

1 Antimicrobial silver inhibits bacterial movement and stalls 2 flagellar motor

3
4

5 Benjamin Russell¹, Ariel Rogers¹, Matthew Kurilich¹, Venkata Rao Krishnamurthi¹, Jingyi Chen^{2,3},
6 Yong Wang^{1,3,4*}

7

8 ¹Department of Physics, ²Department of Chemistry and Biochemistry, ³Microelectronics-
9 Photonics Program, ⁴Cell and Molecular Biology Program, University of Arkansas, Fayetteville, AR,
10 72701, USA.

11

12 *To whom correspondence should be addressed: yongwang@uark.edu (YW).

13

14

15 Abstract

16

17 Silver (Ag) has been gaining broad attention due to their antimicrobial activities and the
18 increasing resistance of bacteria to commonly prescribed antibiotics. However, various aspects
19 of the antimicrobial mechanism of Ag have not been understood, including how silver affects the
20 motility of bacteria, a factor that is intimately related to bacterial virulence. Here we report our
21 study on the antibiotic effects of Ag⁺ ions on the motility of *E. coli* bacteria using swimming and
22 tethering assays. We observed that the bacteria slowed down dramatically when subjected to
23 Ag⁺ ions, providing direct evidence showing that Ag inhibits the motility of bacteria. In addition,
24 through tethering assays, we monitored the rotation of flagellar motors and observed that the
25 tumbling frequency of bacteria increased significantly in the presence of Ag⁺ ions. Furthermore,
26 the rotation of bacteria in the tethering assays were analyzed using hidden Markov model
27 (HMM); and we found that Ag⁺-treatment led to a significant decrease in the tumbling-to-running
28 transition rate of the bacteria, suggesting that the rotation of bacterial flagellar motors was
29 stalled by Ag⁺ ions. This work provided a new quantitative understanding on the mechanism of
30 Ag-based antimicrobial agents in bacterial motility.

31

32

33 **Keywords:** hidden Markov model, antibiotics, *E. coli*, motility, tethering assay.

34

35 Introduction

36

37 The rising prevalence of antibiotic-resistance in harmful microbes due to overuse of conventional
38 antibiotics has become a serious global concern for public health[1, 2, 3], posing the need for
39 different approaches for fighting against drug-resistant microbes[4, 5]. Recent research in the
40 past two decades revisited the antimicrobial activities of noble metals, such as silver (Ag), in
41 different forms – including ions and nanoparticles – and has uncovered their strong capacity for
42 suppressing bacterial growth and killing bacteria[6, 7, 8]. Exciting progress has been made
43 towards understanding the antimicrobial mechanism of Ag, suggesting that Ag caused
44 multidirectional damages to bacteria, including DNA damage, membrane disruption, free radical
45 generation (ROS), and loss of ATP production[7, 9, 10, 11, 12, 13]. However, various aspects of
46 the antimicrobial mechanism of Ag remain elusive, especially that the temporal resolution for
47 understanding the Ag-caused damages in bacteria Ag is still limited[7, 14, 15]. This includes how
48 silver affects the motility of bacteria, which is tightly coupled to bacterial virulence[16].

49

50 Motility is essential to many bacteria for detecting and pursuing nutrients, as well as avoiding
51 and fleeing from toxicants. Certain bacteria, such as *Escherichia coli* (*E. coli*), use flagella to move
52 in aqueous environments[17]. *E. coli* flagella are filaments extending outward from the
53 bacteria[18]. The flagella are connected to and driven by motors embedded in the bacterial
54 membrane through hooks[19]. For *E. coli* – peritrichous bacteria with flagella covering their entire
55 surfaces, their movement depends on the rotation direction of their flagella[17, 19]. When
56 flagella rotate counterclockwise (CCW), they are bundled and propel the bacteria to move
57 directionally (i.e., running) for purposeful movement toward chemical attractants or away from
58 repellents[17, 20]; when flagella rotate clockwise (CW), they are splayed out, resulting in
59 reorientation (i.e., tumbling) of the bacteria[17, 20]. The *E. coli* flagella contains mainly three
60 parts: the filament, the hook, and the basal body[17, 20]. The basal body consists of several rings,
61 some of which (e.g., MS ring and C ring) are essential components of the flagellar motor for
62 driving the rotation of the flagella[17]. Structurally, the flagellar motor involves both the stator
63 proteins (e.g., MotA and MotB) and the rotor proteins (e.g., FliG, FliM, and FliN), which also play
64 critical roles in the torque generation of the motor[17]. Functionally, the CW/CCW direction of
65 the flagellar motor's rotations relies on another set of chemotaxis proteins (e.g., CheY, CheZ,
66 CheA, CheW, CheR, and CheB). For example, the flagellar motor switches from CCW rotation to
67 CW rotation when the phosphorylated response regulator CheY binds to the flagellar motor[21].

68

69 As Ag in various forms (e.g., ions, nanoparticles) suppresses and kills bacteria, we hypothesized
70 that the motility of bacteria is significantly affected by Ag. This hypothesis is indirectly supported
71 by evidence from previous studies. For example, Ivask et al. performed liquid-culture-based high-
72 throughput growth assays for a library of single-gene-deletion strains of *E. coli*, and found that a
73 series of flagella-related mutants (e.g., *fliG*, *fliM*, *flgF*, *flgG*, etc., which are involved in the
74 assembly and function of flagella) were sensitive to Ag⁺ ions and Ag nanoparticles[13]. Also, plate-
75 based chemical genetic screening assays on a similar library identified and confirmed some
76 flagella-related genes (e.g., *flgA*, *flgD*, *flgJ*, *flgK*, *fliC*, *fliE*, *fliL*, *fliP*, *fliR*, and *motB*)[22]. In addition,
77 recent work by us and others showed that Ag affects the organization and function of certain

78 universal regulatory proteins in bacteria, such as histone-like nucleoid structuring (H-NS)
79 proteins, which regulate bacterial chemotaxis and motility[14, 22, 23, 24, 25]. Furthermore,
80 although mixed results were present, plate-based swimming and swarming motility assays
81 suggested that Ag could change the motility of bacteria under certain conditions[26]. On the
82 other hand, Ag⁺ ions have been used for staining bacterial flagella for decades[27], implying that
83 Ag⁺ ions interact with flagella.

84
85 However, few studies on real-time observation and quantification of Ag's effects on bacterial
86 movement are presented in the literature[7, 28]. In this work, we investigated the antibiotic
87 effects of Ag⁺ ions on the swimming behavior of *E. coli* bacteria based on microscopic imaging,
88 with a temporal resolution of 15–50 ms. Ag⁺ ions were chosen for two reasons. First, Ag⁺ ions are
89 effective at suppressing and killing bacteria[6, 10, 29]. Second, the release of Ag⁺ ions from AgNPs
90 is one major contribution to the toxicity of AgNPs[7]. Through the swimming assays, we provided
91 direct evidence showing that Ag inhibits the motility of bacteria. In addition, we monitored the
92 rotation of flagellar motors of *E. coli* bacteria through tethering assays in the absence and
93 presence of Ag⁺ ions, directly observing that Ag⁺ ions increased the frequency of bacterial
94 tumbling. Furthermore, based on hidden Markov model (HMM) analysis, we found that Ag⁺-
95 treatment caused bacterial transition rate from the tumbling state to the running state to
96 decrease significantly, suggesting that the rotation of bacterial flagellar motors was stalled by Ag⁺
97 ions. This real-time quantification analysis by high temporal resolution microscopic imaging
98 provides direct evidences of Ag effects on bacterial mobility.

99

100 **Materials and Methods**

101

102 **Bacterial strain and growth**

103

104 An *E. coli* K12-derived strain from Refs.[15, 23, 30, 31] was used in this study. The strain has been
105 used in previous investigations of the antimicrobial activities of Ag⁺ ions and AgNPs[15, 23, 31].
106 This strain has the *hns* gene knocked out from the chromosomal DNA, but supplemented with a
107 plasmid encoding for the H-NS protein fused to mEos3.2 fluorescent protein[32] and for
108 resistance to kanamycin and chloramphenicol[15, 23, 30, 31].

109

110 Each experiment started with inoculating a single bacterial colony into 5 mL of Luria Broth (LB)
111 medium supplemented with kanamycin and chloramphenicol (50 µg/mL and 34 µg/mL,
112 respectively)[23]. The liquid culture was grown at 37°C in a shaking incubator (250 RPM)
113 overnight. On the second day, the overnight culture was diluted by 5000× into 5mL of fresh LB
114 medium with the antibiotics. The new culture was grown at 32°C[33, 34, 35] in the shaking
115 incubator until the bacterial culture reached the mid-exponential phase ($OD_{600} \approx 0.3$), followed
116 by measurements as described below.

117

118 **Phase contrast microscopy**

119

120 Measurements in the swimming and tethering assays were done at room temperature using
121 phase contrast microscopy on an Olympus IX-73 inverted microscope equipped with a 100×,
122 NA=1.25 phase-contrast, oil-immersion objective (Olympus) and an EMCCD camera (Andor
123 Technology). The microscope and data acquisition was controlled using Micro-Manager[36, 37].
124 The effective pixel size of recorded images/movies was 0.16 μm.

