

Swimming Kinematics of *Enterobacter sp.* SM3

By

Silverio Gabriel Johnson

B.S., University of Massachusetts Lowell, Lowell, MA, 2019

M.Sc., Brown University, Providence, RI, 2021

Thesis

Submitted in partial fulfillment of the requirements for the Degree of
Doctor of Philosophy in the Department of Physics at Brown University

PROVIDENCE, RHODE ISLAND

May 2025

© Copyright 2025 by Silverio Gabriel Johnson

This dissertation by Silverio Gabriel Johnson is accepted in its present
form by the Department of Physics as satisfying the
dissertation requirement for the degree of Doctor of Philosophy.

Date _____
Prof. Jay X. Tang, Advisor

Recommended to the Graduate Council

Date _____
Prof. James M. Valles, Jr., Reader

Date _____
Prof. Robert A. Pelcovits, Reader

Date _____
Prof. Thomas R. Powers, Reader

Approved by the Graduate Council

Date _____
Thomas A. Lewis, Dean of the Graduate School

Curriculum Vitae

Education

2019–2025 Ph.D., (Physics)

Brown University (Providence, RI, USA)

2019-2021 M.Sc., (Physics)

Brown University (Providence, RI, USA)

2015–2019 Honors B.S., (Physics)

University of Massachusetts (Lowell, MA, USA)

Publications

Papers

S. G. Johnson, B. Freedman, and J. X. Tang, Run-and-Tumble Kinematics of *Enterobacter* sp. SM3, *Phys. Rev. E* 109, 064402 (2024).

J. Bell, **S. G. Johnson**, B. Pugno, and J. X. Tang, Bacteria Can Rotate While Body-Tethered to a Solid Surface, *Accepted at Biophys. J.*, (2025).

C. Pawul, T. Dutta, **S. G. Johnson**, and J. X. Tang, Mucin Promotes Bacterial Swarming by Making the Agar Surface More Slippery, *Langmuir*, (2024).

S. G. Johnson, J. X. Tang, and E. Lushi, Cell length affects the swimming trajectory of flagellated bacteria near a solid surface, *In Preparation*, (2025).

Presentations

S. G. Johnson, Swimming Kinematics of *Enterobacter* sp. SM3. *Invited Talk*, Syracuse University, New York (April 2025).

S. G. Johnson, E. Lushi, J. Hall, and J. X. Tang, Effects of Cell Length on the Swimming Behavior of Flagellated Bacteria Near a Solid Surface. *Proceedings of the APS*, Anaheim, California (March 2025).

S. G. Johnson, B. Freedman, and J. X. Tang, Dilute Swimmers Have Different Run-and-Tumble Motility Than Planktonic Swimmers in *Enterobacter* sp. SM3. *Proceedings*

of the *Dr. Samuel M. Nabrit Conference for Early Career Scholars*, Providence, Rhode Island (June 2024).

S. G. Johnson, B. Freedman, and J. X. Tang, Dilute Swimmers Have Different Run-and-Tumble Motility Than Planktonic Swimmers. *Proceedings of the APS*, Minneapolis, Minnesota (March 2024).

S. G. Johnson, B. Freedman, S. Mani, and J. X. Tang, Run-and-Tumble Analysis of *Enterobacter sp.* SM3. *Proceedings of the APS*, Las Vegas, Nevada (March 2023).

S. G. Johnson, M. Yu, P. Moroshkin, R. M. Osgood III, and J. Xu, Towards Magnetically Tunable Extraordinary Optical Transmission. *Proceedings of the APS*, virtual during COVID-19 pandemic (March 2021).

Software

S. G. Johnson and B. Freedman, Bacterial Tracking Algorithm, *Zenodo*, doi: 10.5281/zenodo.8066767, (2024).

Teaching

Spring 2025

APS Squishy Science Sunday, (APS March Meeting, Anaheim, CA)

Community outreach featuring activities, demos, and opportunities to meet physicists

Summer 2024

Summer Mentor for PROBE Initiative, (Brown University, Providence, RI)

Mentor for students from the University of Puerto Rico in conducting summer research

Spring 2024

APS Squishy Science Sunday, (APS March Meeting, Minneapolis, MN)

Community outreach featuring activities, demos, and opportunities to meet physicists

Summer 2023/2024

NSF Undergraduate Mentor, (Brown University, Providence, RI)

Mentor for NSF-funded undergraduate student visiting from Tougaloo University

Spring 2022

Teaching Assistant, (Brown University, Providence, RI)

Course: Physics 0040

Summer 2021

Summer Leadership Alliance Mentor, (Brown University, Providence, RI)

Mentor for student-leadership-alliance-funded undergraduate student visiting from Caltech

2017-2018

Supplemental Instructor, (University of Massachusetts, Lowell, MA)

Course: Physical Properties of Matter (Physics III)

2017-2018

Departmental Tutor, (University of Massachusetts, Lowell, MA)

Tutor for students across all physics courses and topics

Professional Development

Workshops

2024, New England Complex Fluids

Brown University, (Providence, RI)

2024, New England Complex Fluids

Tufts University, (Medford, MA)

2023, New England Complex Fluids

Brandeis University, (Waltham, MA)

2022, New England Complex Fluids

Brandeis University, (Waltham, MA)

Awards and Honors

2022: Award of Excellence as a Graduate Teaching Assistant

Brown University

2019-2020: NIH Initiative for Maximizing Student Development Scholarship

Brown University

2018: Lorenzo J. Cabrera Scholarship

University of Massachusetts Lowell

2017: Russell V. Leclaire Endowed Scholarship

University of Massachusetts Lowell

Acknowledgments

First and foremost, I dedicate this thesis to my two parents—my mother, Sandra Cora Patenaude, and my uncle, Joseph Maurice Patenaude—who, through their undying love and dedication, instilled in me a strong love of learning and provided me with the means to achieve this highest of degrees. I love you with all my heart.

I also dedicate this thesis to the memories of those deceased. To my father, Silverio Alberto Johnson, who, although our time together was cut short, watched over me on this journey and kept my path straight. To my abuela, Mercedes Johnson, who always believed in my education and wished for my continued success, and who continues to provide for me, even in death. To my mentor and friend, Dr. Ruizhe Yao, who passed shortly after receiving his doctorate in physics, and to whom I promised that I would receive my doctorate; I have fulfilled my promise. To my dear friend, Mikhaela Gillman, who also passed while pursuing her doctorate; I always believed in your success, and I will carry the torch for you in your stead.

I thank my advisor, Dr. Jay X. Tang, for believing in me and providing me with the guidance and tools to become a fine researcher. His genuine care for and love of his students has inspired me to, someday, show the same compassion for my students. Thank you for believing in me, even when I doubted myself.

I thank my committee—Dr. James M. Valles, Jr., Dr. Robert A. Pelcovits, and Dr. Thomas R. Powers—for their valuable conversations and shining examples, both as

scientists and as people.

I thank all of those whom I have collaborated with over the years, especially Dr. Enkeleida Lushi, who provided me with valuable feedback for this thesis while in the process of drafting a paper with me.

I thank my academic mentors at the University of Massachusetts Lowell, especially Dr. Wei Guo, Dr. Nikolay Lepeshkin, Dr. Jayant Kumar, and Dr. James Egan. I would not have made it to Brown University without your superb example.

I thank my academic and spiritual advisor, Sr. Irene Martineau. Our God has gotten us both this far.

I thank my peers—Brandon Pugnet, Danielle Germann, and Thomas Dutta—for their valuable scientific conversations and for their friendship. I could not have asked for better co-workers. I hope for your continued success.

