a treadmilling FtsZ filament persistently and
169 unidirectionally for ≥ 4 seconds. Because of the stochastic nature of Brownian
170 ratcheting, if 50% or more of simulated FtsI trajectories displayed such a persistent

171 directional movement, we characterized the state of FtsI under this parameter set
172 condition as persistent end-tracking in the phase diagram.

173

174 As shown in the phase diagram in Fig. 2A, the model showed that when the binding
175 potential between FtsZ and FtsI was weak ($< 5 k_B T$, \sim mM Kd), FtsI largely displayed
176 random diffusion without directional movements along the septum. When the attraction
177 potential was sufficiently strong ($> 5 k_B T$), strong binding quenched free diffusions and
178 confined FtsI to the end of a FtsZ filament. As the FtsZ subunit at the shrinking end of
179 the filament fell off, the next one in the row attracted and coupled FtsI, which pulled FtsI
180 to the right by ~ 5 nm. With the subsequent FtsZ subunits falling off one after the other
181 from the shrinking end, the FtsI molecule ratcheted forward and stably tracked the end
182 of the treadmilling FtsZ filament. These consecutive movements resulted in a persistent
183 and directional trajectory of FtsI with 5-nm sub-steps (Fig. 2B). Consequently, the speed
184 of FtsI directional movement was tightly coupled to FtsZ's treadmilling speed (Fig. 2C),
185 recapitulating the experimentally measured nearly linear correlation between FtsI's
186 directional moving speed with FtsZ's treadmilling speeds in both wildtype and FtsZ
187 GTPase mutants¹³.

188

189 The phase diagram also showed that at a constant binding potential between FtsZ and
190 FtsI, persistent end-tracking of FtsI required an appropriate range of diffusion constant
191 (Fig. 2A). If FtsI diffused too rapidly, it could not be confined by the binding potential of
192 the shrinking end of the FtsZ filament. Conversely, when FtsI diffused too slowly, it
193 would not be able to keep up with the speed of departing FtsZ subunits at the shrinking

194 end. Once falling behind, the FtsI molecule would lose the contact with the left most
195 FtsZ subunits permanently.

196

197 Taken together, the phase diagram analysis showed that the Brownian-ratchet
198 mechanism was able to couple FtsI's directional movement to FtsZ's treadmilling within
199 the parameter range that is well consistent with experimentally measured data, *i.e.*,
200 0.001 to 0.1 $\mu\text{m}^2/\text{s}$ for FtsI's diffusion coefficient, $> 5 k_{\text{B}}T$ for the binding potential
201 between FtsI and FtsZ, and at the *in vivo* FtsZ treadmilling speed of ~ 25 nm/s. Further,
202 the same model could explain the nondirectional movement of the other divisome
203 protein FtsN in a recent *in vitro* study¹⁹. In that study, the cytoplasmic tail of FtsN was
204 reported to follow the tracks of treadmilling FtsZ filaments on a supported lipid bilayer at
205 the ensemble level. At the single molecule level, however, the FtsN tail only binds and
206 unbinds FtsZ filaments transiently but does not exhibit directional movement²⁶. Such a
207 scenario could be explained by to our Brownian ratchet model in that the diffusion of
208 free FtsN cytoplasmic tail anchored on the membrane was too large (0.3 – 0.6 $\mu\text{m}^2/\text{s}$)¹⁹
209 to be confined by the binding potential of FtsZ.

210

211 **FtsZ's treadmilling speed modulates processivity of FtsI's end-tracking**

212

213 Next, we investigated how FtsZ's treadmilling speed impacts the processivity of FtsI's
214 directional movement. Addressing this question will help us understand the role of
215 FtsZ's treadmilling dynamics in the spatial organization and/or regulation of sPG
216 synthesis activity. We focused on three features that collectively defined the processivity

217 of FtsI's end-tracking: (1) the propensity, (2) the run distance, and (3) the duration of
218 persistent end-tracking trajectories.

219

220 We first examined how the propensity of FtsI's persistent end-tracking was modulated
221 by FtsZ treadmilling speed. Here we define the propensity as the percentage of FtsZ
222 trajectories that showed > 4s directional movement in all simulated trajectories under
223 each parameter set. Keeping the diffusion constant of FtsI at $0.01 \mu\text{m}^2/\text{s}$ and the binding
224 potential at $10 k_B T$, stochastic simulations of the Brownian ratchet model predicted that
225 when the FtsZ treadmilling speed was < 50 nm/s, nearly all simulated FtsI trajectories
226 displayed persistent end-tracking (Fig. 3A). However, as the FtsZ treadmilling speed
227 increased beyond 50 nm/s, the percentage of persistent end-tracking trajectories of FtsI
228 dropped off sharply to 60% at 75 nm/s, and further to 20% at 100 nm/s (Fig. 3A). That is,
229 when the FtsZ treadmilling was too fast, FtsI could not couple FtsZ shrinking end in
230 most cases and became largely diffusive.

231

232 To further this point, we calculated the phase diagram of FtsI's persistent end-tracking
233 propensity as a function of both FtsI's diffusion constant and FtsZ treadmilling speed
234 (Fig. 3B), while keeping the binding potential fixed at $10 k_B T$. Again, we used a 50% FtsI
235 persistent end-tracking trajectories as the criterium for the phase boundary. As shown in
236 Fig. 3B, for a fixed diffusion constant of FtsI, there was an upper limit of FtsZ's
237 treadmilling speed that FtsI could persistently couple. Conversely, for a fixed FtsZ
238 treadmilling speed, persistent end-tracking of FtsI required an appropriate range of
239 diffusion constants. Importantly, a very large diffusion constant of FtsI ($> 0.1 \mu\text{m}^2/\text{s}$) did

240 not support persistent end-tracking irrespective of FtsZ's treadmilling speed. These
241 results were consistent with the phase diagram in Fig. 2A and the recent *in vitro* study of
242 FtsN's cytoplasmic tail²⁶.