125

126 **Swimming assay**

127

128 In swimming assay experiments, *E. coli* bacteria at $OD_{600} \approx 0.3$ were treated with Ag^+ ions at 30
129 μM or 40 μM for 1, 2, and 4 hr, which clearly showed suppressed growth. At each time point, 2
130 mL of the bacterial culture were transferred to a cleaned glass-bottom Petri-dish, followed by
131 monitoring and recording the free swimming of the bacteria using phase-contrast microscopy.
132 The swimming of untreated bacteria (i.e., before the addition of Ag^+ ions, or 0 hr) was monitored
133 and used as negative controls. The exposure time was set to 30 ms, while the actual time interval
134 between adjacent frames of the acquired movies was 54 ms. The acquired movies of freely
135 swimming bacteria were processed in ImageJ by inversion, smoothing, and background
136 subtraction[38, 39], followed by automated identification and localization of the bacteria using
137 custom-written MATLAB programs[40]. The localizations of the bacteria were then linked into
138 trajectories following standard algorithms[40, 41, 42], using a maximum displacement between
139 adjacent frames of 1.92 μm (12 pixels), a memory of 0 frame (i.e., no gap), and a minimum length
140 of 12 frames. The identified trajectories further went through a manual quality control process
141 by removing the bacteria that were stuck on the glass surface or formed large clumps.

142

143 The trajectories of the bacteria in the freely swimming assays were further analyzed using
144 custom-written or open-source Python programs. For example, the instantaneous velocities
145 were calculated from the trajectories $\mathbf{r}(t)$ of the bacteria, $v(t) = \left| \frac{\mathbf{r}(t+\Delta t) - \mathbf{r}(t)}{\Delta t} \right|$, where $\Delta t = 54$
146 ms. In addition, we estimated the maximum chord-to-arc ratio (γ_{CA}^M) for each trajectory, inspired
147 by TumbleScore[43], $\gamma_{CA}^M = \frac{\max_{i,j}(|\mathbf{r}_i - \mathbf{r}_j|)}{\sum_i |\mathbf{r}_{i+1} - \mathbf{r}_i|}$, where \mathbf{r}_i and \mathbf{r}_j were positions of a single trajectory.
148 Furthermore, the changing rates of swimming directions Ω were estimated directly from the
149 trajectories[43, 44, 45], $\Omega_i = \cos^{-1} \left(\frac{\mathbf{v}_{i+1} \cdot \mathbf{v}_i}{|\mathbf{v}_{i+1}| |\mathbf{v}_i|} \right)$. Lastly, we calculated the ensemble mean-square-
150 displacement (MSD) for each sample using the *trackpy* Python package[42], $MSD(\tau) =$
151 $\langle (\mathbf{r}(t + \tau) - \mathbf{r}(t))^2 \rangle$, where τ is the lag time.

152

153 **Tethering assay**

154

155 In tethering assay experiments[46, 47], *E. coli* bacteria in the mid-exponential phase ($OD_{600} \approx 0.3$)
156 were tethered to glass-bottom Petri-dishes through their flagella. The tethering was achieved by
157 coating the glass surface with biotinylated BSA, neutravidin, and biotinylated anti-FliC antibody
158 sequentially[48, 49]. *E. coli* flagella bind to the anti-FliC antibody[50, 51], immobilizing the
159 bacteria. The rotations of the tethered bacteria were monitored and recorded under phase
160 contrast microscopy with an exposure time of 5 ms for 10000 frames without Ag^+ ions (the actual

161 time interval between adjacent frames was 14.1 ms). Then Ag^+ ions were directly added to the
162 Petri-dish at a final concentration of 40 μM , followed by recording the rotations of the same
163 bacteria for 50,000 to 100,000 frames. For negative controls, LB medium (instead of Ag^+ ions)
164 was added to the Petri-dish and the rotations of the bacteria were recorded similarly. More than
165 10 replicate experiments were performed independently on different days.

166
167 Bacteria in the tethering assays were identified and characterized in each frame of the recorded
168 movies using custom-written Python programs based on the *scikit-image* package[52]. From the
169 primary axis of the identified bacteria, the orientation θ of the bacteria were obtained[53, 54],
170 followed by estimating the angular velocities of the bacterial rotation $\omega = \Delta\theta/\Delta t$, where $\Delta t =$
171 0.0141 s. Note that frames containing other non-tethered bacteria invading the region of the
172 tethered ones were removed from further analysis to ensure accuracy. The ω -trajectories were
173 analyzed using the hidden Markov model (HMM)[55], in which two states of the bacteria (running
174 and tumbling) were assumed. In addition, Gaussian emission distributions were applied for the
175 emission from the two states to the observable (i.e., angular velocities ω)[55]. The HMM analysis
176 was done using the *hmmlearn* Python package. For each bacterium in the tethering assay, we
177 fitted the HMM model using the ω -trajectory before the addition of Ag^+ ions (or LB medium).
178 Then the fitted model was used to predict the states for the data after the addition of Ag^+ ions
179 (or LB medium), from which the probabilities of the two states and the transition rates were
180 estimated[56].

181

182 Results

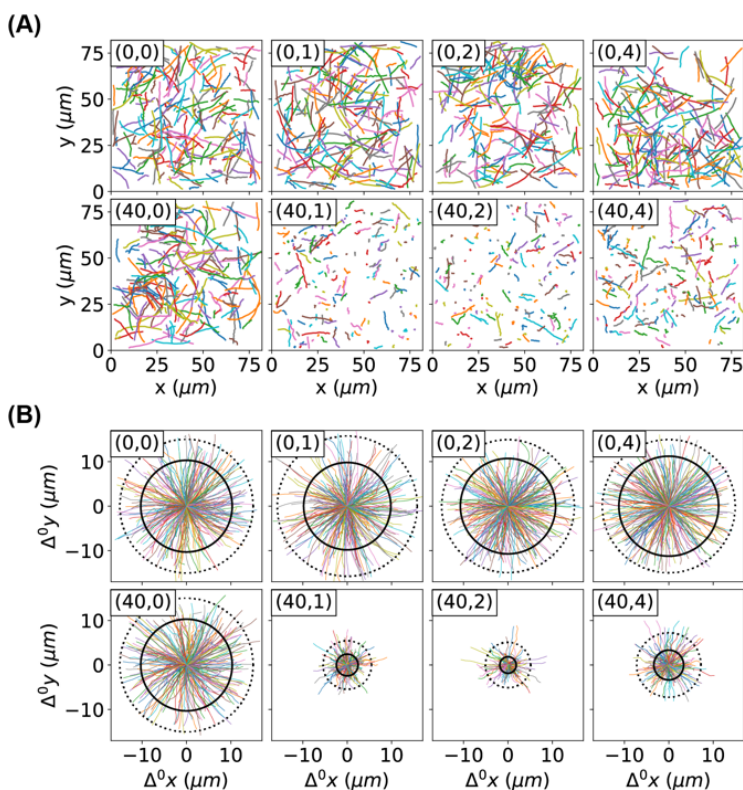
183

184 Lower motility of bacteria caused by Ag^+ ions

185

186 We first examined the effects of Ag^+ ions on the motility of *E. coli* bacteria using swimming
187 assays[57, 58, 59]. When *E. coli* bacteria at $\text{OD}_{600} \approx 0.3$ were treated with Ag^+ ions at 40 μM for
188 1, 2, and 4 hr, the cell density did not increase and the bacterial growth was suppressed. At each
189 time point, 2 mL of the bacterial culture were taken to a glass-bottom Petri-dish, followed by
190 monitoring and recording the free swimming of the bacteria using phase-contrast microscopy.
191 Untreated bacteria (i.e., 0 hr) were measured as negative controls, and we observed that the
192 treated bacteria were much slower (Movies M1 and M2). From the movies of the freely
193 swimming bacteria, the trajectories $\mathbf{r}(t)$ of individual bacteria were obtained. 200 randomly
194 chosen examples of trajectories for each experimental condition were shown in Fig. 1A, where
195 longer traveling distances were observed for the untreated bacteria compared to the ones
196 treated with Ag^+ ions. To see this difference more clearly, we plotted the corresponding rose
197 graphs[43], in which the displacements of the bacteria from their individual initial positions were
198 drawn, $\Delta\mathbf{r}^0(t) = \mathbf{r}(t) - \mathbf{r}(0)$. 300 randomly chosen examples were shown in Fig. 1B, where the
199 first 12 frames of the trajectories were shown to eliminate the differences due to different
200 lengths of trajectories[43]. It is obvious that the motility of bacteria decreased significantly after
201 the treatment with Ag^+ ions. Note that, although the trajectories are longer (up to ~ 70 frames),
202 only the first 12 frames were used in the rose graphs (Fig. 1B) to make direct comparisons. We
203 quantified the mean and 90th percentile of the displacements of the first 12 frames of all the

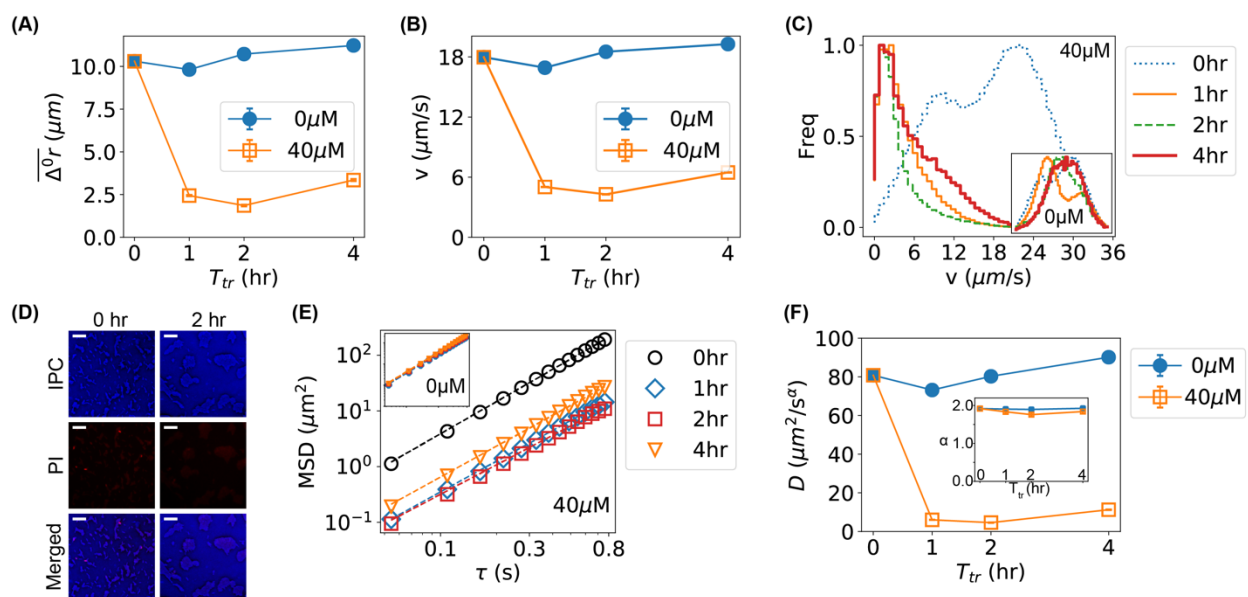
204 trajectories in each condition, shown as solid and dotted circles in the rose graphs (Fig. 1B),
 205 respectively. We found that the two circles for untreated bacteria did not change significantly
 206 from 0 to 4 hr, indicating that the motility of the bacteria remained similar. In contrast, the
 207 treated bacteria showed much smaller radii for both the mean ($\overline{\Delta^0 r}$) and 90th percentile circles,
 208 indicating Ag⁺-treatment led to lower bacterial motility. We also note that the radii slightly
 209 increased for longer treatment time, implying possible recovery of the bacteria as reported by
 210 our previous results[6, 15].
 211