Finally, I thank all those who have gone unmentioned, especially the many undergraduate and master students who help with the research in our lab. Science is never completed in a vacuum—many bright minds are needed.

Contents

Acknowledgments	viii
1 INTRODUCTION	1
1.1 A World Within Our Own	1
1.2 A Brief Physiology of Bacteria	3
1.3 Life at the Microscopic Scale	6
1.3.1 The Issue of Water	6
1.3.2 Life in the Slow Lane: Low Reynolds Number	8
1.3.3 The Navier-Stokes Equation for Low Re Fluids	9
1.4 The Physics of Bacteria Swimming	12
1.4.1 The Flagellar Filament	13
1.4.2 The Flagellar Motor	15
1.4.3 Chemotaxis	17
1.4.4 Run-and-Tumble	18
1.4.5 Hydrodynamics of Microswimmers	19
1.4.6 Near-Surface Swimming	21
1.5 Other Modes of Motility	23
1.5.1 Swarming	23
1.5.2 Living on the Edge: Biofilms	25
1.6 In Summary	25

2	METHODS	38
2.1	Bacterial Culture Preparations	38
2.1.1	Overnight Growth	38
2.1.2	Hard Agar Plate Colonies	39
2.1.3	Fresh Growths	40
2.1.4	Swarm Assays	40
2.2	Other Chemical Preparations	41
2.2.1	Motility Buffer	41
2.2.2	Chemoattractant Solutions	41
2.3	Microscopy	42
2.3.1	Sample Preparations	42
2.3.2	Phase Contrast and Dark-Field Microscopy	43
2.3.3	Digital Holographic Microscopy	44
2.4	Bacteria Detection and Tracking	45
2.4.1	Image Processing and Bacteria Tracking Using Fiji	45
2.4.2	Image Processing and Bacteria Tracking Using Using HoloPro	48
2.5	Analysis of Trajectories	51
2.5.1	Calculation of Kinematic Variables	52
2.5.2	Detection of Parameters in 2D	54
2.5.3	Detection of Parameters in 3D	56
2.6	Hydrodynamic Simulations	57
2.6.1	Mathematical Model for a Swimmer in Free Space	58
2.6.2	Swimmer Above a Planar No-Slip Wall	60
2.6.3	Parameter Values for the Model	61
3	RUN-AND-TUMBLE KINEMATICS OF <i>ENTEROBACTER</i> SP. SM3	66
3.1	Introduction	66
3.2	Results	69

3.2.1	SM3 Exhibits Run-and-Tumble Behavior	69
3.2.2	SM3 Alters Its Behavior in Response to Chemoattractants	73
3.2.3	SM3 Swimmers Have Different Run-and-Tumble Parameters . . .	74
3.2.4	Flagellum-Driven Tumble Model	75
3.3	Discussion	77
4	CELL LENGTH AFFECTS THE SWIMMING TRAJECTORY OF FLAGELLATED BACTERIA NEAR A SOLID SURFACE	86
4.1	Introduction	86
4.2	Results	89
4.2.1	SM3 Swims in Circular Trajectories Near the Surface	89
4.2.2	The Radii of Circular Trajectories Depend on the Cell Length . .	92
4.2.3	Simulations Show that Circular Trajectories Emerge From the Head- Tail Counter-Rotation	93
4.3	Discussion	95
5	THREE-DIMENSIONAL DIGITAL HOLOGRAPHIC TRACKING OF BACTERIA	101
5.1	Introduction	101
5.2	Results	104
5.2.1	SM3 Performs Run-and-Tumble in Three Dimensions	104
5.2.2	Run-and-Tumble Parameters Are Similar in 2D and 3D Experiments	107
5.2.3	SM3 Chemotaxes in 3D	108
5.3	Discussion	109
6	CONCLUDING REMARKS	114

List of Figures

1.1	Simplified drawing of the physiology of bacteria based on the species <i>Vibrio cholerae</i> , which is Gram negative and has a single flagellum located at one pole. This figure is adapted from [6].	3
1.2	The complex internal structure of <i>Escherichia coli</i> . This colored illustration was produced by Dr. David S. Goodsell.	4
1.3	Experimental demonstration of the viscous forces in a fluid winning out over the inertial forces. From left to right: an ink droplet is injected into a vat of glycerin and stirred continuously until it disappears. Upon stirring in the opposite direction, the ink droplet reforms. From reference [13]. . .	7
1.4	The lattice structure of L-type and R-type flagellar filaments from reference [19].	12
1.5	A sinking infinitesimal rod segment experiences more drag perpendicular to its length than parallel, resulting in a drift to the left. From reference [13].	14
1.6	Schematic of the flagellar motor embedded in the cell wall of a Gram negative bacterium. (Left) Simplified depiction of the motor-flagellum system from reference [31]. (Right) Realistic depiction of the flagellar motor with proteins color-coded from reference [32].	15

1.7	Schematic drawing of run-and-tumble. The bacterium runs in one direction while all motors rotate in unison and form a bundle. Once one or more flagella rotate opposite to the rest, the bundle disassociates and the bacterium tumbles. Sometime later, the bundle reforms and the bacterium continues on a new trajectory. From reference [65].	18
1.8	The flow field around <i>E. coli</i> as measured experimentally (Bottom) and modeled theoretically (Top). The flow field for bulk swimmers (Left) has unbound flow lines whereas the field for swimmers next to a surface (Right) wrap back into the swimmer. Adapted from the original figure in reference [75].	21
1.9	A bacterium close to the surface swimming with a curved trajectory. The opposing torques of the cell body and flagellum (black) exerts opposing forces on each part (red), resulting in a trajectory with a curvature, κ (blue).	22
1.10	A typical swarm of <i>Enterobacter</i> SM3. Bacteria spread radially to fill one-half to three-quarters of the Petri dish six hours after the point of inoculation. The result is a circularly symmetric growth with the initial footprint of inoculation appearing as an opaque, white disk in the center. See the Methods for an explanation of the swarming assay procedure. Image acquired using the method described in reference [85].	23

2.1	Schematic of the digital holographic microscopy system. A laser illuminates a cuvette from the left, which is fixed to an adjustable imaging platform. The laser light scatters from the bacteria in the sample and interferes with the unscattered background light, creating an interference. The light is gathered by a microscope objective to the right of the cuvette and recorded digitally with a CMOS camera concealed within the chassis. Note the coordinate axes, with the positive y-direction being against gravity and the positive z-direction perpendicular to the cuvette and pointing along the laser direction.	44
2.2	(a) Schematic of the two-bead micro-swimmer model. The cell body and flagellar bundle are modeled as two beads of diameter $2a$ connected by a spring, and counter-rotating with equal and opposite torques T and $-T$. The flagellar force F^f on the cell body is prescribed on the bead tail. The swimming direction, which is along the tail-to-head vector, is $\vec{n}$. (b-c) The fluid flow velocity field $\mathbf{u}$ produced by the locomotion of the two-bead micro-swimmer. The color plots $\log(\mathbf{u})$. Whereas in (b) we can only see the effect of the force-dipole, cross-sections along the tail, center, and head beads reveal the effect of the torque-dipole.	57
3.1	(Top) A segment of an SM3 trajectory. The segment was taken at 100 FPS for 0.7 s, resulting in 70 consecutive frames. Images were selected seven frames apart and combined into an overlay image, showing the trajectory with visual clarity. (Bottom) Frame-by-frame sequence of the tumbling event from the above trajectory. The event lasts 12 frames, corresponding to a tumble time of 0.12 s. The tumble angle of this event is 63° . Red dots denote the two frames that are included in the image overlay above, which overlap at the position of the tumble. A movie of this tumbling event is available at [46].	68