243

244 Next, we investigated how FtsZ's treadmilling speed modulates the run distance and
245 duration of FtsI's persistent end-tracking. The Brownian ratchet model predicted that
246 both the run length and duration of FtsI's persistent end-tracking should display broad
247 distributions due to the stochastic nature of FtsI's diffusion and the interaction between
248 FtsI and FtsZ. Moreover, the model predicts that when FtsZ's treadmilling speed
249 increase, the duration of FtsI's persistent end-tracking will decrease (Fig. 3C), whereas
250 the run distance will display a biphasic dependence that peaks around an intermediate
251 FtsZ treadmilling speed (~ 50 nm/s at the current parameter setting, Fig. 3D).
252 Importantly, such distinctive dependences of duration and run distance on FtsZ
253 treadmilling speed is a natural consequence of the Brownian ratchet mechanism
254 (Supplementary Info). Qualitatively speaking, when a FtsZ subunit falls off from the
255 shrinking end of the FtsZ filament, the associated FtsI molecule will either diffuse away
256 or catch up with the next FtsZ subunit in the row to continue end-tracking, the latter
257 depending on how fast FtsZ treadmills. When FtsZ treadmills too fast (for example > 50
258 nm/s), it will be difficult for FtsI to catch up (Fig. 3A), resulting in early termination of
259 end-tracking, and hence both the persistence run distance and duration will be short.
260 When FtsZ treadmills relatively slowly (< 50 nm/s), the probability of FtsI catching up
261 with the shrinking end of the FtsZ filament is high (Fig. 3A). Therefore, the persistence
262 run distance will be proportional to the FtsZ treadmilling speed as predicted in Fig. 3D.

263 Within the same time window, however, when FtsZ treadmills slowly, an end-tracking
264 FtsI molecule would face fewer number of dissociation events, and hence there is a
265 lower chance for FtsI to diffuse away to terminate the trajectory, leading to a longer
266 duration of persistence run compared to the regime when FtsZ treadmills relatively fast
267 (but still < 50 nm/s). One can imagine in the extreme case where FtsZ does not
268 treadmill at all ($V_z = 0$), the duration of persistence runs would then be mainly dictated
269 by the intrinsic dissociation rate of FtsI from FtsZ, and the persistence run distance
270 would be the shortest (i.e., the size of a single FtsZ subunit). An analytical proof of
271 these relationships was provided in Supplementary Information and Extended Data Fig.
272 1.

273

274 **Single-molecule tracking of FtsI confirms model predictions**

275 To experimentally examine the model's predictions as described above, we performed
276 single-molecule tracking (SMT) of a functional sandwich fusion protein FtsI-Halo^{SW}
277 labeled with JF646 in live *E. coli* cells^{31,32}. To avoid disrupting the cytoplasmic
278 interactions of FtsI's N-terminal tail with other divisome proteins, we inserted the Halo
279 tag between the last residue (18) of the N-terminal cytoplasmic tail and the first residue
280 of the inner membrane helix (19) of FtsI (Fig. 4Ai). We integrated the *ftsI-halo*^{SW} fusion
281 gene into the chromosome replacing the endogenous *ftsI* gene and showed that it was
282 expressed as a full-length fusion protein and supported normal cell division
283 indistinguishable from wild-type (WT) cells (Extended Data Fig. 2).

284

285 To obtain precise measurements of the persistence run distance and duration of single
286 FtsI-Halo^{SW} molecules, we trapped individual *E. coli* cells vertically in agarose
287 microholes made using cell-shaped nanopillar molds as previously described^{33,34} so that
288 the entire circumference of the septum could be visualized at the same focal plane (Fig.
289 4Aii). To determine whether a FtsI-Halo^{SW} molecule was at a septum, we labeled the
290 FtsZ-ring using an ectopically expressed GFP-ZapA fusion protein, which we and others
291 have previously shown as a faithful marker of the Z-ring localization and dynamics³⁵.
292 The GFP-ZapA image also allowed us to unwrap the circular trajectories of FtsI-Halo
293 molecules along the septum to linear displacements along the circumference of the
294 septum, from which we could measure the persistent run speed, distance, and duration
295 (Fig. 4Aiii-v).

296
297 As shown in Fig. 4B, the directional moving speed of FtsI-Halo exhibited a wide
298 distribution as we previously showed for FtsZ's treadmilling. Consistent with our
299 prediction (Fig. 3A), the occurrence of FtsI's directional movement decreases
300 significantly as the speed is > 40 nm/s. Most excitingly, the persistence run distance and
301 duration exhibited precisely the same trends as what was predicted by the model: while
302 the run duration decreased monotonically (Fig. 4C), the persistence run length
303 increased and then decreased when FtsI's speed increased (Fig. 4D). Note here that
304 we inferred FtsZ's treadmilling speed from FtsI's directional moving speed due to the
305 difficulty in the two-color co-tracking experiment, although we have demonstrated
306 previously that these two were linearly coupled¹³. Also note that one potential caveat in
307 these experiments was that a very fast FtsZ treadmilling speed (*i.e.*, > 80 nm/s) is rare

308 in wildtype *E. coli* cells as we showed previously and here by the FtsI speed distribution.
309 Therefore, given the relatively small dataset for high speed FtsZ treadmilling, our data
310 may not be definitive to distinguish whether the FtsI couldn't effectively end-track fast
311 FtsZ treadmilling or there were simply not many fast FtsZ treadmilling events in the first
312 place. Nevertheless, the agreement of our experimental measurements with theoretical
313 predictions supported the validity of the Brownian ratchet model.