212
 213 **Figure 1.** Motion of bacteria. (A) Trajectories of bacteria, untreated or treated by Ag⁺ ions at 30
 214 μM or 40 μM. Each sub-figure contains 200 randomly chosen trajectories, and is labeled by (c_{Ag},
 215 T_{tr}), where c_{Ag} is the concentration of Ag⁺ ions, and T_{tr} is the treatment/incubation time. (B) Rose
 216 graphs of the first 12 frames of trajectories of bacteria, untreated or treated by Ag⁺ ions at 30 μM
 217 or 40 μM. Each sub-figure is labeled similar as in panel A. Under each condition, 300 randomly
 218 chosen examples of the trajectories were shown in color, while the mean and 90th percentile of
 219 the displacements of the first 12 frames of all the trajectories were shown as solid and dotted
 220 circles, respectively.
 221
 222

223 The slower motion of bacteria caused by Ag⁺ ions was further visualized in Fig. 2A by plotting the
 224 radii of the mean circles in the rose graphs ($\overline{\Delta^0 r}$, Fig. 1B) as functions of treatment time. To
 225 further confirm that the Ag⁺ ions inhibits the movement of bacteria, we calculated the
 226 instantaneous velocities of the bacteria directly from the trajectories, $v = |\mathbf{v}| = |\Delta \mathbf{r} / \Delta t|$ where
 227 $\Delta t = 0.054$ s is the time interval between adjacent frames. The dependence of the mean velocity

228 on the treatment time is shown in Fig. 2B, showing the same trends as $\overline{\Delta^0 r}$. In addition, we
 229 examined the distributions of the bacterial velocities (Fig. 2C), and observed a double-peak
 230 distribution (centered around 10 and 22 $\mu\text{m/s}$) for the untreated bacteria ($t = 0$ hr), while Ag^+ -
 231 treatment moved the peak to ~ 2 $\mu\text{m/s}$ (Fig. 2C). Such significant shift in the velocity-distribution
 232 was absent in the negative controls (inset of Fig. 2C). We also found that a second peak/shoulder
 233 (7–10 $\mu\text{m/s}$) emerged in the distributions of bacterial velocities at 4 hr (Fig. 2C), consistent with
 234 the previously observed recovery of the bacteria[6, 15].
 235



236
 237 **Figure 2.** Lower motility of bacteria caused by Ag^+ ions. (A) The dependence of the mean
 238 displacements ($\overline{\Delta^0 r}$) of the first 12 frames of all trajectories of bacteria on incubation/treatment
 239 time in the absence (0 μM) and presence of Ag^+ ions (40 μM). (B) The dependence of the mean
 240 bacterial velocity on incubation/treatment time in the absence (0 μM) and presence of Ag^+ ions
 241 (40 μM). (C) Distributions of bacterial velocities in the presence of Ag^+ ions at 40 μM for 0, 1, 2,
 242 and 4 hr. Inset: the corresponding result for untreated bacteria (0 μM). (D) Cell viability assay
 243 based on propidium iodide (PI) staining for untreated (0 hr, left column) and treated (2 hr, right
 244 column) bacteria. Top: inverted phase-contrast (IPC) images; Middle: fluorescence images due to
 245 PI staining; Bottom: merged IPC/PI images. Scale bar = 16 μm . (E) Log-log plot of mean-square-
 246 displacements (MSD) vs. lag time (τ) for trajectories of treated bacteria by Ag^+ ions at 40 μM for
 247 0, 1, 2, and 4 hr. Inset: the corresponding result for untreated bacteria (0 μM). (F) Dependencies
 248 of the generalized diffusion coefficient D and the anomalous scaling exponent α (inset) on the
 249 incubation/treatment time T_{tr} .
 250
 251

252 As the bacterial velocities after Ag^+ -treatment were close to 0 (peaked at ~ 2 $\mu\text{m/s}$), one
 253 possibility is that the bacteria were killed by the bacteria at the given concentrations (40 μM) of
 254 Ag^+ ions. However, this possibility was not favored for the following reasons. First, our previous
 255 work showed that the majority of bacteria treated with 60 μM Ag^+ ions were alive, fighting
 256 against damages caused by Ag^+ ions and showing oscillations in their cell-lengths within 12 hr[15].

257 Second, cell viability assay based on propidium iodide staining[60] showed that the majority of
258 treated bacteria were alive at 40 μM Ag^+ ions (Fig. 2D). Third, if the bacteria were killed, they
259 would display random diffusion (Brownian motion) and the corresponding mean-square-
260 displacement (MSD) would be proportional to the diffusion coefficient (D) and the lag time (τ)
261 and shows a slope of 1 in the log-log plot of MSD vs. τ (Fig. 2E) [61, 62]; however, fitting the
262 experimental MSD curves (Fig. 2E) with $MSD = 4D\tau^\alpha$ (α is the anomalous scaling exponent)
263 showed that α remained ≈ 2 in the presence of Ag^+ ions for various amounts of time (inset of Fig.
264 2F), indicating that the bacteria retained active motion after Ag^+ -treatment[31, 63]. In contrast,
265 the diffusion coefficient decreased significantly (Fig. 2F), following the same dependence on
266 treatment time as the mean velocity of the bacteria (Figs. 2A and 2B).

267

268 **Comparison of bacterial movement before and after Ag^+ -treatment**

269

270 We quantitatively compared the movement of bacteria before and after Ag^+ -treatment by
271 examining the velocity autocorrelation. Briefly, we calculated the autocorrelations of the x and y
272 components of bacterial velocities, $C_{v_i}(\tau) = \frac{\langle v_i(t+\tau)v_i(t) \rangle}{\langle v_i(t)v_i(t) \rangle}$, where $v_i = v_x$ or v_y and τ is the lag
273 time. For the untreated bacteria, the velocity autocorrelation did not change at different
274 incubation time (insets of Figs. 3A and 3B); in contrast, treating the bacteria with Ag^+ ions resulted
275 in shifts to the left in the velocity autocorrelation (Figs. 3A and 3B). The left-shift of the velocity
276 autocorrelation suggested that the “persistence” time of the bacterial movement became
277 shorter after Ag^+ -treatment, and the movement of bacteria became not as straight as that before
278 treatment.