3.2	Analysis of SM3 trajectories performed using a tracking algorithm. (a) A representative trajectory. The green circle marks the beginning of the trajectory. The blue dots indicate tumbles detected by the algorithm. A movie of this trajectory is available at [47]. (b) Linear speed versus frame number. (c) Angular speed versus frame number. Opaque curves in (b) and (c) show the unsmoothed data, with the solid lines after smoothing. (d) Overlay of the linear and angular speeds on the same plot. Each blue line notes the alignment of a dip in the smoothed linear speed with a peak in the smoothed angular speed, indicating a tumble.	69
3.3	Histograms of the measured tumble times, run times, and tumble angles for SM3 swimmers (a)-(c) and swarmers (d)-(f). The total counts and average values are listed in Table 3.1.	71
3.4	The effect of serine (black) and aspartate (red) on the run-and-tumble behavior of SM3. The data for dilute swarmers (green) is included for comparison. Plots include the (a) average tumble time, (b) average run time, (c) average tumble angle, and (d) average bacterium speed as functions of concentration. Dotted lines serve to guide the eye to apparent trends. Error bars are standard errors to the mean.	72
3.5	Histograms of the measured bacterium length. Bacteria were measured for (a) growth incubated overnight for 16 hours, (b) fresh growth incubated from overnight growth for 4 hours, and (c) bacteria extracted from the swarm front after 6 hours of growth on a Petri dish (refer to Figure 1.10 for an example swarm). Histograms represent 200, 200, and 203 data points, respectively. Average values for each data set are reported in the upper right of each panel with the reported error being the standard deviation.	73

3.6	Schematic illustration of a tumble event in SM3. When one flagellum switches rotation direction from CCW to CW (colored red), it leaves the bundle and causes the bundle to become uncoordinated, initiating a tumble. During the tumble, swimming motility is reduced as the bacterium reorients itself. This diagram can be directly compared with the observed behavior in Figure 3.1. (Inset) The net torque exerted by the flagella drives the rotation of the cell body with viscous drag opposing the rotation direction. Flagella were not observed in this study.	74
4.1	(a) Illustration of the forces and torques acting on a bacterium above a surface. The counter-rotation of the cell body and flagellar bundle (red arrows) produce forces in opposite directions (black arrows), causing a net torque that rotates the system. (b) Schematic of a circular trajectory with labeled kinematic parameters. (c) Representative circular trajectories of SM3 near the surface with length 3 and 10 μm . The colormap indicates the instantaneous linear speed. The average radius of curvature and linear speed of the trajectories are noted on each plot. Movies of these two trajectories are available at [23] and [24].	88
4.2	An example of SM3 swimming at the surface with multiple circular loops. A tumble occurs where the trajectory is kinked and the linear speed decreases sharply, as shown by the colormap. The start of the trajectory is indicated by a red circle. A movie of this trajectory is available at [25].	89

4.3	Average (a) radius of curvature, (b) linear speed, and (c) angular speed as functions of cell length for 97 different bacteria. Two fit lines are plotted for each data set: a weighted best fit (magenta), that accounts for the greater number of data points at shorter lengths, and an unweighted fit (cyan). The weighted fit is the linear fit to the average values of data points within each 1 μm length bin. Error bars are based on a sample standard deviation of the data used to produce a single data point.	91
4.4	(a) A simulated microswimmer with only force-dipole hydrodynamics. Once at the surface, it swims in a straight trajectory. (b) A simulated microswimmer with force- and torque-dipole hydrodynamics. It swims in a circle at the surface. (c)-(e) Top-down view of three circular trajectories for simulated microswimmers 1, 3, and 10 μm in length.	93
4.5	Numerical results for the radius of curvature as a function of length for constant force and torque. Numerical data points are shown with the corresponding trend line based on a linear fit.	94
4.6	Simulated results for the radius of curvature as a function of length for four combinations of increasing force and torque.	95

5.1	(a) Schematic of the sample cuvette with coordinate system. The laser travels along the z-direction, perpendicular to the cuvette's face, while gravity acts along the y-direction, parallel to the cuvette's length. (b) Three-dimensional plot of 828 trajectories obtained from a single digital holographic microscopy measurement. The hologram was recorded at 25 FPS for 60 s, resulting in 1500 frames, and was digitally reconstructed. Each trajectory is at least three seconds long. The hologram from which these trajectories were extracted is available at [14]. (c) The longest trajectory among the 828 shown in (b), spanning 352 frames over 14.1 s. The trajectory starts at the end closest to the top of the plot. Black stars denote tumbles detected by the algorithm. (d) Overlay of the instantaneous linear speed and trajectory angle—the angle between successive velocity vectors of the bacterium—on the same plot. Each blue line marks the alignment of a dip in the linear speed with a peak in the trajectory angle, indicating a tumble.	104
5.2	Jitter plots for the (a) tumble time, (b) run time, and (c) tumble angle using both two dimensional (2D) and three dimensional (3D) measurement techniques. Corresponding violin plots illustrate the data distributions. The mean is represented by a red data point, and the standard deviation is indicated by a red vertical line in each plot. The p-value, based on a two-sided Welch's t-test, is displayed at the top of each plot. The 2D jitter plots are based on 209, 199, and 209 data points, respectively, and were obtained using phase-contrast microscopy. The 3D jitter plots were based on 232, 207, and 232 data points, respectively, and were obtained using digital holographic microscopy.	106

5.3	(a) Three-dimensional tracks of SM3 exposed to 10 mM glucose. The hologram was recorded at 25 FPS for 60 s, resulting in 1500 frames, and was digitally reconstructed. Each trajectory is at least three seconds long.	
	(b) Jitter plot of the average linear speed for 25 trajectories in both MB and glucose. The mean is represented by a red data point, and the standard deviation is indicated by a red vertical line in each plot. The p-value, based on a two-sided Welch's t-test, is displayed at the top of the plot.	108

List of Tables

3.1	Run-and-tumble parameters of SM3. The results include measurements of swimmers in motility buffer, swimmers with added serine or aspartate, and swarmers in motility buffer. Tabulated data includes the average tumble time, run time, tumble angle, and run speed based on the number of data points shown in brackets. Reported errors are standard deviations, showing a large range of values for each measured parameter.	71
-----	---	----

CHAPTER 1

INTRODUCTION

“If you want to master something, teach it. The more you teach, the better you learn.”

—Richard P. Feynman

1.1 A World Within Our Own

Bacteria dominate the world around us; they exist in the darkest depths of the ocean [1], help plants to grow by populating the soil beneath our feet [2], allow us to digest food and get the most out of every bite [3], and may even be the first glimmers of life among the stars as we search for worlds beyond our own [4]. In fact, many estimates predict that there are 100 million more bacteria (13×10^{28}) in our oceans than there are stars in the known universe [5]. Despite their overwhelming numbers, bacteria generally occupy relatively little space in our collective psyches—that is, unless you are a bacteria-obsessed PhD student—and the microscopic world that they inhabit is quite unlike anything that we experience in our everyday lives.

The purpose of this thesis is to catalog and communicate novel research findings; however, more importantly, it hopes to instill an amazement of the marvel of the microscopic world into the reader.