314

315 **Brownian ratchet mechanism explains the differential dependence of sPG**
316 **synthesis activity on FtsZ treadmilling speed in different bacterial species**

317

318 In *E. coli*, the total amount of septal PG synthesis and the septum constriction rate are
319 insensitive to perturbations in FtsZ's treadmilling speed from ~ 8 nm/s to ~ 30 nm/s in a
320 series of FtsZ GTPase mutants¹³. This insensitivity suggests that end-tracking,
321 directional moving FtsI molecules were unlikely active in sPG synthesis. Thus, the
322 functional role of FtsZ's treadmilling in *E. coli* is proposed to spatially control the location
323 of sPG synthesis by acting as a shuttle to transport sPG synthase molecules along the
324 septum without affecting their enzymatic activity¹³. In *B. subtilis*, however, it was shown
325 that the constriction time positively correlated with FtsZ's treadmilling speed, suggesting
326 that the faster FtsZ treadmills, the more sPG synthesis activity there is¹². Many factors
327 have been proposed to explain why there is such a difference, such as cell wall
328 synthesis precursor levels, species-specific protein-protein interactions, septal cell wall
329 compositions or cell wall constriction stages, but none has been confirmed. As we show
330 below, the interplay between the diffusion of synthase molecules and FtsZ's treadmilling

331 speed in the Brownian ratchet model determines the fraction of active sPG synthases,
332 which naturally gives rise to the differential dependence of sPG synthesis activity on
333 FtsZ's treadmilling speed.

334
335 To illustrate the interplay between the diffusion of synthase molecules and FtsZ's
336 treadmilling, we first determined the fraction of time a FtsI molecule spent in persistent
337 end-tracking (termed "fraction of end-tracking") at different FtsZ treadmilling speeds.
338 The fraction of end-tracking is defined as the time a FtsI molecule continuously end-
339 tracks the shrinking end of a FtsZ filaments divided by the total time simulated, which is
340 typically 60 s. As shown in Fig. 5A, when FtsI diffused relatively fast ($\sim 0.05 \mu\text{m}^2/\text{s}$,
341 dashed line with triangle), the fraction of time FtsI molecules spent in end-tracking was
342 largely insensitive to FtsZ's treadmilling speed so that it only decreased from 99% to 91%
343 when the corresponding FtsZ treadmilling speed increased 3-fold from $\sim 8 \text{ nm/s}$ to 25
344 nm/s . In contrast, when FtsI diffused relatively slowly, the fraction of time FtsI
345 molecules spent in end-tracking not only reduced significantly as compared to that of
346 faster diffusion but was critically dependent by FtsZ treadmilling speed. For example, at
347 a diffusion constant of $0.003 \mu\text{m}^2/\text{s}$, the fraction of end-tracking FtsI molecules
348 decreased from 73% to 24% when FtsZ treadmilling speed increased from $\sim 8 \text{ nm/s}$ to
349 25 nm/s (continuous line with circle). The physical reason behind this drastic difference
350 between fast and slow FtsI diffusion lies at the core of Brownian ratchet mechanism. A
351 fast diffusion will allow FtsI to catch up with the shrinking end of a FtsZ filament in very
352 short time (Fig. 5B). When FtsI's diffusion becomes slower and slower, it eventually
353 becomes the rate-limiting factor in the Brownian ratchet—more than often the slow FtsI

354 molecules falls behinds the FtsZ shrinking end and takes a long time to catch up with
355 the shrinking end of a departing FtsZ filament, or simply just diffuses away and become
356 lost (Fig. 5C). As such, further increasing the FtsZ treadmilling speed in the latter case
357 will significantly reduce the chance of FtsI keeping up with the FtsZ shrinking end and,
358 hence the percentage of end-tracking.

359

360 Exploiting the above results could allow us to explain the differences between *E. coli*
361 and *B. subtilis*. Consider that the fraction of time a FtsI molecule end-tracks reflects the
362 overall fraction of all FtsI molecules that are associated with treadmilling FtsZ filaments
363 at the septum. As we previously showed in *E. coli* that the FtsZ treadmilling-driven
364 population of FtsI are not active in sPG synthesis, it follows that the active FtsI
365 population would be proportional to the fraction released from treadmilling FtsZ
366 filaments. Therefore, FtsZ's treadmilling speed could modulate the amount of sPG
367 synthases that would be available for synthesis in different bacterial species depending
368 on the diffusion of sPG synthase molecules.

369

370 In *E. coli*, we observed that the sPG synthesis activity was insensitive to FtsZ's
371 treadmilling speed from 8 nm/s to ~ 30 nm/s in a series of FtsZ GTPase mutants¹³. This
372 insensitivity could be explained by the Brownian ratchet model if the diffusion of FtsI in *E.*
373 *coli* is fast enough so that there is negligible change in the fraction of end-tracking FtsI
374 molecules when FtsZ's treadmilling speed changes as that predicted in Fig. 5A (dash
375 line with triangle). To examine this possibility, we performed single-molecule tracking of
376 FtsI again using a faster frame rate (20 Hz) in order to capture freely diffusing molecules.