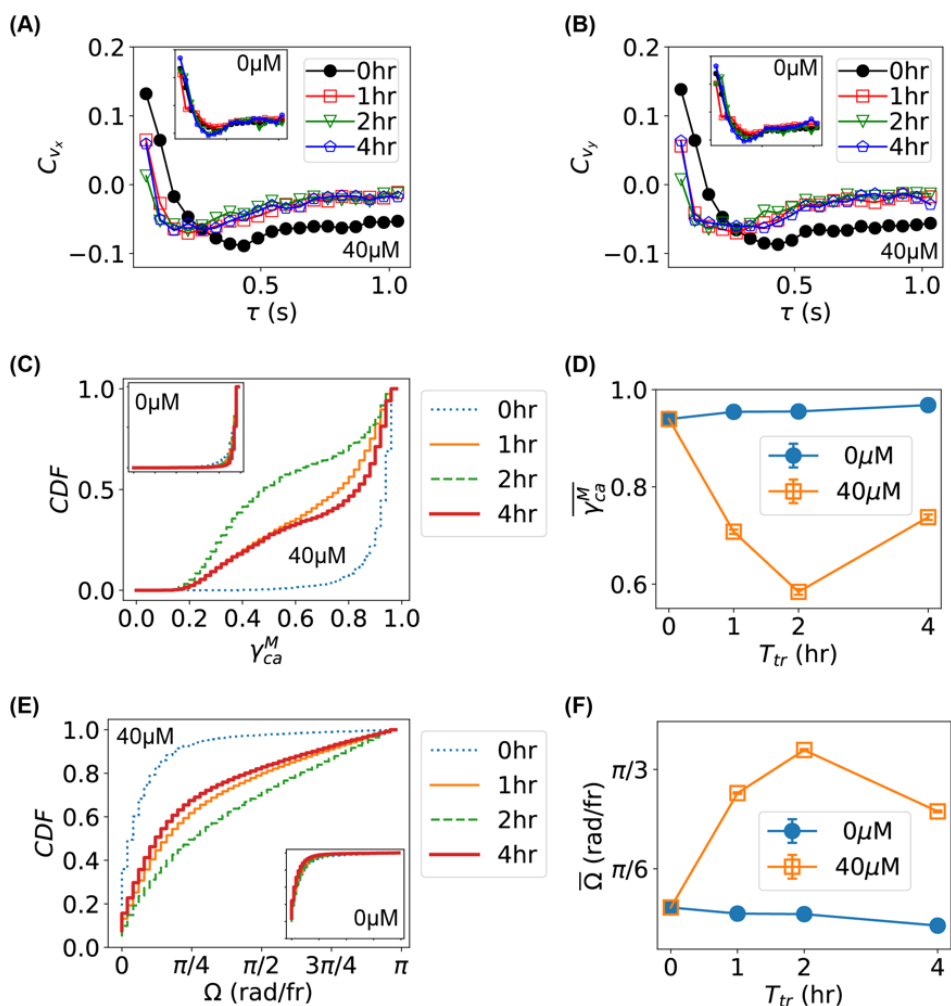
279

280 We also examined the maximum chord-to-arc ratio (γ_{CA}^M) of the trajectories (inspired by
281 TumbleScore[43]), $\gamma_{CA}^M = C^M/A$, where $C^M = \max_{i,j}(|\mathbf{r}_i - \mathbf{r}_j|)$ is the maximum chord length of
282 a trajectory and $A = \sum_i |\mathbf{r}_{i+1} - \mathbf{r}_i|$ is the “arc” length of the trajectory. If a trajectory is straight,
283 $\gamma_{CA}^M \approx 1$, while a trajectory dominated by directional changes gives $\gamma_{CA}^M \approx 0$; therefore, the
284 maximum chord-to-arc ratio could be used as another indicator of the persistence of the
285 trajectories. The cumulative distributions (CDF) of the γ_{CA}^M of all the trajectories for bacteria
286 untreated (0 μM and/or 0 hr) or treated with Ag^+ ions for 1, 2, and 4 hr are shown in Fig. 3C.
287 Compared to the untreated bacteria, the CDFs for treated bacteria rose up at lower γ_{CA}^M values,
288 indicating that Ag^+ ions led to higher fractions of lower γ_{CA}^M . This change was obvious by
289 examining the time dependence of the mean values of γ_{CA}^M (Fig. 3D). Note that a similar result
290 was observed for the normalized maximum chord-to-arc ratio $\beta_{CA}^M = \gamma_{CA}^M/N$ where N is the
291 length of the trajectory.

292

293 Furthermore, we estimated the changing rate of moving directions directly from the trajectories,
294 $\Omega = \cos^{-1}(\mathbf{v}_{i+1} \cdot \mathbf{v}_i / v_{i+1}v_i)$ [43, 44, 45]. The CDFs of Ω for all the trajectories of bacteria
295 untreated (0 μM and/or 0 hr) or treated with Ag^+ ions for 1, 2, and 4 hr are shown in Fig. 3E. We
296 found that the CDFs lowered down after Ag^+ -treatment, indicating increased fraction of higher Ω
297 values. This was confirmed by the time dependence of the mean values of Ω (Fig. 3F). All the
298 three quantifications (C_v , γ_{CA}^M , and Ω) showed consistent result that the movement of bacteria
299 became less persistent (i.e., less straight) after subjecting the bacteria to Ag^+ ions.

300



301
 302 **Figure 3.** Characterization of bacterial movement and comparison between untreated and
 303 treated bacteria. (A, B) Autocorrelation of velocities (A: v_x ; B: v_y) for bacteria treated with Ag^+
 304 ions at $40 \mu\text{M}$ for 0, 1, 2, and 4 hr. Insets: the corresponding results for untreated bacteria. (C)
 305 Cumulative distribution function (CDF) of the maximum chord-to-arc ratio (γ_{CA}^M) for the
 306 trajectories of bacteria untreated (0 hr) or treated with $40 \mu\text{M}$ Ag^+ ions for 1, 2, and 4 hr. (D)
 307 Dependence of the mean of γ_{CA}^M on treatment time. (E) CDF of the changing rate of swimming
 308 directions (Ω) for bacteria untreated (0 hr) or treated with $40 \mu\text{M}$ Ag^+ ions for 1, 2, and 4 hr. (F)
 309 Dependence of the mean of Ω on treatment time.

310

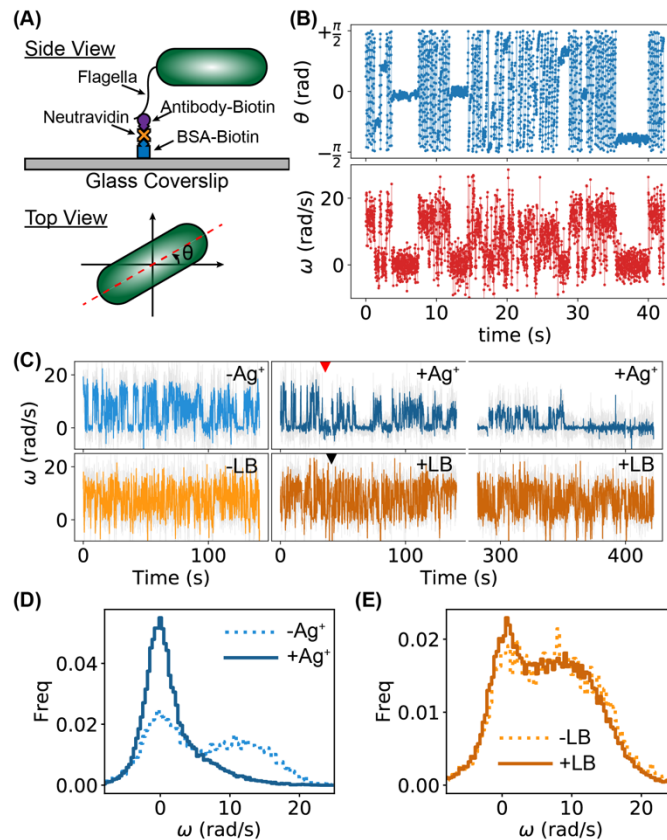
311

312 Higher frequency of bacterial tumbling caused by Ag^+ ions

313

314 To further understand the underlying mechanism of the inhibition of bacterial motility by Ag^+
 315 ions, we exploited the tethering assay on individual bacteria[46, 47]. Briefly, bacteria were
 316 tethered to clean glass coverslips through their flagella using biotinylated Anti-FliC antibody,
 317 neutravidin, and biotinylated bovine serum albumin (BSA) (Fig. 4A)[48]. The tethered bacteria

318 would rotate on the glass surfaces as the flagellar motors rotate (Movie M3)[48, 64]. Between
 319 continuous rotations (i.e., running), occasional pauses and reversed rotations were observed,
 320 corresponding to the tumbling of the bacteria (Movie M4)[65, 66]. After monitoring the rotation
 321 of the bacteria for 10,000 frames, Ag^+ ions were added into the samples at a final concentration
 322 of $40 \mu\text{M}$. The rotation of the bacteria was then monitored for 50,000 to 100,000 frames. It was
 323 observed that the rotation of the bacteria slowed down, and that the frequency of pauses
 324 increased (Movie M4).
 325



326
 327 **Figure 4.** Tethering assay for investigating the running and tumbling of individual bacteria. (A)
 328 Tethering of a bacterium on a glass coverslip (side view), and orientation of a bacterium θ (top
 329 view). (B) Examples of trajectories of orientation θ and angular velocity ω of a bacterium for
 330 3000 frames (or 42.3 s). (C) Examples of ω -trajectories for two bacteria. The top one was treated
 331 (blue curves) with Ag^+ ions; the red arrow indicates the time of adding Ag^+ ions. The bottom
 332 trajectories (orange curves) were for a bacterium without treatment. LB medium was added into
 333 the sample at the time indicated by the black arrow. (D) Distributions of ω for a bacterium
 334 treated by Ag^+ ions: pre- Ag^+ (dotted) and post- Ag^+ (solid). (E) Distributions of ω for an untreated
 335 bacterium: pre-LB (dotted) and post-LB (solid).
 336

337 To quantify the results of the tethering assay, we first extracted the orientation of the bacteria,
 338 $\theta \in (-\pi/2, +\pi/2]$, in each frame of the movies; then the angular velocities of the bacterial
 339 rotations were calculated, $\omega = \Delta\theta/\Delta t$, where $\Delta\theta$ and $\Delta t = 0.0141$ s were the change of the
 340 bacterial orientation and time interval between adjacent frames, respectively. Examples of

341 trajectories of θ and ω for 3,000 frames (or 42.3 s) for a bacterium before Ag^+ -treatment are
342 shown in Fig. 4B. Two distinct states were observed in the ω -trajectory, presumably
343 corresponding to the running and tumbling states[65, 66]. The full ω -trajectory (10,000 frames)
344 of Fig. 4B is shown in Fig. 4C ($-\text{Ag}^+$, light blue), while two segments (each with 10,000 frames) of
345 the ω -trajectory of the same bacterium during and after Ag^+ -treatment are also presented (Fig.
346 4C, $+\text{Ag}^+$, dark blue), where the red arrow indicates the time of adding Ag^+ ions. It is clear that
347 the tumbling state (i.e., lower angular velocity) became more frequent after Ag^+ -treatment. In
348 contrast, untreated bacteria (adding LB medium instead of Ag^+ ions) did not show observable
349 differences in the ω -trajectories (Fig. 4C, $\pm\text{LB}$, light and dark orange). This observation was
350 quantified by the distribution of the angular velocities. For the control, double peaks were
351 observed for both before and after the addition of LB medium (Fig. 4E); in contrast, the tumbling
352 peak (lower ω) became dominant after the addition of Ag^+ ions (Fig. 4D). The observed increase
353 in the tumbling frequency is consistent with a previous report based on swimming assays for the
354 effect of Ag nanoparticles[28].

