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2 **Detailed analyses of stall force generation in *Mycoplasma mobile* gliding**

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4 **Masaki Mizutani^a, Isil Tulum^{a,b}, Yoshiaki Kinosita^c, Takayuki Nishizaka^c and**
5 **Makoto Miyata^{a,b}**

6

7 ^a Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku,
8 Osaka 558-8585, Japan

9 ^b The OCU Advanced Research Institute for Natural Science and Technology
10 (OCARINA), Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka
11 558-8585, Japan

12 ^c Department of Physics, Faculty of Science, Gakushuin University, 1-5-1 Mejiro,
13 Toshima-ku, Tokyo 171-8588, Japan

14

15 Corresponding author: (Makoto Miyata, Graduate School of Science, Osaka City
16 University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan,
17 +81-6-6605-3157, miyata@sci.osaka-cu.ac.jp)

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19 conversion

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21

22 **Abstract**

23 *Mycoplasma mobile* glides on solid surfaces at a velocity of up to 4.5 $\mu\text{m/s}$ with a
24 unique mechanism. The gliding machinery composed of hundreds of units generates
25 the force for gliding based on the energy of ATP, and catches and pulls the sialylated
26 oligosaccharides fixed on solid surfaces. In the present study, we measured the stall
27 force of a wild type and mutant strains of *M. mobile* carrying a bead manipulated by
28 using optical tweezers. The strains enhanced for binding showed weaker stall force
29 than the wild-type strain, indicating that the stall force is related to the step of force
30 generation rather than binding. The stall force of the wild-type strain decreased
31 linearly from 113 to 19 pN by the addition of free sialyllactose, a sialylated
32 oligosaccharide from 0 to 0.5 mM with decreasing the number of working units. In the
33 0.5 mM sialyllactose conditions, the cells carrying a bead loaded by the optical tweezers
34 showed stepwise movements with force increments. The force increments distributed
35 from 1 to 2 pN. Considering the 70-nm large step size, this small unit force may be
36 explained by the large gear ratio involved in the gliding machinery of *M. mobile*.

37

38 **Significance**

39 *Mycoplasma* is a group of bacteria parasitic for animals. Dozens of species glide on
40 their host tissues for infection. The gliding machinery of *Mycoplasma mobile*, the
41 fastest species includes intracellular motors and many legs on the cell surface. In the
42 present study, we measured the force generation precisely by using strongly focused
43 laser beam, called optical tweezers, in different conditions. The measurements
44 suggested that the gliding machinery has a large gear ratio to achieve the fast gliding
45 speed.

46

47 **Introduction**

48 Class *Mollicutes*, represented by *Mycoplasma* is parasitic and occasionally
49 commensal bacteria that have small cell size, genomes, and no peptidoglycan layer (1,
50 2). Dozens of *Mycoplasma* species featured by protrusions, such as the fish pathogen
51 *Mycoplasma mobile* (3-5) and the human pathogen *Mycoplasma pneumoniae* (6-8)
52 exhibit gliding motility in the direction of the protrusion on solid surfaces, which is

53 involved in the parasitism of mycoplasmas. Interestingly, mycoplasma gliding has no
54 relation with flagella, pili, other bacterial motility systems or conventional motor
55 proteins that are common in eukaryotic motility.
56 *M. mobile*, isolated from the gills of a freshwater fish, is a fast-gliding mycoplasma
57 (9-13). It glides smoothly and continuously on glass at an average speed of 2.0 to 4.5
58 $\mu\text{m/s}$, or three to seven times the length of the cell per second. The gliding mechanism
59 of *M. mobile* has been studied and proposed a working model, called centipede or
60 power-stroke model, by which the cells repeatedly catch, pull drag and release sialylated
61 oligosaccharides (SOs) on solid surfaces (3-5, 14). The gliding machinery is
62 composed of internal and surface structures (15-19). The internal structure includes
63 the α - and β -subunit paralogs of F-type ATPase/synthase and generates the force for
64 gliding, based on the energy of ATP (18-22). The force should be transmitted across
65 the cell membrane to the surface structure, which is composed of at least three huge
66 proteins, Gli123, Gli521 and Gli349 (15-17, 23). Gli521, the crank protein transmits
67 the force from the cell inside to Gli349 with actual structural changes (17, 24). Gli349,
68 the leg protein extends after thermal fluctuation and catches SOs which is the major
69 structures on animal cell surfaces (Fig 1A) (9, 25-27). The cells always glide in the
70 direction of the machinery, which may be caused by the directed binding of cells on
71 solid surfaces (3, 28). Hundreds of gliding units existing on the cell should function
72 cooperatively to achieve smooth gliding (Fig. 1B) (15, 27, 29). To examine this
73 working model in detail, the behaviors of individual units should be clarified.
74 Recently, the discrete movements of gliding motility, possibly single leg movement was
75 observed by controlling the working leg number through addition of free SOs (22, 27).
76 Now, we focus on the pulling force exerted to the solid surface in the movements,
77 because the force generation was not analyzed carefully for other than the final stall
78 force of the wild-type strain (ref). In the present study, we quantitatively measured the
79 force of whole cell of various strains under various conditions by using optical tweezers
80 (11, 28, 30-32), and characterized the force generated by the gliding machinery.