377 The mean-square-displacement (MSD) analysis showed that the apparent diffusion
378 constant of FtsI in wildtype *E. coli* cells was $\sim 0.024 \mu\text{m}^2/\text{s}$ (Fig. 5D), well within the
379 predicted range in Fig. 5A and similar to the measured diffusion constant of PBP1b¹⁸.
380 In contrast, the TPase PBP2B diffused ~ 10 -fold slower in *B. subtilis* ($\sim 0.003 \mu\text{m}^2/\text{s}$)
381 than that in *E. coli*^{17,18}, likely due to more viscous septal environment in *B. subtilis* than
382 that in *E. coli*. The slow diffusion of PBP2B leads to the highly sensitive curve of the
383 fraction of time of PBP2B spent in end-tracking on FtsZ's treadmilling speed as shown
384 in Fig. 5A (continuous line with circle). As FtsZ's treadmilling speed increases, there will
385 be reduced levels of end-tracking PBP2B molecules, leading to increased levels of
386 available PBP2B molecules for active sPG synthesis, and hence faster septal cell wall
387 constriction.

388

389 Discussion

390

391 We note that other mechanisms (but not necessarily exclusive from the Brownian
392 Ratchet model) could also be at play. For instance, detailed molecular interactions
393 among the septal ring complexes might be regulated distinctly to fulfil the different
394 functional requirements in different systems. After all, the sPG synthesis in *B. subtilis*
395 needs to produce a cell wall of ~ 10 times thicker than *E. coli* ($\sim 50 \text{ nm}$ vs. $\sim 5 \text{ nm}$)^{36,37}.
396 This may relate to the fact that the septal ring components are typically several-fold
397 more abundant in *B. subtilis* than those in *E. coli*¹². Nevertheless, our results in *E. coli*
398 points to a mechanistic possibility that the same Brownian-ratchet machinery may be at
399 work in *B. subtilis* but operate in a different regime of the parameter space. This

400 possibility precipitates further questions: What are the differences in sPG synthesis and
401 cell division between *E. coli* and *B. subtilis*? How does the coupling between sPG
402 synthesis complexes and FtsZ treadmilling adapt to the different systems? There could
403 be many possible evolutionary routes revolving around these issues. For instance, a
404 thicker cell wall and/or a more crowded periplasm – perhaps due to the presence of
405 more abundant PG synthesizing-proteins – may slow down FtsI diffusion. A slower FtsI
406 diffusion will in turn reduce the percentage of end-tracking FtsI which are inactive in
407 sPG synthesis (Fig. 5A). Reciprocally, there will be more fraction of FtsI molecules
408 active in PG-synthesis, meeting the requirement for producing a thicker cell wall. From
409 evolutionary standpoint, is a thicker cell wall the cause or the result of a slower FtsI
410 diffusion? Is it an emergent phenomenon of sPG synthesis being proportional to FtsZ
411 treadmilling speed in *B. subtilis*? After all, FtsZ not only localizes the sPG-synthesizing
412 enzymes to the septum but could also regulate the available level of enzymes for sPG
413 synthesis by controlling the FtsZ treadmilling speed (Figs. 5A-C). This way, the
414 chemical energy of FtsZ GTPase hydrolysis is harnessed “purposely” for bacterial cell
415 division.

416

417 We further note that in addition to *E. coli* and *B. subtilis*, other bacteria seem to adopt
418 more diverse strategies of exploiting FtsZ treadmilling in sPG synthase activity and cell
419 division. For instance, in *S. aureus*, it was observed that septum constriction was
420 dependent on FtsZ's treadmilling initially but becomes independent at a later stage³⁸. In
421 *S. pneumoniae*, the directional movement of the synthase complex was found to be
422 completely independent of FtsZ's treadmilling¹⁵. It will be in our future work to

423 interrogate whether and how the Brownian-ratchet mechanism plays out under these
424 different contexts. In a broader scope, given the lack of linear stepper motors in
425 prokaryotic world, Brownian-ratcheting appears to be an ancient mechanism for directed
426 cargo transportation in bacteria – another salient example is ParA-mediated DNA
427 partitioning³⁹⁻⁴¹. Interestingly, a similar Brownian-ratchet mechanism also underlies
428 directional movements of mitotic chromosomes by end-tracking spindle microtubule in
429 eukaryotes⁴². Can we distill unified fundamental principle(s) by which evolution shapes
430 the same Brownian-ratchet mechanism to meet distinct needs under different contexts?
431 We will relegate these exciting quests in our near-future study.

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433

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442

443 **Author Contributions**

444

445 J.W.M., X.Y and Z.L performed experiments. J.L. carried out theoretical modeling. All
446 authors contributed to concept development, data analysis, and manuscript writing.