355

356 **Stalling of flagellar motors caused by Ag^+ ions**

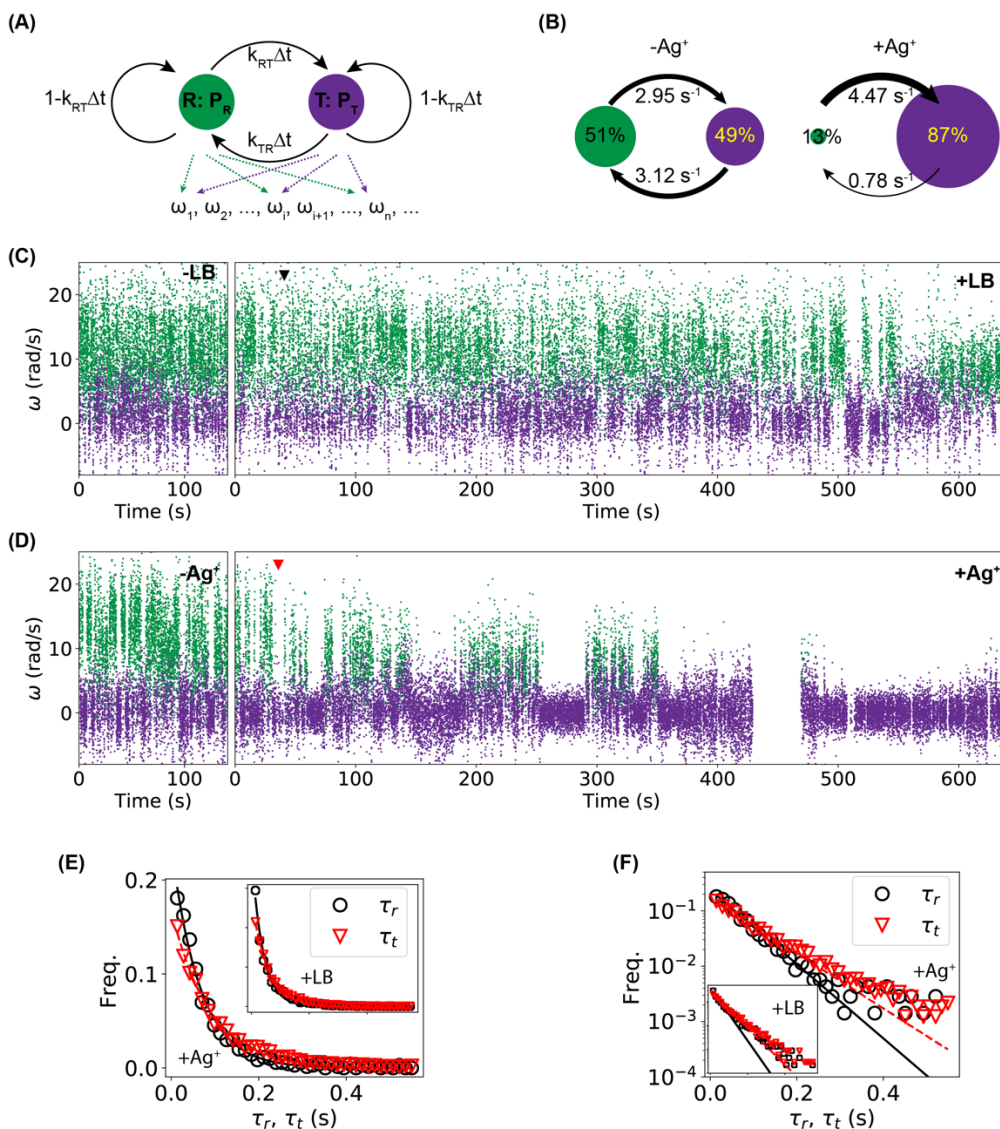
357

358 To obtain a deeper understanding of why Ag^+ ions inhibit the bacterial movement and induce
359 higher tumbling frequency, we performed hidden Markov model (HMM) analysis[55] on the
360 trajectories of angular velocities from the tethering assay. It is noted that hidden Markov model
361 is necessary because the motility states of the bacteria were not directly measured from the
362 experiments; instead, the observable (i.e., the directly measured quantity) was the angular
363 velocity (ω). Therefore, our hidden Markov model assumes two states: a running state (R) and a
364 tumbling state (T), which emit observations of angular velocities (Fig. 5A). The probabilities for a
365 bacterium to be in the running and tumbling states are P_R and P_T , respectively. The bacterium
366 can switch between the two states, with transition rates of k_{RT} (from R to T) and k_{TR} (from T to
367 R). For a given time interval between observations ($\Delta t = 0.0141$ s between adjacent frames in
368 the tethering assay), the transition probabilities would be $P_{RT} = k_{RT}\Delta t$ and $P_{TR} = k_{TR}\Delta t$,
369 respectively. For each bacterium, we fitted/trained the HMM using the pre- Ag^+ or pre-LB data,
370 and the fitted model was used to predict the states of all the observed angular velocities for that
371 bacterium, which were then used to estimate the HMM parameters (P 's and k 's). As an example,
372 the predicted states and the HMM parameters (P_R , P_T , k_{RT} , and k_{TR}) for the $\pm\text{Ag}^+$ bacterium in
373 Fig. 4C are presented in Figs. 5C and 5B, respectively. Two significant changes were observed.
374 First, the tumbling probability (P_T) increased dramatically from 49% to 87% (correspondingly,
375 $P_R = 1 - P_T$ decreased); second, while the running-to-tumbling transition rate increased
376 slightly, the tumbling-to-running transition rate k_{TR} decreased significantly by > 4 -fold after Ag^+ -
377 treatment (3.12 s^{-1} to 0.75 s^{-1}). These observations suggest that Ag^+ ions lead to higher
378 tumbling frequency by blocking the transition from the tumbling state to the running state.

379

380 As simple and hidden Markov models typically assume exponential distributions for the dwell
381 times (i.e., the time staying in the states), we wondered whether and how this assumption was
382 satisfied in the tethering assay. Briefly, from the predicted states for the control and sample
383 shown in Fig. 5C ($+\text{LB}$ and $+\text{Ag}^+$, respectively), we calculated the running time (τ_r) and tumbling

384 time (τ_t) and found that the distributions of both dwell times followed roughly the exponential
 385 distribution for both the control (+LB) and the sample (+Ag⁺), as shown in Fig. 5D, where the solid
 386 and dashed lines are fittings. This observation indicates that the hidden Markov model is
 387 reasonably suitable for the analysis here. On the other hand, we note that, a closer look on the
 388 distributions of the dwell times in the log-linear scale indicated that a single exponential decay
 389 did not fit the data well (Fig. 5E), suggesting that modified hidden Markov models that assume
 390 arbitrary distributions of the dwell times may improve the analysis.
 391

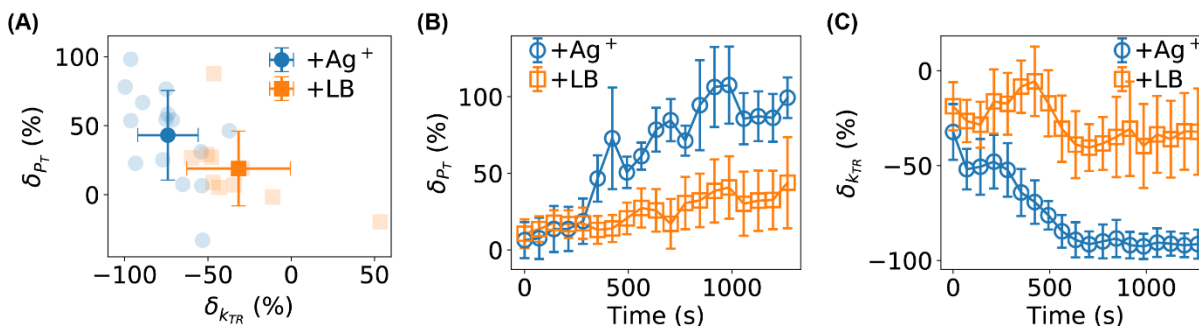


392
 393 **Figure 5.** Hidden Markov model (HMM) analysis. (A) The hidden Markov model with two states
 394 (running (R) vs. tumbling (T)), which emit observations of angular velocities ω_i . The probabilities
 395 for the system to be in the running and tumbling states are P_R and P_T , respectively. The transition
 396 probabilities between the two states are $P_{RT} = k_{RT}\Delta t$ and $P_{TR} = k_{TR}\Delta t$, where k_{RT} and k_{TR}
 397 are the corresponding transition rates and Δt is the time interval between observations. (B)
 398 Predicted parameters (P_R , P_T , k_{RT} and k_{TR}) from the HMM analysis for pre- Ag^+ and post- Ag^+ ω -

399 trajectories of the bacterium in the top row of Fig. 4C. (C, D) Predictions of states from the
 400 fitted/trained HMM model for the angular velocity (ω) trajectories for (C) an untreated bacterium
 401 and (D) an Ag^+ -treated bacterium. Green and purple dots correspond to the running and tumbling
 402 states, respectively. (E, F) Distributions of the dwell times (τ_r for running dwell time and τ_t for
 403 tumbling dwell time) from the (E) untreated and (F) Ag^+ -treated bacteria shown in panels (C) and
 404 (D). Solid and dashed lines are fitted exponential curves. Insets: the same data plotted in log-
 405 linear scale.