Before embarking on our journey, it is helpful to have an idea of the scales that we are dealing with. If you were to shrink by 10 times of your height, you would be, give or take, the size of a banana (~ 15 cm). Reducing by a further factor of 10, you would be standing at the height of a US quarter (~ 25 mm); another factor, the height of the edge of the quarter (~ 1.75 mm). From here, we begin to enter the microscopic world and the organisms that inhabit it. Tardigrades—the nigh-indestructible water bear that feasts on many smaller microorganisms—stand 10 times smaller than the edge of a coin ($\sim 0.5 - 1.5$ mm) and are an order of magnitude larger than a red blood cell (~ 5 μm). At this scale, objects can not be easily viewed by the naked eye and special tools, such as microscopes, become necessary for us to see them. Finally, with one more 10 times reduction in size, we would be standing, perhaps not-so-comfortably, next to a cell of *Escherichia coli* (*E. coli*) ($\sim 0.5 - 2$ μm); a bacteria whose strains both help (e.g, by allowing us to properly digest food) and hinder (e.g., by releasing toxins that can have adverse effects on our health) us. It is this length scale, one million (10^6 $\mu\text{m} = 1$ m) times smaller than a human, that we are most interested in¹.

The microscopic world is quite unlike our own and does not compare to our everyday experience of common substances, such as water. Bacteria, for example, are ~ 1000 times larger than a single water molecule, which are ~ 0.3 nm in diameter. Going by the previous scale progression, that would be comparable to the edge of a coin or a grain of sand. So, for bacteria, loosely speaking, being in water is like being surrounded by thick, viscous quicksand. Bacteria need to live in liquid environments, so they have evolved to meet the challenges of their environment. Before addressing how bacteria have “figured out” a way to move around, their physiology must first be understood.

¹Thank you “Epic Spaceman” for this useful mnemonic for remembering microscopic length scales. (See on YouTube, as of the writing of this thesis: “I shrink 10x every 21s until I’m an atom - The Micro Universe”).

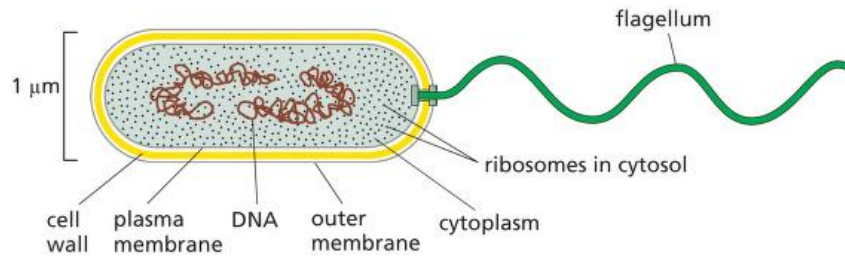


Figure 1.1: Simplified drawing of the physiology of bacteria based on the species *Vibrio cholerae*, which is Gram negative and has a single flagellum located at one pole. This figure is adapted from [6].

1.2 A Brief Physiology of Bacteria

Bacteria are very complicated; so complicated, in fact, that whole books of multi-part volumes have been written about their simple, yet beautifully-evolved physiology. However, to better understand some of the discussion that will occur later, a brief introduction is in order.

Since the advent of the microscope technology that gave us the ability to peer into a previously unknown microscopic world, it was known that cells could be grouped according to their structure. The bacteria (eubacteria) animal kingdom is a subset of the much larger domain prokaryota. Prokaryotic cells, or prokaryotes, are among the simplest of single-celled organisms; unlike the eukaryotic cells that make up our bodies, they do not have a nucleus: a separate compartment that houses the genetic material of life—the double-helix of deoxyribose nucleic acid, DNA. However, their simplicity allows them to be one of the most diverse and ecologically varied organisms on earth [6].

A basic drawing of a bacteria is shown in Figure 1.1. Bacteria are simple in their shape; they are typically either rods or spheres, and some have appendages that they use to move through their environment. The internal biochemical processes and materials (DNA, ribosomes, etc.) of the cell are separated from the external environment by a cell wall and plasma membrane, allowing the cell to function as its own isolated chemical system. The cell wall is rigid and helps to protect the cell from the environment; for example, from changes in the osmolarity (salt concentration) of the surrounding environment. It



Figure 1.2: The complex internal structure of *Escherichia coli*. This colored illustration was produced by Dr. David S. Goodsell.

is composed of peptidoglycan, a mesh-like substance comprised of polysaccharides and amino acids [6].

Historically, bacteria have been separated into two categories based on the thickness of their cell wall. The Gram staining method exposes bacteria to the stain crystal violet. Bacteria with a relatively thick cell wall retain the stain when exposed to ethanol and are Gram-positive, as opposed to Gram-negative bacteria, which have thinner cell walls that do not retain the stain [7]. Gram-positive bacteria have a single thick cell wall that surrounds the plasma membrane. Gram-negative bacteria, on the other hand, are more complex; they have two plasma membranes—an inner and an outer—separated by a thin layer of peptidoglycan within the periplasmic space [8]. Examples of Gram-positive bacteria genera include *Staphylococcus* and *Bacillus*, while Gram-negative genera include *Escherichia* and *Enterobacter*. This thesis will be concerned with Gram-negative species.

The plasma membrane is comprised of amphipathic molecules—that is, molecules that have both a hydrophilic (water-loving) head and hydrophobic (water-hating) tail. These molecules spontaneously arrange themselves into a bilayer, with the hydrophilic heads

pointing out towards the aqueous environment and the hydrophobic tails protected within. The cell wall and plasma membrane, however, cannot be completely impermeable, as the cell needs a way to exchange molecules and nutrients with the environment [6]. To this end, proteinaceous structures—those consisting of protein—are positioned in the cell wall and allow the passage of materials. These channels are remarkable in their transport capabilities. For example, some only allow certain ions to pass through, and in only one direction, creating a potential difference across the cell that can be used to do work [9, 10]. Bacteria live by separating their inner workings from the external environment, supporting many biochemical and electrochemical processes.

A simple diagram (Figure 1.1) does not do the complexity of the bacterial physiology justice. Figure 1.2 is a beautifully illustrated depiction of the inside of a bacterium by Dr. David S. Goodsell of Rutgers University. The illustration is specifically of *E. coli*, a Gram-negative bacteria. There are two plasma membranes, colored in green, with a light-blue, mesh-like structure between them, which is the peptidoglycan layer. Embedded in the cell wall is a flagellum, also in green, with its distinct ring-like motor structure just below the surface. A tunnel-like structure (top right) allows for the passage of materials across the cell wall. Within the cell, there is a dense collection of macromolecules, other small molecules, and ions suspended in water—a mixture called cytoplasm. There is a clear order to the inside of the cell, despite it lacking a nucleus: the genetic material and machinery of the cell is near the center in a yellow, tangled mass, with purple, protein-synthesizing machines called ribosomes on the periphery. Our understanding of the arrangement of the cytoplasm has come a long way. In fact, researchers have recently begun exploring why it is the case that the genetic material concentrates itself closer to the center of the cell [11]. Needless to say, bacteria, although “simple”, are already incredibly complex!

This is, of course, an overly-simplified summary of bacterial physiology. However, it will serve as valuable context for some of the discussion going forward. We will

concern ourselves primarily with Gram-negative bacteria, such as *E. coli*, and the one studied extensively in this thesis, *Enterobacter* sp. SM3—or SM3 for short. Of the many components of the cell, the motility appendages—specifically flagella—and how they are powered will be more closely detailed. First, however, we must understand why bacteria have evolved the specific shape and function for their flagella, which has everything to do with their physical environment.

1.3 Life at the Microscopic Scale

1.3.1 The Issue of Water

Bacteria can thrive in many different environments. However, energy, nutrition, and water are paramount to survival. In this way, bacteria are not quite different from us; we also need food, water, and a proper temperature range to live comfortably. The length scale at which bacteria live, however, makes acquiring these vital components more challenging than what we may suspect.