447

448 **Competing interests**

449

450 The authors declare no competing interests.

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455 Reference

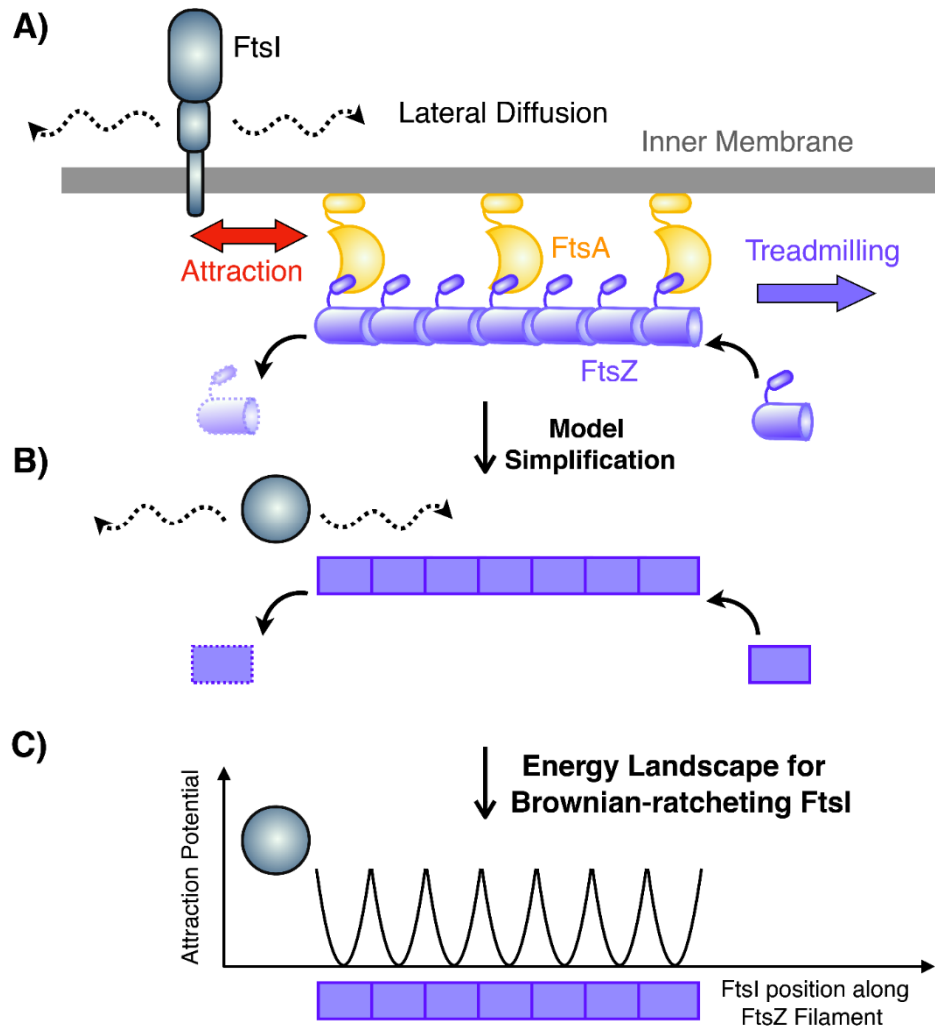
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- 582