406
 407
 408 We replicated the tethering assay experiments and HMM analysis on 10 untreated ($\pm\text{LB}$) and 15
 409 treated ($\pm\text{Ag}^+$) bacteria. We observed large variations in the absolute values of the angular
 410 velocities for different bacteria, which could be attributed to differences in the cell length, the
 411 number of tethered flagella per bacterium, and the location of tethering points on the flagella[48,
 412 67]. To compare among different bacteria, we used the relative changes in the HMM parameters,
 413 $\delta_{P_T} = (P_T^+ / P_T^- - 1) \times 100\%$ and $\delta_{k_{TR}} = (k_{TR}^+ / k_{TR}^- - 1) \times 100\%$, where the superscripts (+
 414 and -) stand for after and before the addition of Ag^+ ions (or the addition of LB medium for the
 415 controls), respectively. The relative changes for the untreated (orange squares) and Ag^+ -treated
 416 bacteria (blue circles) from the full-length trajectories are shown in Fig. 6A. Performing one-
 417 sample t -test showed that the increase in P_T and decrease in k_{TR} were much more statistically
 418 significant for the Ag^+ -treated bacteria (p -values: 2.1×10^{-4} and 4.1×10^{-10} for P_T and k_{TR} ,
 419 respectively) than the untreated cells (p -values: 0.065 and 0.014, respectively). Two-sample t -
 420 test showed that the differences between the treated and untreated samples were also
 421 statistically significant (e.g., the p -value for k_{TR} was 4.2×10^{-4}).

422



423
 424 **Figure 6.** (A) Statistics of the relative changes in P_T and k_{TR} for 10 untreated (orange squares)
 425 and 15 Ag^+ -treated bacteria (blue circles). Error bars stand for standard deviation. (B, C) Time
 426 dependencies of the relative changes in (B) P_T , and (C) k_{TR} for untreated (orange squares) and
 427 Ag^+ -treated (blue circles) bacteria. Error bars stand for the standard error of the mean.

428
 429
 430 Finally, we examined the dependence of the HMM parameters on the treatment time (Figs. 6B
 431 and 6C), which was done by analyzing individual segments of the full-length ω -trajectories
 432 (window-size = 10,000 frames, stride between segments = 5,000 frames) using the fitted/trained
 433 HMM models. We observed that both δ_{P_T} and $\delta_{k_{TR}}$ started from ≈ 0 , which is reasonable as it
 434 takes time for the Ag^+ ions to diffuse to the bacteria and affect the bacteria. More interestingly,

435 the effects of Ag^+ ions became more and more significant after ~ 300 s compared to the controls
436 (Figs. 6B and 6C). After ~ 750 s, the relative change of k_{TR} reached $\sim -90\%$, suggesting that Ag^+
437 ions prevented the flagellar motor of the bacteria from rotating effectively and efficiently.

438

439 **Conclusions and Discussions**

440

441 To conclude, we directly visualized and investigated the antibiotic effects of Ag^+ ions on the
442 motility of *E. coli* bacteria based on swimming and tethering assays. From the swimming assay,
443 we observed that the bacteria slowed down dramatically when subjected to Ag^+ ions.
444 Characterization of the swimming trajectories showed higher changing rates of swimming
445 directions. In addition, we tethered the bacteria on glass surfaces through bacterial flagella (i.e.,
446 the tethering assay) and monitored the rotation of flagellar motors directly, from which we
447 observed an increase in the tumbling frequency due to Ag^+ -treatment. We performed hidden
448 Markov model (HMM) analysis on the trajectories of angular velocities of the bacterial rotation
449 and compared the bacteria before and after Ag^+ -treatment. It was found that treated bacteria
450 stayed in the tumbling state with much higher probability and that the transition rate from the
451 tumbling state to the running state decreased in the presence of Ag^+ ions, suggesting that Ag^+
452 ions stalled the flagellar motors and prevented them from rotating.

453

454 The observed inhibition of bacterial movement and higher frequency of tumbling caused by Ag^+
455 ions confirmed our hypothesis that the motility of bacteria is significantly affected by Ag. This
456 work provides direct visualization of the Ag's effects on the bacterial movements and advances
457 quantitatively our understanding on the mechanism of Ag-based antimicrobial agents in terms of
458 bacterial motility. More importantly, it raises more interesting questions worth further
459 investigations. For example, what is the molecular basis for the observed slower swimming, more
460 frequent tumbling, and motor stalling, when subjecting bacteria to Ag^+ ions? To what extent the
461 observed effects on the bacterial motility are Ag-specific? How will the bacteria adapt to, or
462 become resistant against, the Ag-induced damages on the bacterial movement? How will
463 bacterial death be related to the observed lower motility? Addressing these biological questions
464 experimentally is expected to be of great importance and interest for understanding the
465 fundamental antimicrobial mechanism of Ag and further exploring their potential biomedical
466 applications.

467

468 Our data suggest that the observed effects of Ag^+ ions on the bacterial motility are likely due to
469 direct interactions between the bacterial flagella and Ag^+ ions, which can be seen from the
470 response time of the rotation of bacteria to the addition of Ag^+ ions (Fig. 6) in the tethering assays.
471 In our tethering experiments, as the Ag^+ ions were added to the top surface of the liquid medium
472 in the Petri-dish above the bacteria under observation, the distance that Ag^+ ions need to travel
473 to the bacteria is roughly $\Delta x = 0.2$ cm (estimated from the volume of the culture medium, 2 mL,
474 and the diameter of the Petri-dish, 3.5 cm). Considering that the diffusion coefficient of Ag^+ ions
475 in water[68] is in the order of $D = 1.5 \times 10^{-5}$ cm²/s, the time scale for the Ag^+ ions to reach the
476 bacteria is in the order of $\Delta t = \frac{\Delta x^2}{6D} \approx 400$ s, which is close to the response time (300 – 750 s) of
477 bacteria to the Ag^+ ions that we measured from our tethering assays (Fig. 6B and 6C). If the

478 observed effects of Ag⁺ ions on the bacterial motility were due to indirect interactions, such as
479 those through regulatory proteins and membrane damages, the response time is expected to be
480 longer as time is needed to transduce those indirect effects to the flagellar motor. Therefore, it
481 is suggested to focus on the bacterial flagella when searching for molecular basis for the Ag-
482 caused slower swimming, more frequent tumbling, and motor stalling in future studies.

483

484 **Acknowledgement**

485

486 This work was supported by the University of Arkansas, the Arkansas Biosciences Institute, and
487 the National Science Foundation (Grant No. 1826642 to YW and JC). AR and MK were partially
488 supported by NSF-REU grants (Grant No. 1460754 and Grant No. 1851919).

489

490 References

- 491
- 492
- 493 1. Davies, J. and Davies, D. **“Origins and Evolution of Antibiotic Resistance.”** *Microbiology and*
494 *Molecular Biology Reviews* 74, no. 3 (2010): 417–433. doi:10.1128/MMBR.00016-10,
- 495 2. Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., and Piddock, L. J. V. **“Molecular**
496 **Mechanisms of Antibiotic Resistance.”** *Nature Reviews. Microbiology* 13, no. 1 (2015): 42–51.
497 doi:10.1038/nrmicro3380,
- 498 3. CDC. **“Antibiotic Resistance Threats in the United State”** (2019):
- 499 4. Allen, H. K., Trachsel, J., Looft, T., and Casey, T. A. **“Finding Alternatives to Antibiotics.”** *Annals*
500 *of the New York Academy of Sciences* 1323, (2014): 91–100. doi:10.1111/nyas.12468,
- 501 5. François, B., Jafri, H. S., and Bonten, M. **“Alternatives to Antibiotics.”** *Intensive Care Medicine*
502 42, no. 12 (2016): 2034–2036. doi:10.1007/s00134-016-4339-y,
- 503 6. Haque, M. A., Imamura, R., Brown, G. A., Krishnamurthi, V. R., Niyonshuti, I. I., Marcelle, T.,
504 Mathurin, L. E., Chen, J., and Wang, Y. **“An Experiment-Based Model Quantifying Antimicrobial**
505 **Activity of Silver Nanoparticles on Escherichia Coli”** *RSC Adv.* 7, no. 89 (2017): 56173–56182.
506 doi:10.1039/C7RA10495B,
- 507 7. Durán, N., Durán, M., Jesus, M. B. de, Seabra, A. B., Fávaro, W. J., and Nakazato, G. **“Silver**
508 **Nanoparticles: A New View on Mechanistic Aspects on Antimicrobial Activity.”** *Nanomedicine :*
509 *nanotechnology, biology, and medicine* 12, no. 3 (2016): 789–799.
510 doi:10.1016/j.nano.2015.11.016,
- 511 8. Rai, M., Yadav, A., and Gade, A. **“Silver Nanoparticles as a New Generation of Antimicrobials.”**
512 *Biotechnology advances* 27, no. 1 (2009): 76–83. doi:10.1016/j.biotechadv.2008.09.002,
- 513 9. Jung, W. K., Koo, H. C., Kim, K. W., Shin, S., Kim, S. H., and Park, Y. H. **“Antibacterial Activity**
514 **and Mechanism of Action of the Silver Ion in Staphylococcus Aureus and Escherichia Coli.”**
515 *Applied and Environmental Microbiology* 74, no. 7 (2008): 2171–2178. doi:10.1128/AEM.02001-
516 07,
- 517 10. Feng, Q. L., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N., and Kim, J. O. **“A Mechanistic Study of**
518 **the Antibacterial Effect of Silver Ions on Escherichia Coli and Staphylococcus Aureus.”** *Journal*
519 *of Biomedical Materials Research* 52, no. 4 (2000): 662–668. doi:10.1002/1097-
520 4636(20001215)52:4<662::AID-JBM10>3.0.CO;2-3,
- 521 11. Radzig, M. A., Nadtochenko, V. A., Koksharova, O. A., Kiwi, J., Lipasova, V. A., and Khmel, I. A.
522 **“Antibacterial Effects of Silver Nanoparticles on Gram-Negative Bacteria: Influence on the**
523 **Growth and Biofilms Formation, Mechanisms of Action.”** *Colloids and Surfaces. B, Biointerfaces*
524 102, (2013): 300–306. doi:10.1016/j.colsurfb.2012.07.039,
- 525 12. Marambio-Jones, C. and Hoek, E. M. V. **“A Review of the Antibacterial Effects of Silver**
526 **Nanomaterials and Potential Implications for Human Health and the Environment”** *Journal of*
527 *nanoparticle research : an interdisciplinary forum for nanoscale science and technology* 12, no. 5
528 (2010): 1531–1551. doi:10.1007/s11051-010-9900-y,
- 529 13. Ivask, A., Elbadawy, A., Kaweeteerawat, C., Boren, D., Fischer, H., Ji, Z., Chang, C. H., Liu, R.,
530 Tolaymat, T., Telesca, D., Zink, J. I., Cohen, Y., Holden, P. A., and Godwin, H. A. **“Toxicity**
531 **Mechanisms in Escherichia Coli Vary for Silver Nanoparticles and Differ from Ionic Silver.”** *ACS*
532 *Nano* 8, no. 1 (2014): 374–386. doi:10.1021/nn4044047,