Bacteria are tiny. Water molecules, for example, are large enough to influence the way in which bacteria move. According to the equipartition theorem, in thermal equilibrium, the energy of a system is equally divided among each quadratic degree of freedom, each contributing a value of $k_B T/2$. For a single water molecule, there are at most nine degrees of freedom, giving a thermal energy of $E = 9k_B T/2$, which is the sum contribution of the kinetic and potential energy. Simply put, water molecules are constantly moving and, as they bombard into bacteria, they impart kinetic energy to them. This is called thermal agitation and the displacement of bacteria and other small particles due to this agitation is called Brownian motion. It was a young graduate student, Albert Einstein, who first mathematically quantified this displacement [12]. The displacement is random, as water molecules strike from all directions. However, every so often, a large displacement is made and, on average, the squared displacement of the particle changes. The displacement is

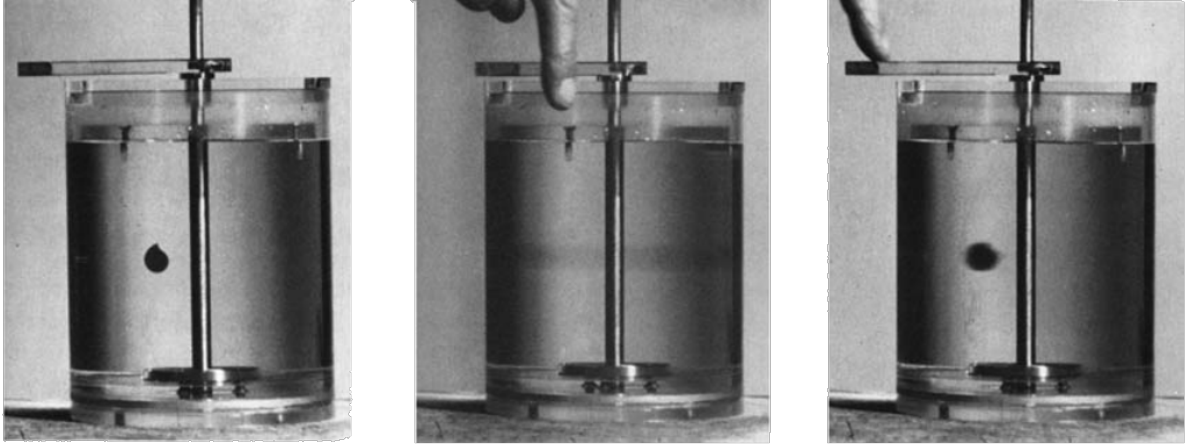


Figure 1.3: Experimental demonstration of the viscous forces in a fluid winning out over the inertial forces. From left to right: an ink droplet is injected into a vat of glycerin and stirred continuously until it disappears. Upon stirring in the opposite direction, the ink droplet reforms. From reference [13].

given by:

$$\langle (r_N^2) \rangle = 6Dt, \quad (1.1)$$

where D is the diffusion constant ($\text{m}^2 \cdot \text{s}^{-1}$) and t is the time over which the displacement occurs. The diffusion constant can be defined mathematically; however, Einstein found a way to calculate it with physical parameters that can be measured. According to Einstein's relation, the diffusion coefficient can be calculated as:

$$\xi D = k_B T, \quad (1.2)$$

where ξ is the viscous friction coefficient ($\text{kg} \cdot \text{s}^{-1}$), k_B is the Boltzmann constant ($1.38 \times 10^{-23} \text{ J} \cdot \text{K}^{-1}$), and T is the temperature (K). Fundamentally, Einstein's relation arises from the fact that, at equilibrium, the collective flow of particles due to an external conservative force balances with the flow due to diffusion, resulting in no net flow. Therefore, to calculate the diffusion coefficient for a particle, we first need to know the viscous friction exerted on it.

1.3.2 Life in the Slow Lane: Low Reynolds Number

For us, swimming through water is usually an enjoyable experience. For bacteria, however, water molecules are large enough to impose significant frictional forces and antagonize motility. One can get an idea of the challenge that bacteria face by conducting a macroscopic experiment. If one were to pour some half-and-half into a cup of black coffee and give it a quick stir, stopping immediately after a full pass around the circumference of the cup, they would notice two things: 1) the half-and-half would continue to mix after being initially perturbed and 2) there would be no way to reverse the process of mixing. This is a good example of a fluid system in which inertial forces are much larger than frictional, or viscous, forces.

Here is a more exotic experiment. A clear, glass cylinder is filled with a thick, viscous substance—in this case glycerin—which is about 1000 times more viscous than water at room temperature (Figure 1.3). If one were to deposit an ink droplet somewhere in the middle of the glycerin and stir it continuously in one direction, the droplet would disappear. The curious feature, however, is that when the system is stirred in exactly the same way, in the opposite direction, the ink droplet reforms in about the same position as it was originally deposited. This is a classic example of a system where viscous forces dominate and inertia is negligible. The property of the ink droplet reforming itself is called time reversibility. Another feature of this system is that, once stirring ceases, so does the motion of the ink droplet. This makes sense: the effects of inertia are damped out by the high viscosity of the glycerin.

The principles of time reversibility and over-damping have important consequences for bacteria. Because of time reversibility, periodic swimming maneuvers result in no net forward displacement—moving an appendage in one direction and then returning back to the original position simply resets the system [14]. If a bacterium does manage to move through internal propulsion, all motion ceases instantly once propulsion is no longer provided due to over-damping. The physical properties of the environment, therefore,

greatly influences the behavior and evolved physiology of bacteria.

The ratio between the inertial and the viscous forces in a fluid is quantified by a dimensionless parameter called the Reynold's number (Re). The Re is scale-dependent, meaning that the size matters when considering the effects of inertia and viscosity. The Re can be calculated from first principles but, as with most dimensionless quantities in fluid mechanics, it is often easier to consider the dimensions of the system. For a spherical particle moving in water, the physical parameters that contribute to the inertial and viscous forces are the speed of the particle, v ($\text{m} \cdot \text{s}^{-1}$), the size of the particle, l (m), the dynamic viscosity of the liquid, μ ($\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-1}$), and the density of the water, ρ ($\text{kg} \cdot \text{m}^{-3}$). The only dimensionless quantity that these parameters can construct is the Re :

$$Re = \frac{vl\rho}{\mu}. \quad (1.3)$$

Approximating the bacteria as a sphere of characteristic length $1 \mu\text{m}$ moving at $30 \mu\text{m/s}$ in water of density 1000 kg/m^3 and viscosity $0.001 \text{ Pa} \cdot \text{s}$ ($\text{Pa} = \text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-2}$), the Re is approximately 3×10^{-5} . This minuscule Re indicates that, for bacteria, water may as well be glycerin!

1.3.3 The Navier-Stokes Equation for Low Re Fluids

The best mathematical formulation that we have to describe hydrodynamics are the Navier-Stokes equations. It is a testament to the complexity of fluids that the unreduced form of these equations are rarely analytically solvable. However, at low Re , the Navier-Stokes equations reduce to a form that yields analytical solutions.