583

584 **Figure 1**

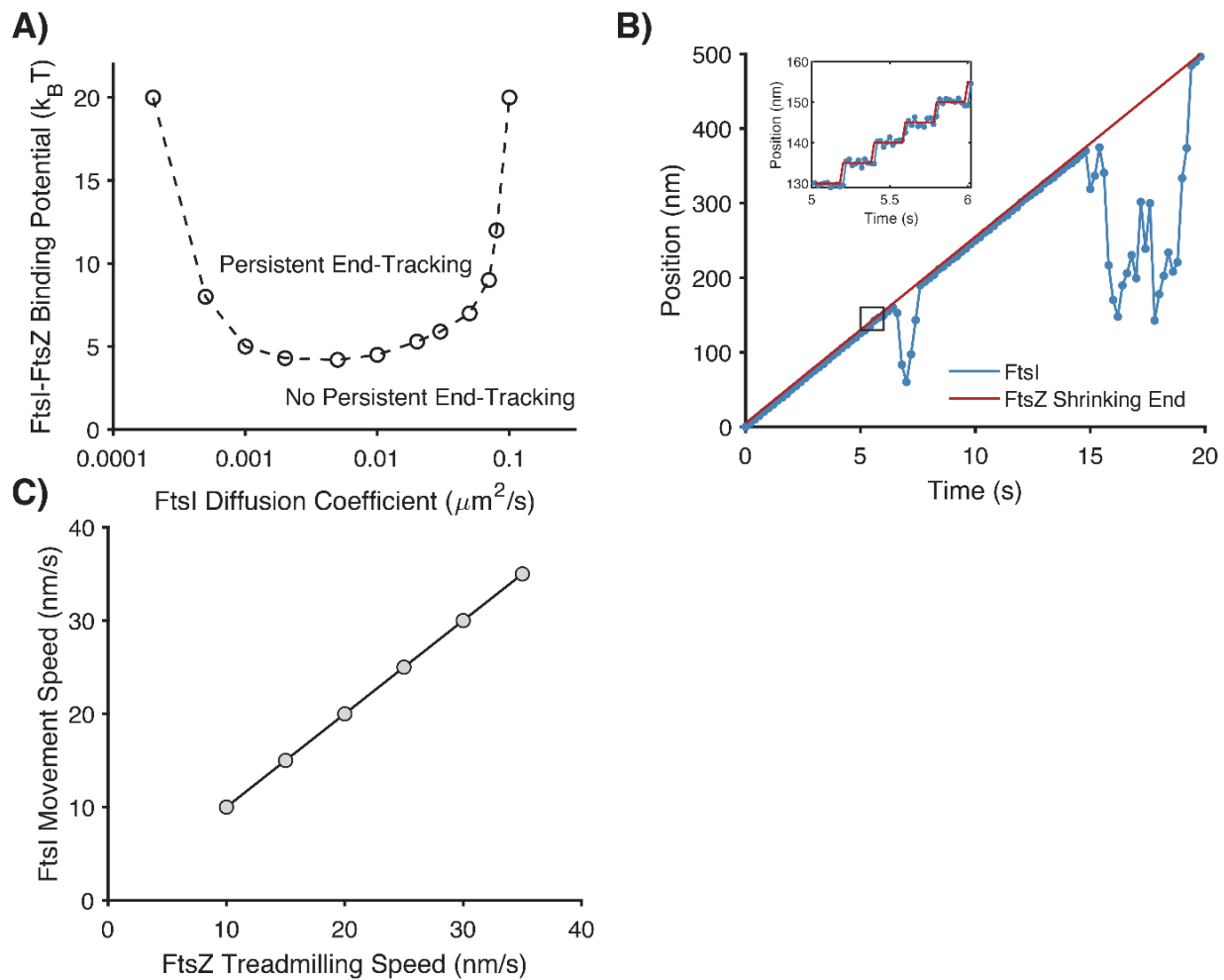


585

586 **Figure 1.** Model description. A) Schematic representation of sPG synthase complex's
587 interaction with FtsZ treadmilling. B) Model simplification of FtsZ – FtsI interaction at the
588 septum. The FtsZ filament (purple) undergoes treadmilling by dissociating FtsZ subunit
589 from the left end and associating new ones from the right end. While the FtsI complex
590 (grey) intrinsically diffuses around, it has binding affinity to FtsZ subunits. C) Schematics
591 of FtsZ – FtsI binding potentials. Here, the binding potential is assumed to be harmonic
592 and short-ranged (~ 5 nm), which is about the size of the individual FtsZ subunit.

593

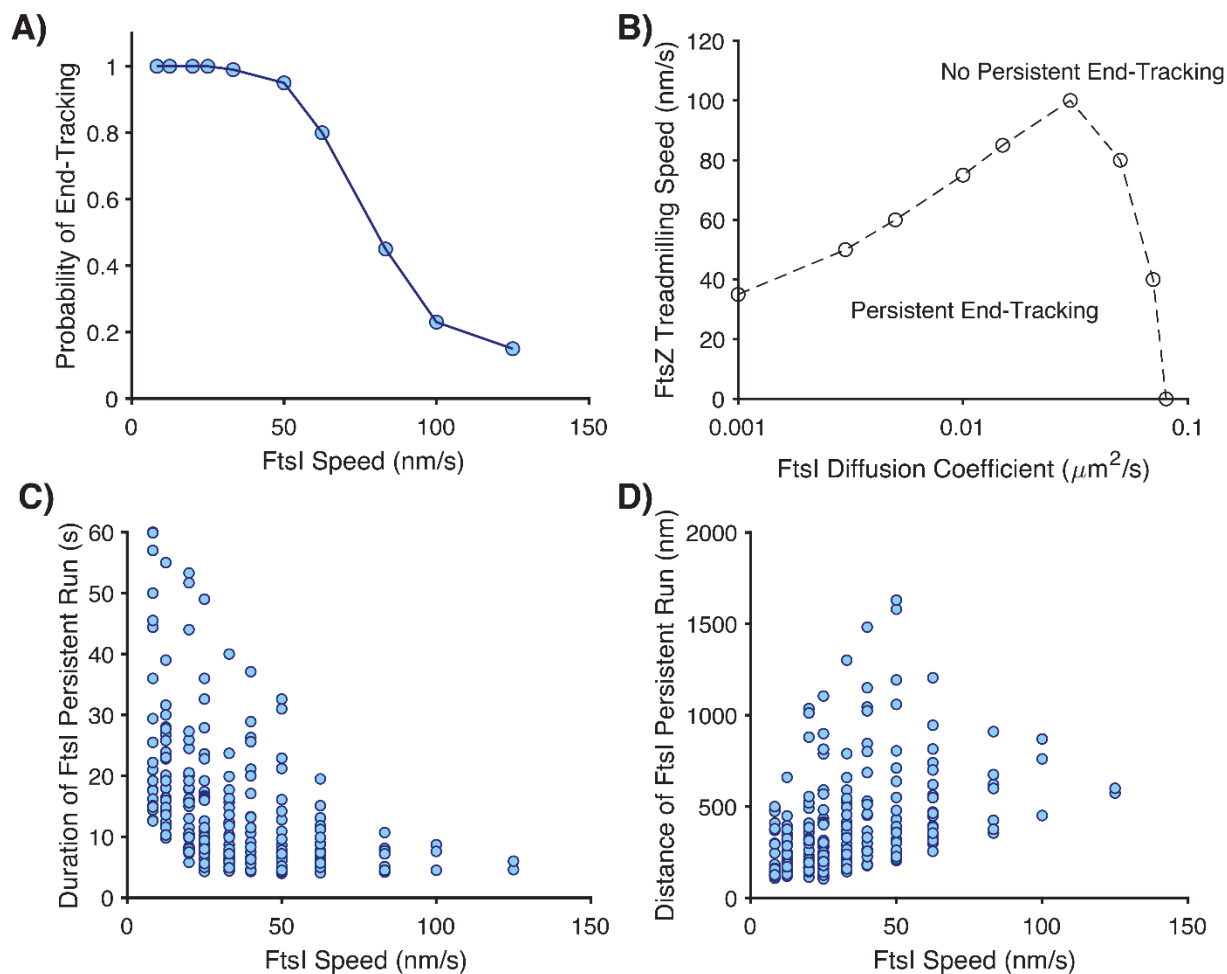
594 **Figure 2**



595
 596 **Figure 2.** FtsZ treadmilling-mediated Brownian ratchet mechanism drives FtsI
 597 directional movement. A) Calculated phase diagram – Dependence of FtsI motilities on
 598 FtsI diffusion constant and FtsI-FtsZ binding potential. B) A representative simulated
 599 trajectory of FtsI persistent end-tracking with FtsZ treadmilling. Here, the model is
 600 simulated with following parameters: FtsZ treadmilling speed is 25 nm/sec, the FtsI
 601 diffusion constant is $0.01 \mu m^2/s$, and the FtsZ–FtsI binding potential is $10 k_B T$. Inset: A
 602 zoom-in view of the trajectory. Here, the simulation time step is 10^{-5} s, the model results
 603 are plotted every 10^{-1} s, and those in the zoom-in inset are plotted every 2×10^{-2} s. C)
 604 FtsI directional speed tightly couples with FtsZ treadmilling speed. The model calculated