- 533 14. Sadoon, A. A., Khadka, P., Freeland, J., Gundampati, R. K., Manso, R., Ruiz, M., Krishnamurthi,
534 V. R., Thallapuram, S. K., Chen, J., and Wang, Y. **“Faster Diffusive Dynamics of Histone-like**
535 **Nucleoid Structuring Proteins in Live Bacteria Caused by Silver Ions.”** *Applied and Environmental*
536 *Microbiology* (2020): doi:10.1128/AEM.02479-19,
537 15. Krishnamurthi, V. R., Chen, J., and Wang, Y. **“Silver Ions Cause Oscillation of Bacterial Length**
538 **of Escherichia Coli.”** *Scientific Reports* 9, no. 1 (2019): 11745. doi:10.1038/s41598-019-48113-4,
539 16. Josenhans, C. and Suerbaum, S. **“The Role of Motility as a Virulence Factor in Bacteria.”**
540 *International Journal of Medical Microbiology* 291, no. 8 (2002): 605–614. doi:10.1078/1438-
541 4221-00173,
542 17. Sowa, Y. and Berry, R. M. **“Bacterial Flagellar Motor.”** *Quarterly Reviews of Biophysics* 41, no.
543 2 (2008): 103–132. doi:10.1017/S0033583508004691,
544 18. Zhao, Z., Zhao, Y., Zhuang, X.-Y., Lo, W.-C., Baker, M. A. B., Lo, C.-J., and Bai, F. **“Frequent**
545 **Pauses in Escherichia Coli Flagella Elongation Revealed by Single Cell Real-Time Fluorescence**
546 **Imaging.”** *Nature Communications* 9, no. 1 (2018): 1885. doi:10.1038/s41467-018-04288-4,
547 19. Minamino, T. and Imada, K. **“The Bacterial Flagellar Motor and Its Structural Diversity.”**
548 *Trends in Microbiology* 23, no. 5 (2015): 267–274. doi:10.1016/j.tim.2014.12.011,
549 20. Parkinson, J. S., Hazelbauer, G. L., and Falke, J. J. **“Signaling and Sensory Adaptation in**
550 **Escherichia Coli Chemoreceptors: 2015 Update.”** *Trends in Microbiology* 23, no. 5 (2015): 257–
551 266. doi:10.1016/j.tim.2015.03.003,
552 21. Sourjik, V. and Wingreen, N. S. **“Responding to Chemical Gradients: Bacterial Chemotaxis.”**
553 *Current Opinion in Cell Biology* 24, no. 2 (2012): 262–268. doi:10.1016/j.ceb.2011.11.008,
554 22. Gugala, N., Lemire, J., Chatfield-Reed, K., Yan, Y., Chua, G., and Turner, R. J. **“Using a Chemical**
555 **Genetic Screen to Enhance Our Understanding of the Antibacterial Properties of Silver.”** *Genes*
556 9, no. 7 (2018): doi:10.3390/genes9070344,
557 23. Alqahtany, M., Khadka, P., Niyonshuti, I., Krishnamurthi, V. R., Sadoon, A. A., Challapalli, S. D.,
558 Chen, J., and Wang, Y. **“Nanoscale Reorganizations of Histone-like Nucleoid Structuring Proteins**
559 **in Escherichia Coli Are Caused by Silver Nanoparticles.”** *Nanotechnology* 30, no. 38 (2019):
560 385101. doi:10.1088/1361-6528/ab2a9f,
561 24. Wang, H., Ayala, J. C., Benitez, J. A., and Silva, A. J. **“RNA-Seq Analysis Identifies New Genes**
562 **Regulated by the Histone-like Nucleoid Structuring Protein (H-NS) Affecting Vibrio Cholerae**
563 **Virulence, Stress Response and Chemotaxis.”** *Plos One* 10, no. 2 (2015): e0118295.
564 doi:10.1371/journal.pone.0118295,
565 25. Bertin, P., Terao, E., Lee, E. H., Lejeune, P., Colson, C., Danchin, A., and Collatz, E. **“The H-NS**
566 **Protein Is Involved in the Biogenesis of Flagella in Escherichia Coli.”** *Journal of Bacteriology* 176,
567 no. 17 (1994): 5537–5540.
568 26. Garuglieri, E., Cattò, C., Villa, F., Zanchi, R., and Cappitelli, F. **“Effects of Sublethal**
569 **Concentrations of Silver Nanoparticles on Escherichia Coli and Bacillus Subtilis under Aerobic**
570 **and Anaerobic Conditions.”** *Biointerphases* 11, no. 4 (2016): 04B308. doi:10.1116/1.4972100,
571 27. West, M., Burdash, N. M., and Freimuth, F. **“Simplified Silver-Plating Stain for Flagella.”**
572 *Journal of Clinical Microbiology* 6, no. 4 (1977): 414–419.
573 28. Ortega-Calvo, J.-J., Molina, R., Jimenez-Sanchez, C., Dobson, P. J., and Thompson, I. P.
574 **“Bacterial Tactic Response to Silver Nanoparticles.”** *Environmental microbiology reports* 3, no. 5
575 (2011): 526–534. doi:10.1111/j.1758-2229.2011.00252.x,

- 576 29. Choi, O., Deng, K. K., Kim, N.-J., Ross, L., Surampalli, R. Y., and Hu, Z. **“The Inhibitory Effects**
577 **of Silver Nanoparticles, Silver Ions, and Silver Chloride Colloids on Microbial Growth.”** *Water*
578 *Research* 42, no. 12 (2008): 3066–3074. doi:10.1016/j.watres.2008.02.021,
- 579 30. Mazouchi, A. and Milstein, J. N. **“Fast Optimized Cluster Algorithm for Localizations (FOCAL):**
580 **A Spatial Cluster Analysis for Super-Resolved Microscopy.”** *Bioinformatics* 32, no. 5 (2016): 747–
581 754. doi:10.1093/bioinformatics/btv630,
- 582 31. Sadoon, A. A. and Wang, Y. **“Anomalous, Non-Gaussian, Viscoelastic, and Age-Dependent**
583 **Dynamics of Histone-like Nucleoid-Structuring Proteins in Live *Escherichia Coli*”** *Physical Review*
584 *E* 98, no. 4 (2018): 042411. doi:10.1103/PhysRevE.98.042411,
- 585 32. Zhang, M., Chang, H., Zhang, Y., Yu, J., Wu, L., Ji, W., Chen, J., Liu, B., Lu, J., Liu, Y., Zhang, J.,
586 Xu, P., and Xu, T. **“Rational Design of True Monomeric and Bright Photoactivatable Fluorescent**
587 **Proteins.”** *Nature Methods* 9, no. 7 (2012): 727–729. doi:10.1038/nmeth.2021,
- 588 33. Tang, H. and Blair, D. F. **“Regulated Underexpression of the FliM Protein of *Escherichia Coli***
589 **and Evidence for a Location in the Flagellar Motor Distinct from the MotA/MotB Torque**
590 **Generators.”** *Journal of Bacteriology* 177, no. 12 (1995): 3485–3495.
591 doi:10.1128/jb.177.12.3485-3495.1995,
- 592 34. Berg, H. C. and Brown, D. A. **“Chemotaxis in *Escherichia Coli* Analysed by Three-Dimensional**
593 **Tracking.”** *Nature* 239, no. 5374 (1972): 500–504. doi:10.1038/239500a0,
- 594 35. Del Tito, B. J., Ward, J. M., Hodgson, J., Gershater, C. J., Edwards, H., Wysocki, L. A., Watson,
595 F. A., Sathe, G., and Kane, J. F. **“Effects of a Minor Isoleucyl tRNA on Heterologous Protein**
596 **Translation in *Escherichia Coli*.”** *Journal of Bacteriology* 177, no. 24 (1995): 7086–7091.
597 doi:10.1128/jb.177.24.7086-7091.1995,
- 598 36. Edelstein, A., Amodaj, N., Hoover, K., Vale, R., and Stuurman, N. **“Computer Control of**
599 **Microscopes Using MManager.”** *Current Protocols in Molecular Biology* Chapter 14, (2010):
600 Unit14.20. doi:10.1002/0471142727.mb1420s92,
- 601 37. Edelstein, A. D., Tsuchida, M. A., Amodaj, N., Pinkard, H., Vale, R. D., and Stuurman, N.
602 **“Advanced Methods of Microscope Control Using MManager Software.”** *Journal of biological*
603 *methods* 1, no. 2 (2014): doi:10.14440/jbm.2014.36,
- 604 38. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S.,
605 Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K.,
606 Tomancak, P., and Cardona, A. **“Fiji: An Open-Source Platform for Biological-Image Analysis.”**
607 *Nature Methods* 9, no. 7 (2012): 676–682. doi:10.1038/nmeth.2019,
- 608 39. Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. **“NIH Image to ImageJ: 25 Years of Image**
609 **Analysis.”** *Nature Methods* 9, no. 7 (2012): 671–675. doi:10.1038/nmeth.2089,
- 610 40. Crocker, J. C. and Grier, D. G. **“Methods of Digital Video Microscopy for Colloidal Studies”**
611 *Journal of Colloid and Interface Science* 179, no. 1 (1996): 298–310. doi:10.1006/jcis.1996.0217,
- 612 41. Stracy, M., Lesterlin, C., Garza de Leon, F., Uphoff, S., Zawadzki, P., and Kapanidis, A. N. **“Live-**
613 **Cell Superresolution Microscopy Reveals the Organization of RNA Polymerase in the Bacterial**
614 **Nucleoid.”** *Proceedings of the National Academy of Sciences of the United States of America* 112,
615 no. 32 (2015): E4390-9. doi:10.1073/pnas.1507592112,
- 616 42. Allan, D. B., Caswell, T., Keim, N. C., and Wel, C. M. van der. **“Trackpy: Trackpy V0.4.1”** *Zenodo*
617 (2018): doi:10.5281/zenodo.1226458,