The Navier-Stokes equation for incompressible flow with uniform density and viscosity is given by:

$$\rho \left(\frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u} \cdot \nabla) \mathbf{u} \right) = \mu \nabla^2 \mathbf{u} - \nabla p + \mathbf{f}, \quad (1.4)$$

where $\mathbf{u}$ is the flow velocity, ρ is the mass density, μ is the dynamic viscosity, p is the pressure, and $\mathbf{f}$ is an applied force. The left-hand side of this equation consists of the inertial terms (i.e., those resulting from the acceleration of an imaginary parcel of water flowing through the system). In the low Re regime, the left-hand side can be set to zero, which reduces Equation 1.4 to:

$$\nabla p = \mu \nabla^2 \mathbf{u} + \mathbf{f}. \quad (1.5)$$

This result is known as the Stokes equation and describes Stokes flow. The assumption of low Re reduces the Navier-Stokes equations to a linear form that can be analytically solved, resulting in many useful equations for studying the motion of bacteria in liquid environments. For example, the viscous friction coefficient for a sphere is determined by solving Equation 1.5 for the velocity and pressure profile of the fluid around the sphere and calculating the force on the sphere by integration. The result is known as Stokes' law:

$$\xi_{sphere} = 6\pi\mu R, \quad (1.6)$$

where R is the radius of the sphere. The translational drag force is then given by:

$$\mathbf{F}_{drag,sphere} = 6\pi\mu R \mathbf{v}. \quad (1.7)$$

The linearity of the Stokes equation also makes it possible to find analytical solutions for the viscous friction coefficients for ellipsoids, which are useful when modeling rod-shaped bacteria as prolate ellipsoids—ellipsoids longer than they are wide. The viscous drag coefficients for prolate ellipsoids are given by [15, 16, 17, 18]:

$$\mathbf{F}_{drag,prolate} = 6\pi\mu(ab^2)^{1/3}\mathbf{v}F_t \quad (1.8)$$

and

$$\boldsymbol{\tau}_{drag,prolate} = 8\pi\mu ab^2\boldsymbol{\omega}F_r, \quad (1.9)$$

where a is half the semi-major axis and b is half the semi-minor axis. Here, $\mathbf{F}_{drag,prolate}$ is the translational drag on a prolate ellipsoid, averaged around all axes, and $\boldsymbol{\tau}_{drag,prolate}$ is the rotational drag about the short axis. The factors, F_t and F_r , are functions of the ellipsoid geometry and are given by:

$$F_t = \frac{\sqrt{1-q^2}}{q^{2/3} \ln \frac{1+\sqrt{1-q^2}}{q}} \quad (1.10)$$

and

$$F_r = \frac{4(1-q^4)}{3q^2[2q^{-2/3}(2-q^2)/F_t - 2]}, \quad (1.11)$$

where F_r is called the Perrin rotational friction factor and $q = b/a$ is the axial ratio.

Low Re fluid dynamics have profound effects on the evolved physiology of bacteria. For example, consider their foraging behavior. A typical “food” molecule, such as the amino acid serine, has a diameter on the order of nanometers ($1 \text{ nm} = 10^{-9} \text{ m}$). Modeling a food molecule as a sphere and using Einstein’s relation (Equation 1.2), D is calculated to be approximately $200 \text{ } \mu\text{m}^2/\text{s}$ at room temperature ($T = 293 \text{ K}$) in water ($\mu = 0.001 \text{ Pa} \cdot \text{s}$). The mean displacement of the particle due to Brownian motion is then given by Equation 1.1, for a time interval of one second, as approximately $35 \text{ } \mu\text{m}$ —meaning that, purely due to diffusion, the typical food particles that bacteria subsist on travel at roughly $30 \text{ } \mu\text{m}/\text{s}$.

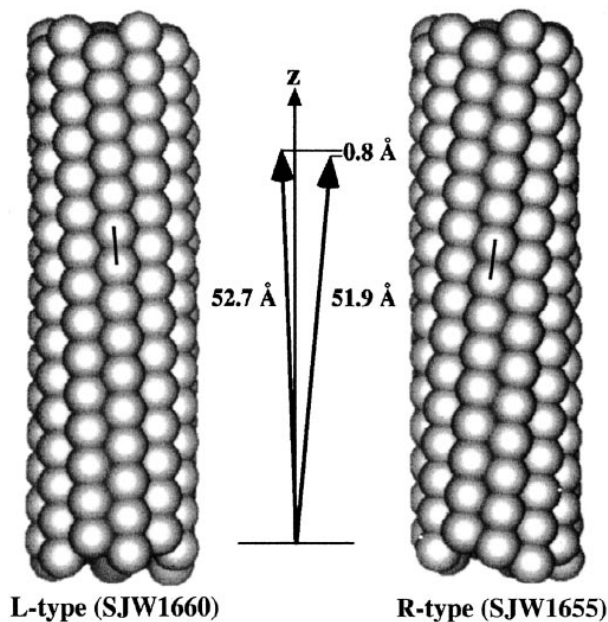


Figure 1.4: The lattice structure of L-type and R-type flagellar filaments from reference [19].

It is no surprise, then, that many bacteria are often observed to swim at least $30 \mu\text{m/s}$, on average. Bacteria need to be fast enough to detect local concentration gradients in food molecules and this puts an evolutionary constraint on their physiology [13].

Another question remains: how do bacteria manage to swim in the first place? As pointed out, in a low Re regime, swimming is made challenging by the fact that periodic swimming maneuvers do not result in any significant translation. The solution is simple, but the biology is anything but: bacteria have evolved non-periodic means of swimming that take advantage of fundamental physics principles.

1.4 The Physics of Bacteria Swimming

Bacteria have more in common with submarines than they do with us. Of course, this is hyperbolic, but there is some truth to it: many motile bacteria are little, living capsules that propel themselves through water with corkscrew-like filaments called flagella (Figure 1.1). The arrangement and number of flagella vary vastly between bacterial species: some are “monotrichous,” having a single flagellum located at one pole of the

cell body (e.g., *Pseudomonas aeruginosa*) [20, 21], while others are “peritrichous” and have them all around the cell body (e.g., *E. coli*) [20, 22, 23]. Scientists have known that bacteria possessed flagella since the 19th century by observing the flagella of large species of bacteria, such as *Chromatium okenii* [24, 25]. However, it was not until the 20th century that innovations in microscope technology, specifically high-intensity dark field condensers, allowed researchers to see the flagellum filament with higher resolution [26, 25]. Since then, there has been extensive work to understand the construction and operation of flagella.

1.4.1 The Flagellar Filament

The flagellar filament is a hollow, cylindrical structure composed of eleven fibrils (rows) of the protein flagellin (*FliC*) arranged in a lattice (Figure 1.4) [27]. The cell grows the filament by ejecting flagellin through the center of a hollow shaft, building it from the distal end of the filament [28]. Each fibril is twisted at an angle relative to the central cylindrical axis, which gives the filament twist. Additionally, flagellins have the ability to pack in two different ways, with R-type flagellins being closer together than L-type ones. For a filament with both types of flagellin, the flagellar filament has both a twist and is helical, resulting in a rigid, propeller-like swimming appendage [19]. The crystalline structure that makes up the flagellar filament is able to change structure, which alters the amplitude, pitch, and even handedness of the helix. For *E. coli*, filaments undergo a polymorphic transformation that changes the flagellum handedness when the flagellar motor switches from counterclockwise (CCW) to clockwise (CW), which allows the flagellum to continue pushing the cell while in the CW state [23].

The flagellar hook connects the flagellar filament to the flagellar motor, which is embedded in the cell wall of the bacterium. The hook is constructed in a similar way as the filament and is made up of the protein *FlgE* [27]; however, the hook is quite flexible compared to the rigid filament [29]. This flexibility allows the flagellar filaments to create

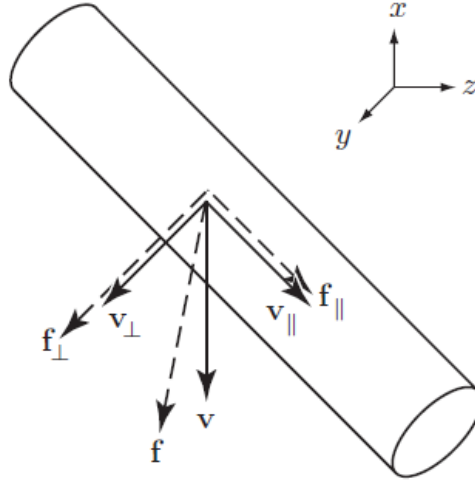


Figure 1.5: A sinking infinitesimal rod segment experiences more drag perpendicular to its length than parallel, resulting in a drift to the left. From reference [13].

a bundle behind the cell body when it swims forward [30].