81

82 **Results**

83 **Stall force measurement.** To examine the force of *Mycoplasma mobile* gliding, we

84 measured a stall force by optical tweezers. Previously, the propelling force of *M.*
85 *mobile* cell was measured by using optical tweezers (11). The bead bound to the cell
86 was trapped by strongly focused laser beam and the force was calculated from the
87 distance between the centers of bead and trap because the force acting on the bead
88 increases linearly with the displacement from the trap center (11, 28, 32). In the
89 present study, *M. mobile* cells, biotinylated and suspended in phosphate-buffer saline
90 with glucose (PBS/G) were inserted into a tunnel chamber. Then, we added
91 1- μm -diameter polystyrene beads coated with avidin to the tunnel. A bead trapped by
92 optical tweezers was attached to the back end of the gliding cell through the
93 avidin-biotin interaction (28, 33, 34). The cell pulled the bead from the trap center
94 (Fig. 2A and Movie S1), and the pulling force increased from 0 s and reached the
95 plateau at around 40 s. The maximum values for average of 25 data points were used
96 as the stall force. The stall force of wild-type strain was 113 ± 32 pN ($n = 50$).
97 To determine the proteins involved in force generation or force transmission, we
98 measured the stall force to compare with that of the wild-type strain, for six strains
99 which were previously isolated and featured for gliding speed and/or binding activity
100 (12, 21, 35). The *gli521* (P476R) mutant was reported to have a single amino acid
101 substitution from proline at 476th to arginine in Gli521 (21), and mutations in the other
102 strains have not been analyzed. The pulling forces of these mutants also increased
103 from 0 s and stalled at 20–50 s. The stall force of m14 strain, 110 ± 29 pN ($n = 29$)
104 was not significantly different from that of the wild-type strain ($P = 0.1 > 0.05$ by
105 Student's *t*-test). The stall forces of the *gli521* (P476R), m6, m27, m29 and m34
106 mutants were significantly reduced to 64%–81% of that of the wild-type strain ($P = 6$
107 $\times 10^{-3}$, 4×10^{-4} , 9×10^{-5} , 8×10^{-9} and 4×10^{-4} , respectively < 0.01 by Student's *t*-test)
108 (Fig. 2B *Inset*). The *gli521* (P476R) mutant featured by enhanced binding showed
109 smaller force than the wild-type strain, suggesting that the legs keep binding, not in
110 repetitive cycle of catch and release, in the stalled state.

111

112 **Genome sequencing of various strains.** To determine the proteins and mutations
113 associated with the decreases in the stall force, we sequenced the genomes of the strains
114 by using a next-generation sequencer, MiSeq. The genome of the *gli521* (P476R)

115 mutant was previously sequenced for a 30,469-bp DNA region encoding four open
116 reading frames (ORFs), *gli123*, *gli349*, *gli521* and *gli42*, however, the other regions
117 have not been sequenced (21). The genome sequencing result of the *gli521* (P476R)
118 mutant was consistent with the previous report for the mutation in *gli521* (21) and also
119 showed additional mutations in the new regions (Table 1). One of the additional
120 mutations caused an amino acid substitution in MvspB, a surface protein (23, 36-39).
121 However, the reduced stall force should be caused by the mutation in Gli521, because
122 MvspB accounts for only 1.2% mass of all surface proteins and the antibody against a
123 closely related abundant protein, MvspI did not influence the gliding motility (23, 39).
124 The genomes of m6, m14, m27, m29 and m34 strains have not been sequenced yet, and
125 we identified various mutations in the present study. The cause of the decreased stall
126 force for m27 strain was suggested to be the mutation in *gli521* identified in the present
127 study. All strains had the same single amino acid substitution from serine to isoleucine
128 as 354th residue in MMOB1700, a homolog of ABC transporter permease based on
129 BLAST search. This mutation may be derived from a substitution caused on the clone
130 used for the reported genome sequencing, because the genome was sequenced for an
131 isolate from the wild-type strain (ATCC 43663) being used in the present study (36).
132 Interestingly, MMOB1700 showed other five different mutations in the 10 strains
133 analyzed, suggesting a special mechanism causing high mutation rate in this gene.
134 Next, we sequenced the genomes of nonbinding strains, m12, m13 and m23 which have
135 been reported to have mutations in *gli123*, *gli349* and *gli349*, respectively (12, 15, 16,
136 35). The genome sequencing showed that the identified mutations were consistent
137 with a previous report (35), although additional mutations were identified in other
138 regions.

139

140 **Binding and gliding of various strains.** To clarify the relation between the features
141 and mutation on the genome systematically, we examined the binding activities and the
142 gliding speeds for the wild-type, *gli521* (P476R), m6, m14, m27, m29 and m34 mutants
143 which can glide. The cell suspensions were adjusted to be the same optical density
144 and inserted into a tunnel chamber. After 15 min, we video recorded the cells to count
145 the numbers on a glass for bound-cell ratio and their gliding speed, as previously

146 reported (Fig. 3A) (29, 35, 40). The binding activities and the gliding speeds were
147 averaged for 20 independent images and 100 cells, respectively. The binding activity
148 and gliding speed of *gli521* (P476R) mutant were consistent with the previous study
149 (35). Other strains have not been analyzed by the method used here. The characters
150 of binding activities allowed classification of the strains into three types (Fig. 3B left).
151 (i) m6 was 44% of that of the wild-type strain. (ii) m14, m27 and m29 were 80%, 92%
152 and 73%, respectively. (iii) *gli521* (P476R) and m34 mutants were 159% and 145%,
153 respectively. We compared these data to the binding activities which were previously
154 estimated from hemadsorption, the adsorption of erythrocytes onto the surface of
155 colonies (12). The hemadsorption of m6, m14, m27, m29 and m34 was 24%, 93%,
156 113%, 96% and 122%, respectively, consistent with the analyses in the present study,
157 except m27. The gliding speed of the wild-type strain was $3.7 \pm 0.2 \mu\text{m/s}$ and the
158 mutants ranged from 80% to 103%, showing that the gliding speed differ less than the
159 binding activities (Fig. 3B right). The strains enhanced for binding, the *gli521*
160 (P476R) and m34 mutants had the reduced stall forces, suggesting that the stall force is
161 not determined simply by binding activity.
162 Interestingly, we noticed that the proportions of nongliding bound cells are much higher
163 in m6, m14, m27 and m29 strains than that of the wild-type strain. The proportions of
164 gliding cells in the cells on glass were 94% for the wild-type strain as previously
165 reported (40), but ranged 38% – 84% in m6, m14, m27 and m29 strains (Fig. S1).
166 This observation can be explained by assuming that the gliding can be achieved by
167 harmonized actions of many molecules and interactions.