605 the FtsI speed only from the part of its trajectory that the FtsI undergoes persistent end-
606 tracking. Therefore, the FtsI speed varies very little as long as FtsZ treadmilling speed is
607 fixed.
608

609 **Figure 3**

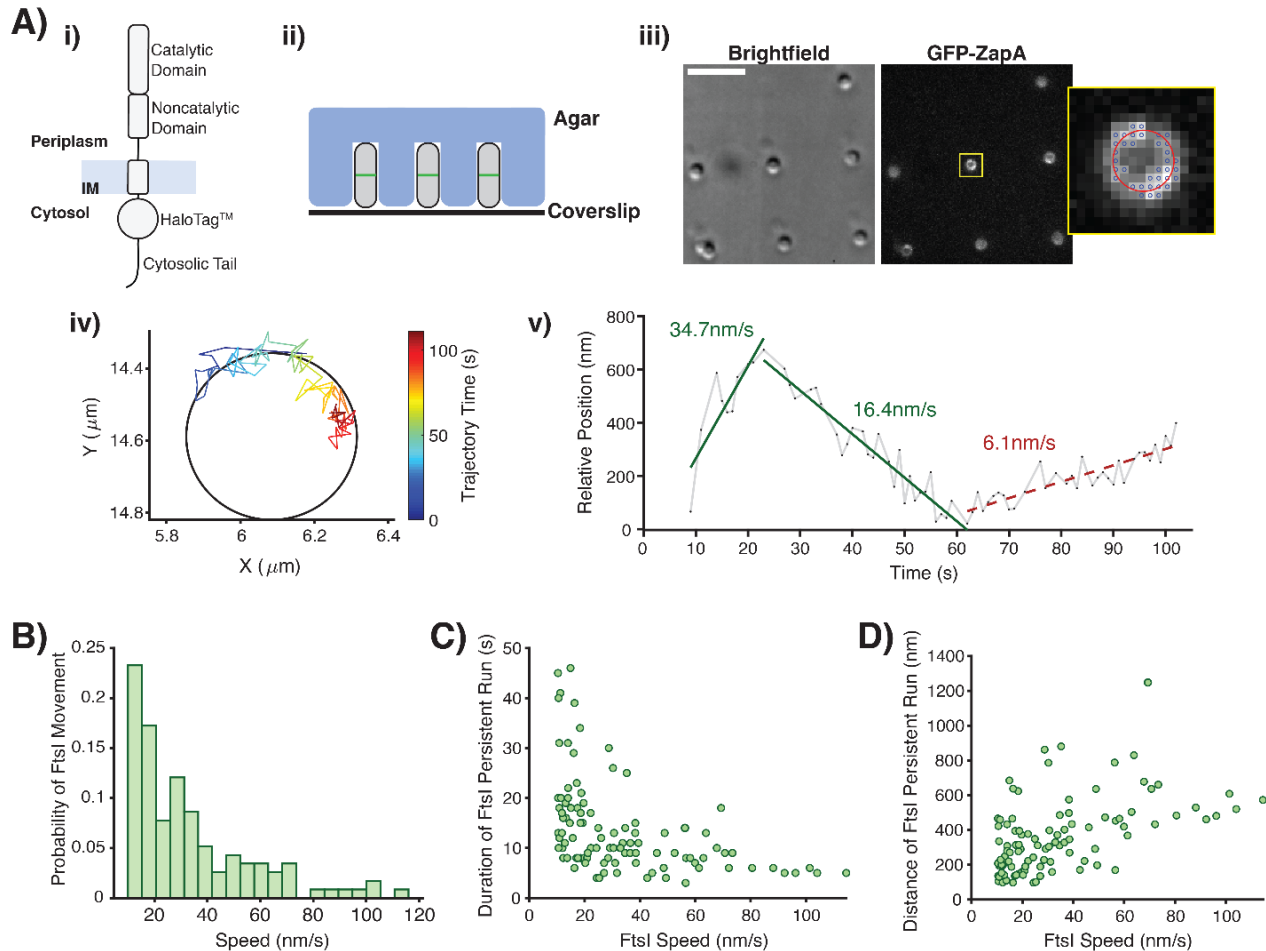


610

611 **Figure 3.** Model predictions on the processivity of FtsI directional movement modulated
612 by FtsZ treadmilling speed. A) Predicted propensity of FtsI persistent end-tracking as a
613 function of FtsZ treadmilling speed. B) Calculated phase diagram of FtsI persistent end-
614 tracking characterized by FtsI diffusion constant and FtsZ treadmilling speed. C)
615 Predicted FtsZ treadmilling speed-dependence of run distance of FtsI persistent end-
616 tracking. D) Predicted FtsZ treadmilling speed-dependence of duration of FtsI/W
617 persistent end-tracking. For the model calculations in (A-D), the FtsZ–FtsI binding
618 potential is set to be $10 k_B T$.