- 618 43. Pottash, A. E., McKay, R., Virgile, C. R., Ueda, H., and Bentley, W. E. **“TumbleScore: Run and**
619 **Tumble Analysis for Low Frame-Rate Motility Videos.”** *Biotechniques* 62, no. 1 (2017): 31–36.
620 doi:10.2144/000114493,
- 621 44. Amsler, C. D. **“Use of Computer-Assisted Motion Analysis for Quantitative Measurements**
622 **of Swimming Behavior in Peritrichously Flagellated Bacteria.”** *Analytical Biochemistry* 235, no.
623 1 (1996): 20–25. doi:10.1006/abio.1996.0086,
- 624 45. Alon, U., Camarena, L., Surette, M. G., Aguera y Arcas, B., Liu, Y., Leibler, S., and Stock, J. B.
625 **“Response Regulator Output in Bacterial Chemotaxis.”** *The EMBO Journal* 17, no. 15 (1998):
626 4238–4248. doi:10.1093/emboj/17.15.4238,
- 627 46. Schniederberend, M., Abdurachim, K., Murray, T. S., and Kazmierczak, B. I. **“The GTPase**
628 **Activity of FlhF Is Dispensable for Flagellar Localization, but Not Motility, in Pseudomonas**
629 **Aeruginosa.”** *Journal of Bacteriology* 195, no. 5 (2013): 1051–1060. doi:10.1128/JB.02013-12,
- 630 47. Schniederberend, M., Williams, J. F., Shine, E., Shen, C., Jain, R., Emonet, T., and Kazmierczak,
631 B. I. **“Modulation of Flagellar Rotation in Surface-Attached Bacteria: A Pathway for Rapid**
632 **Surface-Sensing after Flagellar Attachment.”** *PLoS Pathogens* 15, no. 11 (2019): e1008149.
633 doi:10.1371/journal.ppat.1008149,
- 634 48. Inoue, Y. **“Rotation Measurements of Tethered Cells.”** *Methods in Molecular Biology* 1593,
635 (2017): 163–174. doi:10.1007/978-1-4939-6927-2_12,
- 636 49. Da Silva, S., Grosjean, L., Ternan, N., Mailley, P., Livache, T., and Cosnier, S. **“Biotinylated**
637 **Polypyrrole Films: An Easy Electrochemical Approach for the Reagentless Immobilization of**
638 **Bacteria on Electrode Surfaces.”** *Bioelectrochemistry* 63, no. 1–2 (2004): 297–301.
639 doi:10.1016/j.bioelechem.2003.09.027,
- 640 50. Kim, J.-S., Kim, Y. J., Seo, S., Seong, M.-J., and Lee, K. **“Functional Role of Bdm during Flagella**
641 **Biogenesis in Escherichia Coli.”** *Current Microbiology* 70, no. 3 (2015): 369–373.
642 doi:10.1007/s00284-014-0729-y,
- 643 51. Francis, N. R., Sosinsky, G. E., Thomas, D., and DeRosier, D. J. **“Isolation, Characterization and**
644 **Structure of Bacterial Flagellar Motors Containing the Switch Complex.”** *Journal of Molecular*
645 *Biology* 235, no. 4 (1994): 1261–1270. doi:10.1006/jmbi.1994.1079,
- 646 52. Walt, S. van der, Schönberger, J. L., Nunez-Iglesias, J., Boulogne, F., Warner, J. D., Yager, N.,
647 Gouillart, E., Yu, T., and scikit-image contributors. **“Scikit-Image: Image Processing in Python.”**
648 *PeerJ* 2, (2014): e453. doi:10.7717/peerj.453,
- 649 53. Patteson, A. E., Gopinath, A., Goulian, M., and Arratia, P. E. **“Running and Tumbling with E.**
650 **Coli in Polymeric Solutions.”** *Scientific Reports* 5, (2015): 15761. doi:10.1038/srep15761,
- 651 54. Cheong, F. C., Wong, C. C., Gao, Y., Nai, M. H., Cui, Y., Park, S., Kenney, L. J., and Lim, C. T.
652 **“Rapid, High-Throughput Tracking of Bacterial Motility in 3D via Phase-Contrast Holographic**
653 **Video Microscopy.”** *Biophysical Journal* 108, no. 5 (2015): 1248–1256.
654 doi:10.1016/j.bpj.2015.01.018,
- 655 55. Korobkova, E. A., Emonet, T., Park, H., and Cluzel, P. **“Hidden Stochastic Nature of a Single**
656 **Bacterial Motor.”** *Physical Review Letters* 96, no. 5 (2006): 058105.
657 doi:10.1103/PhysRevLett.96.058105,
- 658 56. Persson, F., Lindén, M., Unoson, C., and Elf, J. **“Extracting Intracellular Diffusive States and**
659 **Transition Rates from Single-Molecule Tracking Data.”** *Nature Methods* 10, no. 3 (2013): 265–
660 269. doi:10.1038/nmeth.2367,

- 661 57. Theves, M., Taktikos, J., Zaburdaev, V., Stark, H., and Beta, C. **“A Bacterial Swimmer with Two**
662 **Alternating Speeds of Propagation.”** *Biophysical Journal* 105, no. 8 (2013): 1915–1924.
663 doi:10.1016/j.bpj.2013.08.047,
- 664 58. Theves, M., Taktikos, J., Zaburdaev, V., Stark, H., and Beta, C. **“Random Walk Patterns of a**
665 **Soil Bacterium in Open and Confined Environments”** *EPL (Europhysics Letters)* 109, no. 2 (2015):
666 28007. doi:10.1209/0295-5075/109/28007,
- 667 59. Lauga, E., DiLuzio, W. R., Whitesides, G. M., and Stone, H. A. **“Swimming in Circles: Motion**
668 **of Bacteria near Solid Boundaries.”** *Biophysical Journal* 90, no. 2 (2006): 400–412.
669 doi:10.1529/biophysj.105.069401,
- 670 60. Boulou, L., Prévost, M., Barbeau, B., Coallier, J., and Desjardins, R. **“LIVE/DEAD BacLight :**
671 **Application of a New Rapid Staining Method for Direct Enumeration of Viable and Total**
672 **Bacteria in Drinking Water.”** *Journal of Microbiological Methods* 37, no. 1 (1999): 77–86.
673 doi:10.1016/S0167-7012(99)00048-2,
- 674 61. Michalet, X. **“Mean Square Displacement Analysis of Single-Particle Trajectories with**
675 **Localization Error: Brownian Motion in an Isotropic Medium.”** *Physical Review. E, Statistical,*
676 *Nonlinear, and Soft Matter Physics* 82, no. 4 Pt 1 (2010): 041914.
677 doi:10.1103/PhysRevE.82.041914,
- 678 62. Metzler, R., Jeon, J.-H., Cherstvy, A. G., and Barkai, E. **“Anomalous Diffusion Models and Their**
679 **Properties: Non-Stationarity, Non-Ergodicity, and Ageing at the Centenary of Single Particle**
680 **Tracking.”** *Physical Chemistry Chemical Physics* 16, no. 44 (2014): 24128–24164.
681 doi:10.1039/c4cp03465a,
- 682 63. Metzler, R. and Klafter, J. **“The Random Walk’s Guide to Anomalous Diffusion: A Fractional**
683 **Dynamics Approach”** *Physics Reports* 339, no. 1 (2000): 1–77. doi:10.1016/S0370-
684 1573(00)00070-3,
- 685 64. Qian, C., Wong, C. C., Swarup, S., and Chiam, K.-H. **“Bacterial Tethering Analysis Reveals a**
686 **‘Run-Reverse-Turn’ Mechanism for Pseudomonas Species Motility.”** *Applied and Environmental*
687 *Microbiology* 79, no. 15 (2013): 4734–4743. doi:10.1128/AEM.01027-13,
- 688 65. Manson, M. D. **“Dynamic Motors for Bacterial Flagella.”** *Proceedings of the National*
689 *Academy of Sciences of the United States of America* 107, no. 25 (2010): 11151–11152.
690 doi:10.1073/pnas.1006365107,
- 691 66. Dominick, C. N. and Wu, X.-L. **“Rotating Bacteria on Solid Surfaces without Tethering.”**
692 *Biophysical Journal* 115, no. 3 (2018): 588–594. doi:10.1016/j.bpj.2018.06.020,
- 693 67. Lele, P. P., Hosu, B. G., and Berg, H. C. **“Dynamics of Mechanosensing in the Bacterial**
694 **Flagellar Motor.”** *Proceedings of the National Academy of Sciences of the United States of*
695 *America* 110, no. 29 (2013): 11839–11844. doi:10.1073/pnas.1305885110,
- 696 68. Johnston, R. R. M. and Spiro, M. **“Diffusion Coefficients of the Silver Ion and the**
697 **Disulfite Silver (I) Ion by the Rotating Disk Method”** *The Journal of physical chemistry* 71, no. 12
698 (1967): 3784–3790. doi:10.1021/j100871a011,
699