168

169 **Effect of SOs on stall force.** An *M. mobile* cell glides as a result of integrated
170 movements for many legs. However, if the number of working legs is decreased, we
171 may detect the pulling force of single unit. Previous studies showed that the number
172 of working legs can be decreased by adding free SOs (9, 22, 25, 27, 40, 41). We
173 therefore added various concentrations of free sialyllactose (SL), an SO, to gliding *M.*
174 *mobile* cells and measured the stall force at 200 and 500 frames/s for the conditions of 0
175 – 0.25 and 0.33 and 0.5 mM free SL, respectively. The cells reached plateau by 30 –
176 40 s, and the time to reach the stall became longer with the concentration of free SL.

177 The stall force decreased from 113 to 19 pN with the addition up to 0.50 mM free SL
178 (Fig. 4A and B). These results suggest that the stall force is sum of the pulling force of
179 many units.

180

181 To detect the pulling force by smaller numbers of unit, we reduced the laser power of
182 optical tweezers to achieve higher resolution of the trace. In this experiment, we
183 applied 0.25 mM SL with the reduction of the trap stiffness of optical tweezers from 0.5
184 –0.7 to 0.1 pN/nm in which the trapped cells could escape from the trap center, and
185 measured the force under these conditions. The force increments with repeated small
186 peaks were detected, and the peaks were measured by a peak-finding algorithm and
187 summarized in Fig. 5A. The distribution of these increments was fitted by the sum of
188 four Gaussian curves whose peaks positioned at 1.1, 2.0, 3.2 and 4.4 pN (Fig. 5B).
189 The peaks positioned at twice, three and four times of the value of the first peak,
190 suggesting that these peaks may reflect single, double, triple and quadruple of the
191 minimum force increment (42). In the same way, the individual increments were
192 analyzed for the *gli521* (P476R) mutant and also fitted by the sum of four Gaussian
193 curves whose peaks positioned at 0.9, 1.8, 2.6 and 3.5 pN (Fig. 5B).

194

195 **Stepwise force increments.** As the force increments were detected under the
196 conditions of limited leg number, these force increments may be derived from the force
197 generation by a single leg or minimum force generation unit. Next, we added 0.50
198 mM SL to limit the number of working legs more with the reduction of the trap stiffness
199 to 0.06–0.07 pN/nm to detect the minimum force increments more precisely. As very
200 small ratio of cells remained on the glass surface under this condition, we attached a
201 bead to the cells by optical tweezers. Fifty one of 63 cells showed displacements.
202 Eight cells glided creeping displacements with occasional discontinuous increments,
203 which were mostly stepwise (Fig. 6A) and sometimes small peaks as shown in Fig 5.
204 Three cells showed continuous stepwise force increments (Fig. 6B). The average value
205 of force increments form 46 steps or peaks in 11 cell trajectories was 1.45 ± 0.44 pN
206 (Fig. 6C). These results suggest that the minimum force increment distributed from 1
207 to 2 pN, which should be the minimum unit force for gliding.

208

209 **Discussion**

210 **Mutations influencing gliding.** In the present study, we sequenced the whole
211 genomes of *M. mobile* mutants featured by binding ability, gliding motility and colony
212 spreading. Based on these results, we propose the proteins responsible or related to
213 these features. The m6 strain featured with reduced binding, slower gliding speed and
214 weaker stall force has a mutation in FtsH, MvspI and SecY (37-39, 43, 44). The
215 substituted amino acid in FtsH is not conserved in other mycoplasmas except
216 *Mycoplasma pulmonis*, a closely related species. The MvspI is unlikely related to their
217 gliding as described above (23). SecY is essential for protein secretion in bacteria,
218 generally (44). The substitution in SecY probably should affect the secretion of
219 gliding proteins, resulting in the reduction of binding activity of cells, because the
220 substituted amino acid is conserved in many mycoplasmas, including *Mycoplasma*
221 *hominis*, *Mycoplasma bovis*, and *M. pulmonis*. The m14 strain featured with reduced
222 binding and gliding, has a substitution of amino acid conserved in glucokinase which
223 phosphorylates glucose to glucose-6-phosphate at the first step of glycolysis (45).
224 Interestingly, the colony of m14 is less dispersed than that of the wild-type strain,
225 suggesting that the glucokinase is related to gliding including chemotaxis, because one
226 of the colony shape determinants is motility in many bacteria (12). The m27 featured
227 with the small proportion of gliding cells has a substitution at the 1461st amino acid of
228 total 4727 in the coded Gli521 protein, suggesting that the structure around this position
229 has an indispensable role for gliding. The m34 strain featured with the enhanced
230 binding has a substitution in the conserved amino acid of β subunit of F-type ATPase
231 (46). The membrane potential may be related to binding activity, because the F-type
232 ATPase is responsible for the membrane potential in *M. mobile*.