619

620 **Figure 4**



621

622 **Figure 4.** Experimental characterization of FtsI directional movements. A) Single

623 molecule tracking experimental setup. i, Schematics of the fully functional sandwich

624 fusion of FtsI. ii, schematics of individual *E. coli* cells loaded in microholes; iii, brightfield

625 and fluorescence images of microholes loaded with *E. coli* cells labeled with GFP-ZapA

626 and FtsI-Halo^{SW} fusion proteins. Inset shows the zoomed image of one cell in the yellow

627 box with a circle fit to its intensity profile. iv, GFP-ZapA circle-fit super-imposed with the

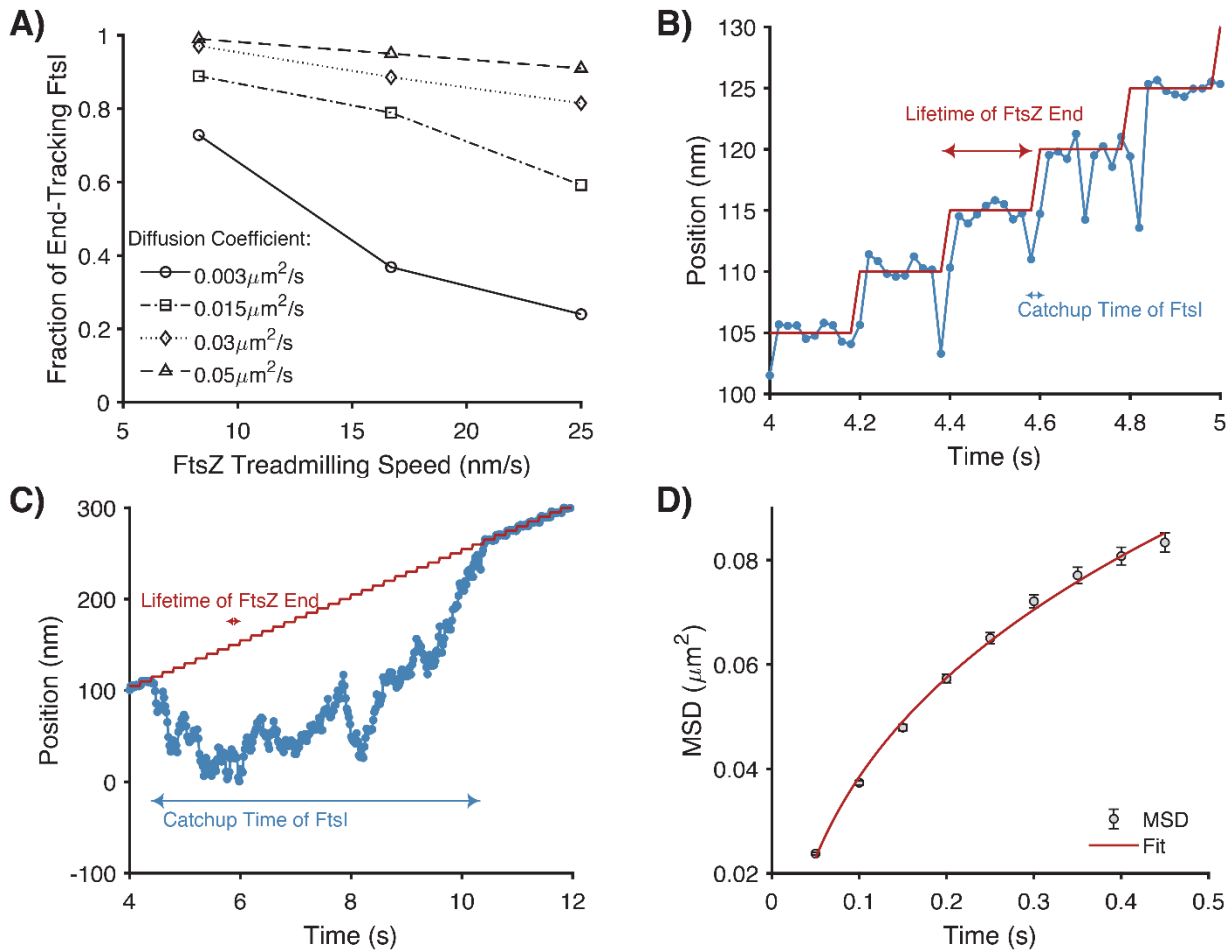
628 trajectory of a single FtsI molecule; v the unwrapped trajectory from iv with fitted lines at

629 each segments to extract directional speeds Fitting the directional mobile events allow

630 us to identify movement states in our trajectories. Note that only movement events >10

631 nm/s were used for analysis in this work (fitted lines) because we recently showed that
632 the slow-moving population (average speed ~ 8 nm/s) is independent of FtsZ's
633 treadmilling¹⁴. B) Histogram of FtsI directional movement speeds. C) Dependence of the
634 duration of FtsI's persistent run on its speed. D) Dependence of the distance of FtsI's
635 persistent run on its speed.
636

637 **Figure 5**



638

639 **Figure 5.** Dependence of FtsI's processivity on its diffusion. A) Predicted dependence
640 of the fraction of end-tracking of FtsI on FtsZ treadmilling speed. The fraction of end-
641 tracking is defined as the percentage of time that FtsI persistently end-tracks FtsZ
642 treadmilling within 60 seconds. B) A representative trajectory of FtsI's persistent end-
643 tracking when FtsI diffusion is fast. C) A representative trajectory of FtsI's persistent
644 end-tracking when the FtsI diffusion is slow. For (B and C), a fast diffusion allows FtsI to
645 catch up with the shrinking end of FtsZ almost immediately, whereas it takes a long time
646 for FtsI to catch up (if it eventually catches up) when it diffuses slowly. Here, the
647 simulation time step is 10^{-5} s, the model results are plotted every 2×10^{-2} s. D)

648 Measured MSD of FtsI as a function of time in wildtype *E. coli* cells. From these data, A
649 diffusion constant of FtsI at $0.036 \pm 0.001 \mu\text{m}^2/\text{s}$ was determined by fitting the MSD
650 curve with function $\text{MSD} = 4Dt^\alpha$ with $\alpha = 0.42 \pm 0.05$ (standard error from fitting).