The way that the flagellum overcomes the issue of locomotion at low Re is an interesting phenomenon that can be understood with fundamental physics concepts. Consider a rod of infinitesimal length, dl , left to sink under its own gravity in a low Re fluid environment (Figure 1.5). In addition to gravity, the rod experiences a drag force from the surrounding fluid. However, the viscous friction coefficient, ξ , is larger in the direction perpendicular to the rod than parallel to it. In a low Re environment, inertial forces are zero and all forces must balance. Thus, the rod experiences a larger force in the perpendicular direction than in the parallel one. The effect is that the rod drifts as it falls. One can try this at home with a straw and a clear bucket of water; it really works.

The flagellum can be thought of as a collection of tiny, infinitesimal rods arranged along a helix. When the flagellum rotates, the components of force from each segment superimpose (add up) along the length of the filament, resulting in a net force away from the cell body. The reactionary force—the fluid pushing back on the flagellum—pushes the cell forward. A question remains, however, and that is how exactly do bacteria manage to rotate their flagella? The answer has been teased: bacteria use motors to rotate their

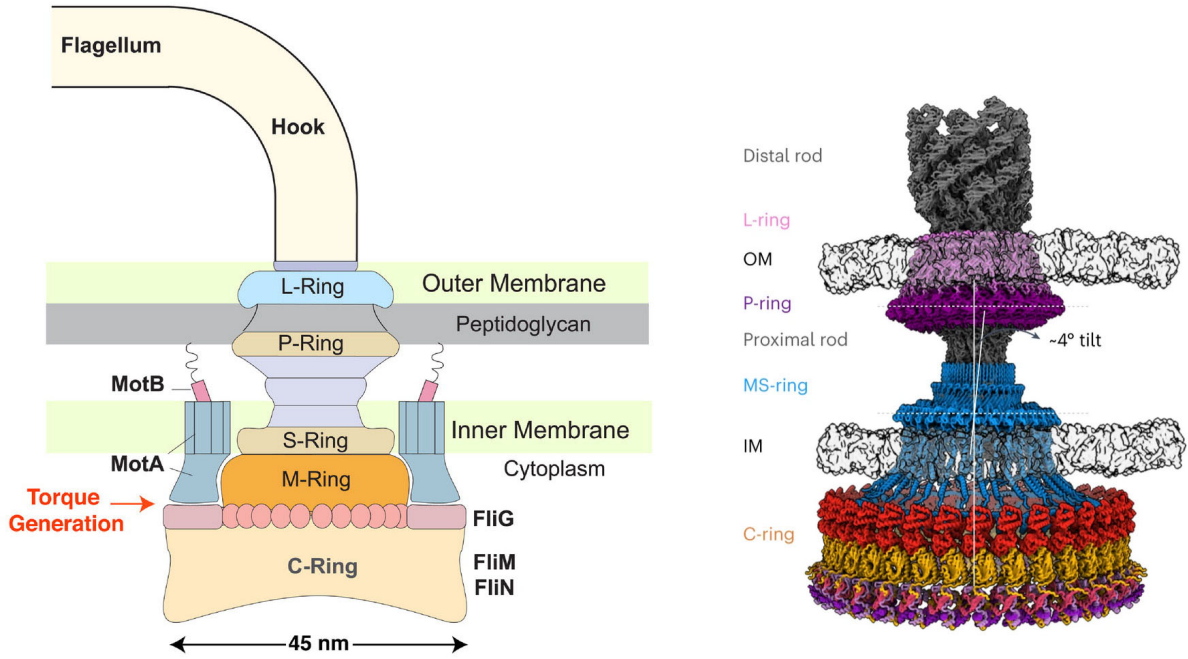


Figure 1.6: Schematic of the flagellar motor embedded in the cell wall of a Gram negative bacterium. (Left) Simplified depiction of the motor-flagellum system from reference [31]. (Right) Realistic depiction of the flagellar motor with proteins color-coded from reference [32].

flagella.

1.4.2 The Flagellar Motor

Since the discovery of bacteria, researchers have always wondered how exactly they swam. The puzzle became more bizarre when our understanding of low Re fluid dynamics improved and it was understood that, however bacteria swam, it had to be nonreciprocal. This realization was the nail-in-the-coffin for ideas such as a wave propagating down the flagellar filament [19].

In 1974, Dr. Michael Silverman and Dr. Melvin Simon designed an experiment that ultimately solidified our understanding of how bacteria move their flagella. By attaching bacterial mutants without flagella to substrates by their hook, they observed that bacteria spun in place when viewed under the microscope [33]. To the surprise of many researchers at the time, it seemed that bacteria rotated their flagella by means of a biological motor.

The idea had been proposed before by other researchers, such as Howard Berg [34], but this experiment made the mechanism conclusive. This finding was revolutionary: never before had a biological motor been observed in nature. And, perhaps not surprisingly, the construction of the motor is a biological marvel that has inspired much research over the decades [32, 35, 36].

A depiction of the flagellar motor is shown in Figure 1.6 for a Gram-negative cell, with a simplified drawing on the left and a realistic rendition on the right. Any motor, and that includes those of the biological variety, is comprised of two components: a stationary stator unit that provides torque and a freely-moving rotor unit. The *MotA* and *MotB* protein complexes, which comprise the stator of the bacterial motor, are embedded in the inner membrane and further anchored to the peptidoglycan matrix. The rotor consists of the MS-ring and the C-ring (cytoplasmic ring), which are free to move when acted upon by the *MotA/MotB* complex. The proteins that make up the MS-ring and C-ring are arranged in a circular, symmetric configuration such that the rotor resembles a turning disk (Figure 1.6, Right). The rotor is connected to a rod (proximal and distal) that passes through the outer membrane and is itself connected to the flagellar hook. As the rotor turns, so does the flagellar hook and filament [19].

The stator complex provides torque to the MS-ring and is powered by either a proton or ion pump. In the case of *E. coli*, protons in the periplasmic space pass through the stator and into the negatively charged cytoplasm, flowing from a region of high potential energy to low—like a dam being let loose—and do work that activates the protein complex. This is called the proton-motive force (pmf) [9, 37, 38]. Upon activation, the *MotA* protein complex takes a “step” around the diameter of the C-ring, facilitated by the protein *FliG*, causing the rotor to rotate [39, 40]. The process of *MotA* binding to *FliG* to take an iterative step can be compared to the well-known example of a kinesin protein “walking” along a microtubule in an animal cell, transporting its cargo [41, 42, 43]. This process occurs as long as a pmf is available and happens for multiple stators at a time. In fact, *E.*

coli is thought to have eleven stators arranged around the diameter of the C-ring, with more stators being recruited depending on the load experienced by the motor [44, 45]. Through this complex motor system, bacteria have evolved a way to utilize rotating flagella for their motility, effectively solving the issue of non-reciprocal swimming.