233

234 **Cell behavior in stall.**

235 The cells were stalled by the optical tweezers focusing to the bead bound to the back
236 end of cell. What events can we expect in the stall? The stall force decreased with
237 the addition of free SL (Fig. 4). This observation suggests that the legs repeat catch,
238 pull and release with SOs in stall rather than generate static force, because the stall force

239 should increase up to the stall force without SOs if the legs do not detach in stall. In
240 our gliding model, we suggest that the leg detaches by the tension caused by the
241 continuous cell displacement in gliding (14, 28, 41). The putative detach in the stall
242 may suggest that the directed detach occurs in much shorter displacement than expected
243 from the leg structure 95 nm in length. This assumption can explain the observation
244 that *gli521* (P476R) and m34 mutants showed smaller stall force than the wild-type
245 strain, although they have higher ratio of bound cell to glass (Fig. 2 and 3). Probably,
246 the higher ratio of bound cell is caused by the smaller force to detach the post stroke
247 legs.

248

249 **The unit number of gliding machinery.** The minimum force increment did not
250 change significantly with the distance from the laser. As the force increments occurred
251 additionally to the previous ones, the gliding unit should generate the same force
252 constantly in a rather long distance ranging 0 to 200 nm (Fig. 6).

253 In the present study, we measured the stall force and the minimum force increments of a
254 cell, which were about 113 and 1.5 pN, respectively (Fig 2B and 6C). Previously, the
255 number of legs in *M. mobile* was estimated to be 450 (16). The number of working
256 units was calculated from the stall force and minimum force increments of a cell, 113
257 over 1.5 pN is calculated to be about 75-fold, suggesting that 75 minimum units can
258 work simultaneously. Assuming that the minimum unit of force corresponds to the
259 single molecule of Gli349, one-sixth of Gli349 molecules are suggested to participate in
260 the force generation in the stalled state simultaneously (Fig. 1B). The friction
261 occurring at the interface between an *M. mobile* cell and water flow in the interlamellar
262 of carp gill is calculated to be 34 pN for the maximum, based on Stokes' law (11, 47).
263 This number is three folds smaller than the stall force of a cell, suggesting that a cell can
264 glide against water flow using the force generated by simultaneous strokes of many
265 legs.

266

267 ***M. mobile* gliding is featured with large step and small force.** We compare the step
268 size and the force of *M. mobile* gliding to those of conventional motor proteins such as
269 myosin, dynein, and kinesin, which perform the stepwise movements along the rail
270 proteins driven by the energy of ATP. The step sizes and the force of myosin-II,
271 cytoplasmic dynein, kinesin and myosin-V have been reported as 5.3, 8, 8 and 36 nm,
272 and 3–5, 7–8, 8 and 2–3 pN, respectively (48-53). Long step and small force of

273 myosin-V should be caused by the lever effect of 26-nm arms (54). The step size of *M.*
274 *mobile* has been reported as 70 nm, much larger than those of the conventional motor
275 proteins (22). The minimum unit force calculated here, 1–2 pN, suggests the gear
276 effect in the gliding machinery. Gli521, the force transmitter forms triskelion with
277 100-nm arms (24) and Gli349, the leg is shaped like an eighth note in musical notation
278 with 50-nm flexible string (26, 55), suggesting that these proteins cause the gear effect.
279

280 **Energy conversion efficiency of *M. mobile* gliding.** The direct energy source of *M.*
281 *mobile* gliding has been shown as ATP, based on the experiments that permeabilized
282 cells, “gliding ghost” can be reactivated by ATP (21). The gliding ghost showed
283 stepwise movement with dwell time dependent on the ATP concentration used,
284 suggesting that the step is coupled with ATP hydrolysis (22). Based on the minimum
285 unit force of 1–2 pN and the spring constant of 0.06–0.07 pN/nm, the work done per
286 step, W_{step} was calculated as 8–33 pN nm from the equation $W_{\text{step}} = 1/2 \times \text{spring}$
287 $\text{constant} \times \text{displacement}^2$. Assuming that one ATP molecule is consumed per step,
288 energy conversion efficiency of *M. mobile* gliding can be calculated around 10–40%
289 because generally about 80 pN nm free energy is available from a hydrolysis of one ATP
290 molecule.

291 F-type ATPase has been reported to reach 100% of energy conversion efficiency (56).
292 The gliding machinery of *M. mobile* has been suggested to be driven by the α - and
293 β -subunit paralogs of F-type ATPase (18). The force transmission from this motor to
294 the solid surfaces through several large components including Gli521 and Gli349 may
295 cause the loss of energy.

296

297 **Materials and methods**

298 **Strains and Cultivation.** *M. mobile* strain 163K (ATCC 43663) as the wild type and its
299 9 mutants were grown in Aluotto medium at 25°C, as previously described (12, 35, 40,
300 57).

301

302 **Surface modifications of *M. mobile* cells and polystyrene beads.** The cultured cells
303 were washed with PBS/G consisting of 75mM sodium phosphate (pH 7.3), 68 mM NaCl,
304 and 20 mM glucose, suspended in 1.0 mM Sulfo-NHS-LC-LC-biotin (Thermo Scientific,
305 Waltham, MA) in PBS/G, and kept for 15 min at room temperature (RT), as previously

306 described (28, 29, 33, 34, 40). Polystyrene beads 1.0 μm in diameter (Polysciences,
307 Warrington, PA) were coated with avidin (Sigma-Aldrich, St. Louis, MO), as previously
308 described (28).

309

310 **Force measurements.** The avidin-coated beads were attached to biotinylated cells in
311 two different ways, according to the concentrations of free SL used in experiments. In
312 force measurements under 0–0.13 mM SL conditions, the biotinylated cells were
313 inserted into a tunnel chamber which was precoated with 10% horse serum (20, 27, 29).
314 Avidin-coated beads were sonicated and inserted into the tunnel chamber with various
315 concentrations of free SL in PBS/G and bound to the cell (32). In force measurements
316 under 0.25–0.50 mM SL conditions, avidin-coated beads were sonicated and mixed with
317 biotinylated cells in a microtube, and kept for 10–30 min at RT. Then, the mixture was
318 inserted into a tunnel chamber and kept 15 min at RT. The chamber was washed with
319 PBS/G and the PBS/G was replaced by various concentrations of free SL in PBS/G.
320 Both ends of the tunnel were sealed with nail polish. The bead movements were
321 recorded at 200 or 500 frames/s and analyzed by the displacement up to 250 nm from the
322 trap center, the linear range of the laser trap, by using ImageJ 1.43u
323 (<http://rsb.info.nih.gov/ij/>) and IGOR Pro 6.33J (WaveMetrics, Portland, OR) (22, 28, 32,
324 58).

325

326 **Genome sequencing of various strains.** All strains were plated and isolated as
327 previously described (19). The genomic DNAs were isolated by using QIAGEN
328 DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany). The isolated genomic
329 DNA were sequenced by MiSeq (Illumina Inc., San Diego, CA) and mapped by CLC
330 Genomics Workbench 8 (QIAGEN, Hilden, Germany).

331

332 **Characterization of binding and gliding of various strains.** All strains were cultured
333 to reach an optical densities at 600 nm of 0.08. They were suspended and inserted into a
334 tunnel chamber (27, 29). The cell behaviors were recorded and analyzed as previously
335 reported (27, 35).

336

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342

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492

493 **Figure legends**

494

495 **Fig. 1.** Schematic illustration of gliding machinery. (A) Magnified illustration of unit.
496 The single unit consists of internal structure (upper blue) and three huge proteins,
497 Gli123 (purple), Gli349 (red) and Gli521 (green) on the cell surface. The force
498 generated by the internal structure based on ATP hydrolysis transmits through Gli521
499 and pulls Gli349 repeatedly. Gli349 catches and pulls SOs on the solid surface, and
500 the unit force was estimated around 1.5 piconewton (pN) in the present study (see Fig.
501 6). (B) About 75 legs (red) sticking out from the cell work simultaneously. The cell
502 glides in the direction of yellow arrow. Only bound legs are illustrated.

503

504 **Fig. 2.** Stall force of various strains. (A) Left: Illustration and micrograph of
505 measurement by using optical tweezers. The polystyrene bead (circle) 1 μm in
506 diameter bound to the cell was trapped by a focused laser beam (yellow hourglass) and
507 glides to the direction of white arrow. Black and pink crosses indicate a focal point of
508 laser and a bead center, respectively. Right: Optical micrographs of trapped cell. The
509 cell with bead (large black ring with white center) was trapped at 0 s and stalled at 80 s.
510 (B) Representative traces. The line colors correspond to the bars in the inset. Inset:
511 Averages were normalized by the WT value, and presented with standard deviations
512 (SD) ($n = 50, 42, 26, 31$ and 35). **, $P < 0.01$ (The difference from WT was supported
513 by Student's t -test).

514

515 **Fig. 3.** Binding and gliding properties of various strains. (A) The cell trajectories
516 were presented as a stack for 5 s, changing its color from red to blue. (B) Averages of

517 binding activity (right) and gliding speed (left) were normalized by the WT value and
518 presented with SD.

519

520 **Fig. 4.** Efficiency of SO on stall force. (A) Representative traces in various
521 concentrations of SL. (B) Concentration dependency on SL. Averages were plotted
522 with SD ($n = 50, 13, 23, 16, 16$ and 11 for $0, 0.05, 0.13, 0.25, 0.33$ and 0.50 mM,
523 respectively).

524

525 **Fig. 5.** Detection of force increments under low load and 0.25 mM SL. (A)
526 Representative traces of force transition in WT and *gli521* (P476R) mutant shown in
527 upper and lower panels, respectively. Green and cyan triangles in each panel indicate
528 small peak positions taken by a peak-finding algorithm. (B) Distributions of peak
529 values detected by the peak-finding algorithm were fitted by the sum of four Gaussian
530 curves. The first, second, third and fourth tops of Gaussian curves are $1.1, 2.0, 3.2$ and
531 4.4 pN in WT, and $1.0, 1.8, 2.6$ and 3.5 pN in *gli521* (P476R) mutant, respectively ($n =$
532 976 and 1067).

533

534 **Fig. 6.** Detection of force increments under low load and 0.50 mM SL. (A) Two
535 representative time courses of force generation. The trajectories in dashed rectangular
536 areas are magnified as insets and marked green and blue lines for increments and dwell
537 times. (B) Representative time course of continuous stepwise trajectory. The
538 histogram of PDF analysis for indicated steps was shown in right inset. (C) The
539 histogram of force increments for 46 steps or peaks. The averaged value was indicated
540 by a black triangle.

541

542 **Fig. S1.** Distributions of gliding speed of various strains in 10 s. Experimental data
543 were fitted by the sum of two Gaussian curves colored in blue.

544

545 **Movie S1.** Stall force of gliding cell carrying a bead measured by optical tweezers.

546 **Movie S2.** Stepwise movement of gliding cell carrying a bead detected with weak
547 trap.

Fig. 1.

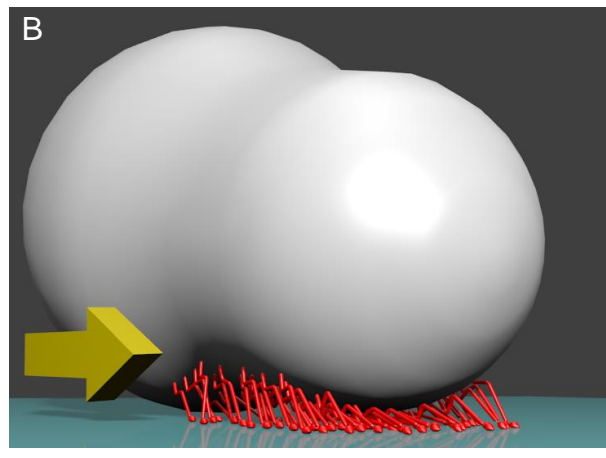
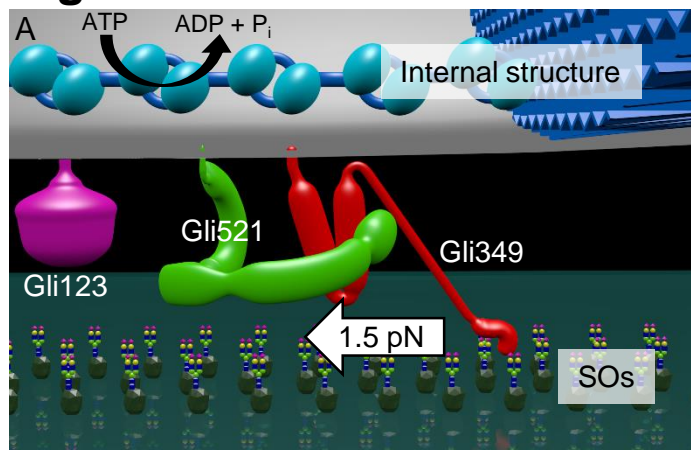


Fig. 2.

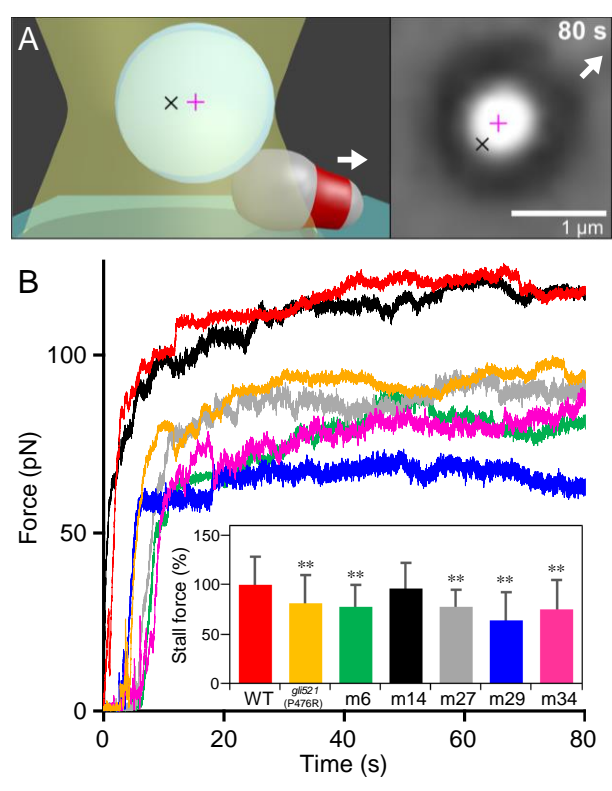


Fig. 3.

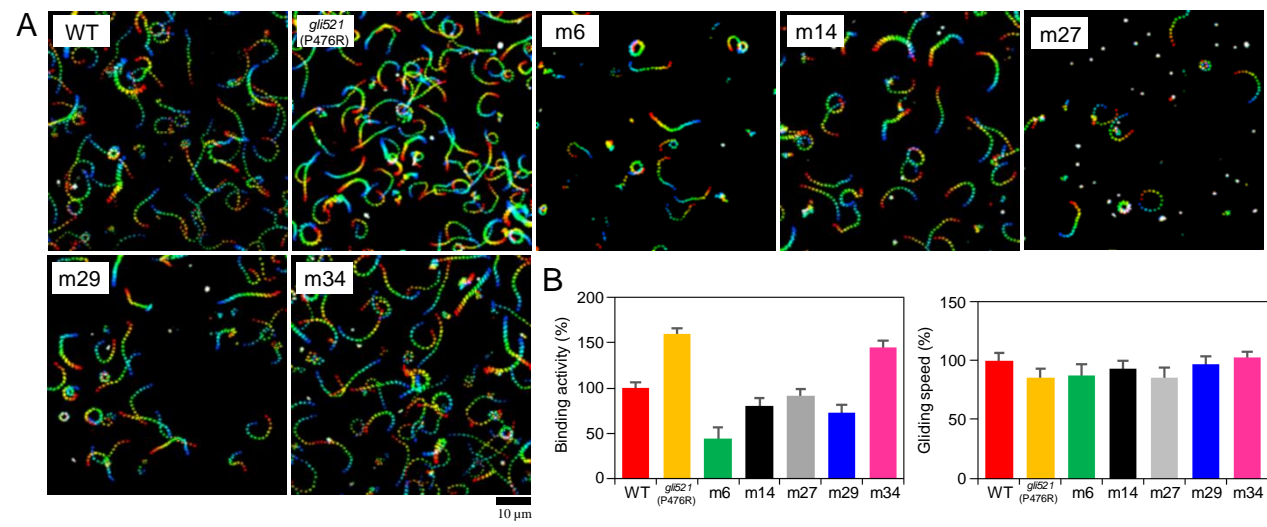


Fig. 4.

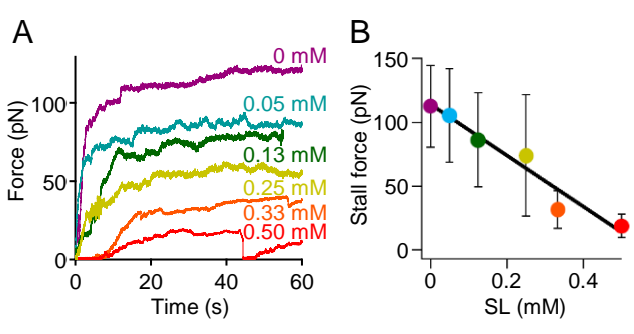


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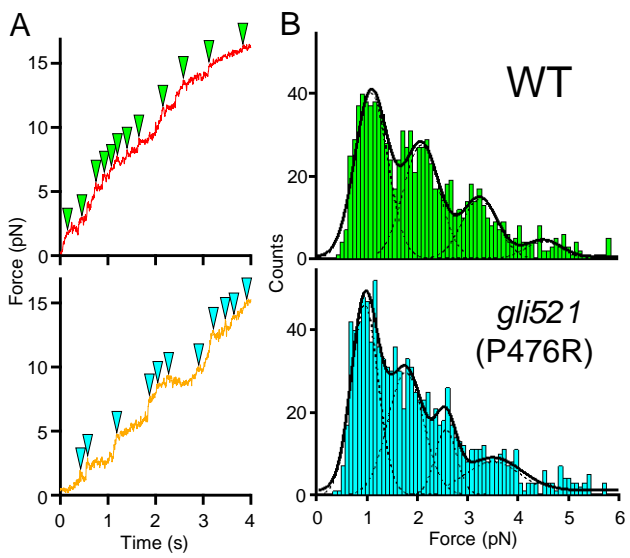


Fig. 6.

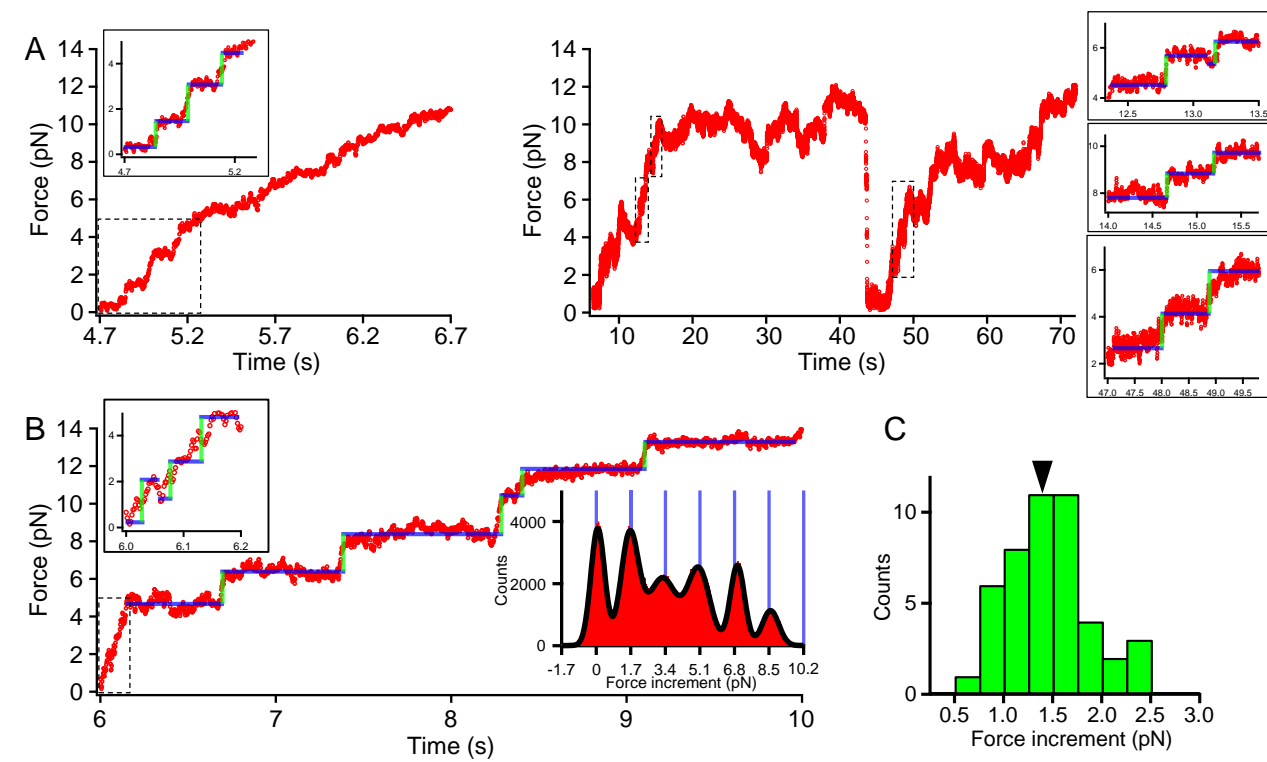


Fig. S1.

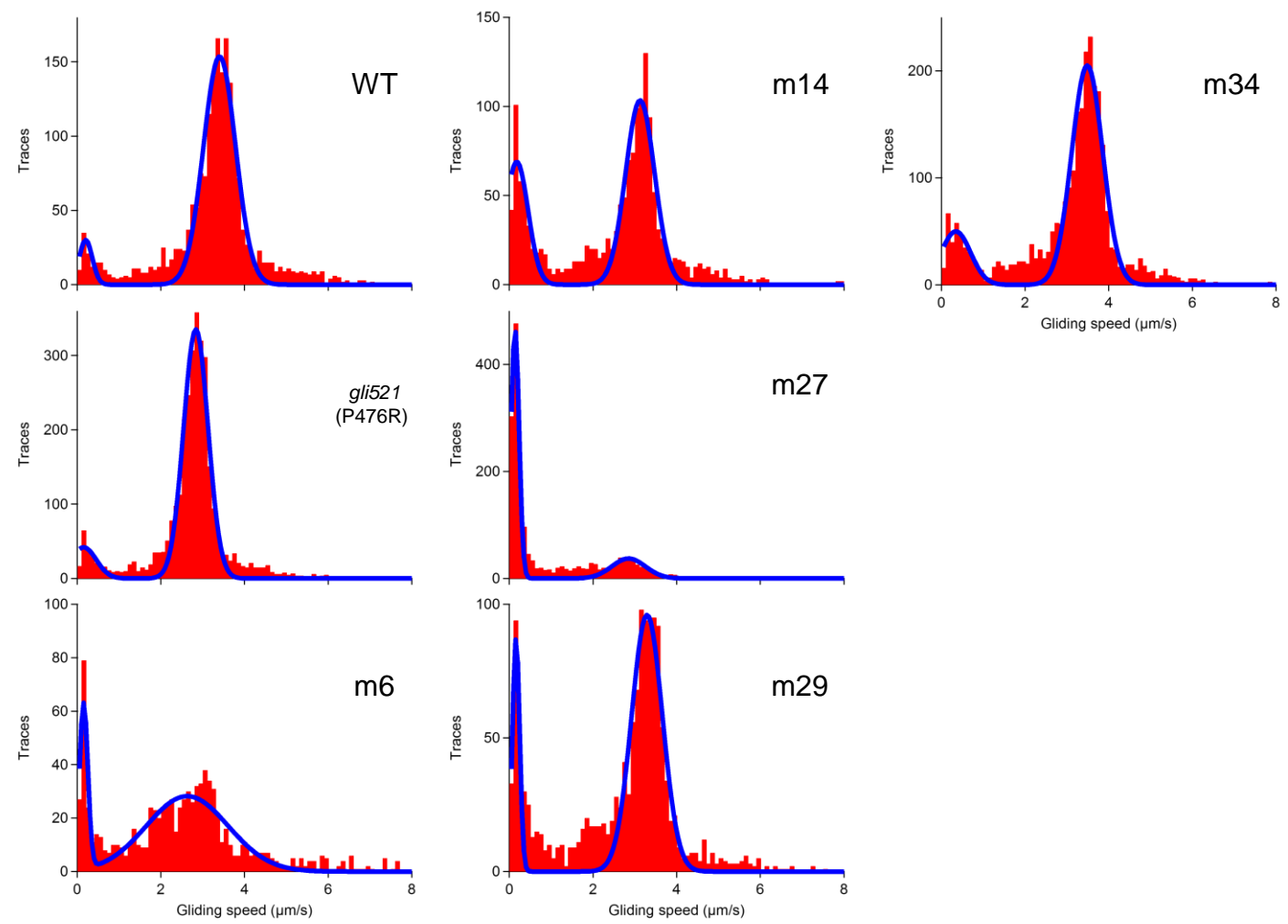


TABLE 1 Whole genome sequence of various strains

Strain	Nucleotide change ^a	Amino acid change ^b	Previously reported	Mutation type	ORF	Annotation ^c
WT	C232735A	S354I		missense	MMOB1700	Hypothetical
<i>gli521</i> (P476R)	C149908G	P476R	P476R	missense	MMOB1040	Gli521
	C232735A	S354I		missense	MMOB1700	Hypothetical
	399903T399904	M8–36*		frame shift	MMOB3220	MvspB
m6	C232735A	S354I		missense	MMOB1700	Hypothetical
	G308962A	G350R		missense	MMOB2540	SecY preprotein translocase subunit
	C423006- G676032C	E225–230* P191R		frame shift missense	MMOB3340 MMOB5400	MvspI FtsH
m12	C136968T	Q523*	Q523*	nonsense	MMOB1020	Gli123
	T214208- C232735A	F672–695* S354I		frame shift missense	MMOB1570 MMOB1700	Oligopeptide ABC transporter binding protein Hypothetical
	A232903T	I298N		missense	MMOB1700	Hypothetical
	C424498A	H195N		missense	MMOB3350	PolC truncated DNA polymerase III
m13	C142646T	Q1257*	Q1257*	nonsense	MMOB1030	Gli349
	C232735A	S354I		missense	MMOB1700	Hypothetical
m14	C232735A	S354I		missense	MMOB1700	Hypothetical
	233458T233459 C272657A	I113–142* P156Q		frame shift missense	MMOB1700 MMOB2030	Hypothetical Glucokinase
	C147186T	S2770L	S2770L	missense	MMOB1030	Gli349
m23	T214208- C232735A	F672–695* S354I		frame shift missense	MMOB1570 MMOB1700	Oligopeptide ABC transporter binding protein Hypothetical
	A233098C	I233S		missense	MMOB1700	Hypothetical
	A345995T	N18Y		missense	MMOB2830	HsdR Type I restriction enzyme r protein
	A73556G	L101L		silent	MMOB0550	Frr ribosome recycling factor
m27	C152863T	P1461L		missense	MMOB1040	Gli521
	C232735A	S354I		missense	MMOB1700	Hypothetical
	C232735A	S354I		missense	MMOB1700	Hypothetical
m29	A587414T	K29N		missense	MMOB4660	TruB tRNA pseudouridine synthase B
	C654504T	W82*		nonsense	MMOB5190	Hypothetical
	A754250- C232735A	I126* S354I		frame shift missense	MMOB6120 MMOB1700	Hypothetical Hypothetical
	A232753G	I348T		missense	MMOB1700	Hypothetical
m34	T365326A	D304V		missense	MMOB2960	AtpD F ₀ F ₁ ATPase beta subunit

^a Numbering is as in the sequence under accession number NC_006908.^b Asterisks indicate stop codons.^c Annotations are based on Molligen 3.0.