Many studies have been conducted on the flagellar motor, one popular method being to attach a particle to a sheared flagellar stub and track it [46, 47]. *E. coli* is a well-studied species with a known motor rotation rate on the order of 100 Hz [19]. Other species, such as *Vibrio alginolyticus*, a marine species that uses sodium ions to power its motor, can rotate much faster [48]. Strains mutated to be deficient in stator units [49, 50] or those broken deliberately under high load [51, 52] are found to produce more torque when working stator units are re-introduced. Optical tweezer experiments have been conducted for *E. coli* cells that were attached to a surface directly by their hook to determine the compliance, or elasticity, of it [29]. Interestingly, studies of bacteria attached to surfaces reveal that the motor can bias itself in either the CCW or CW direction [33, 53]. This is a property that is at the core of bacterial navigation, or taxis.

1.4.3 Chemotaxis

Bacteria need to have the ability to change direction to forage for resources and to escape dangers. If they could not, they would be at the mercy of diffusion due to Brownian motion to change direction, which is not an effective strategy. Rather, bacteria can bias their flagellar motors in either the CCW or CW direction to alter their trajectory in response to their local environment, a behavior called chemotaxis [22, 54, 55].

On the surface of the cell wall, exposed to the extracellular environment, are various receptor complexes. When one of these receptors is triggered, a signal transduction pathway causes the phosphorylation of a cytoplasmic protein called *CheY*, resulting in a higher concentration of *CheY-P* in the cytoplasm [56, 57, 58]. *CheY-P* binds to the protein *FliM*, a sub-component of the C-ring, and causes the ring to undergo a

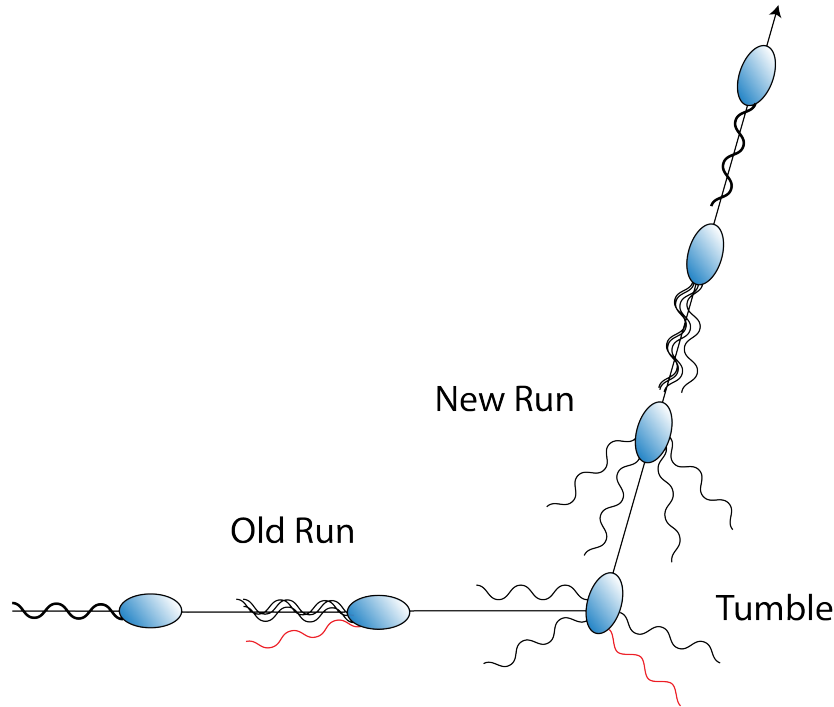


Figure 1.7: Schematic drawing of run-and-tumble. The bacterium runs in one direction while all motors rotate in unison and form a bundle. Once one or more flagella rotate opposite to the rest, the bundle disassociates and the bacterium tumbles. Sometime later, the bundle reforms and the bacterium continues on a new trajectory. From reference [65].

conformational (shape) change [59, 60, 61]. It is believed that *FliM* can exist in two states mediated by *CheY-P*: left- and right-leaning. When *CheY-P* binds to a *FliM* subunit, it causes it to become right-leaning. *MotA* causes the C-ring to rotate CCW or CW when interacting with either the left or right conformation, respectively. As the concentration of cytoplasmic *CheY-P* increases, so does the number of bound *FliM*, and the overall tendency of the rotor to rotate CW [62, 63, 64]. This majority-rules mechanism allows the bacteria to detect and respond to changes in the local environment by biasing their motors accordingly.

1.4.4 Run-and-Tumble

The way that bacteria change direction is species dependent. For peritrichous bacteria, such as *E. coli* [23] and *SM3*, the flexibility of the flagellar hook, coupled with the effects

of hydrodynamic entrainment and drag, allow the flagella to form a bundle when the cell swims forward. This occurs when all motors are rotating CCW [30, 66]. However, when at least one motor switches to a CW rotation, the delicate balance of the flagellar bundle is disturbed and it either fully or partially dissociates. While uncoordinated, the bacterium randomly reorients itself over a short interval of time while remaining relatively localized in space [65]. This reorientation event is known as a tumble. Once the flagellar motors are back in agreement (i.e., all are biased CCW), the bundle gradually reforms and the bacterium begins swimming in a new direction. The periods of smooth swimming, uninterrupted by tumbles, are called runs. This iterative process of swimming (running) and changing direction (tumbling) is known as run-and-tumble and it is characteristic of the motility of peritrichously flagellated bacteria (Figure 1.7) [65, 67, 68].

Run-and-tumble is not a random walk. As discussed, bacteria can bias their motor rotation direction. Therefore, run-and-tumble can be accurately thought of as a biased random walk. In fact, current experimental and theoretical approaches model run-and-tumble as a Levy walk: a type of random walk characterized by small displacements interspersed with occasional large displacements [69, 70, 71, 72]. This results in a run time distribution that is heavy-tailed. Unlike a pure random walk, Levy walk allows for the organism to explore a larger region of space.

1.4.5 Hydrodynamics of Microswimmers

As a bacterium swims, its cell body and flagellar bundle produces a flow, or velocity, field in space. Using solutions to the Stokes equation, the velocity field around a swimming bacterium can be modeled.

Consider a point force, $\mathbf{F} \cdot \delta(\mathbf{r})$, on a low Re fluid that occurs at the origin in a given coordinate system. The Stokes equation, Equation 1.5, then reads:

$$\nabla p = \mu \nabla^2 \mathbf{u} + \mathbf{F} \cdot \delta(\mathbf{r}). \quad (1.12)$$

The Green's function solution of this linear, inhomogeneous, differential equation is known as a Stokeslet [30, 73]:

$$\mathbf{u}(\mathbf{r}) = \mathbf{F} \cdot \frac{1}{8\pi\mu} \left(\frac{\mathbf{1}}{|\mathbf{r}|} + \frac{\mathbf{r}\mathbf{r}}{|\mathbf{r}|^3} \right), \quad (1.13)$$

where $\mathbf{1}$ is the identity matrix and $\mathbf{u}(\mathbf{r})$ is the second-rank tensor describing the velocity flow field. The field diminishes as $1/r$ from the point of the disturbance, similar to the electric potential of a point charge. However, unlike for a point charge, the field described by Equation 1.13 is anisotropic. If one evaluates Equation 1.13 along the parallel and perpendicular directions, the magnitude of the flow velocity in the parallel direction is exactly twice that of the perpendicular direction. This is why, for example, the viscous friction coefficient is larger in the perpendicular direction than in the parallel.

In addition to the Stokeslet, there is a solution to Equation 1.5 for a point torque, $1/2(\nabla \times \mathbf{T})\delta(\mathbf{r})$, at the origin known as the rotlet. The rotlet velocity flow field at the origin is given by the expression [74]:

$$\mathbf{u}(\mathbf{r}) = \frac{1}{8\pi\mu} \frac{\mathbf{T} \times \mathbf{r}}{|\mathbf{r}|^3}, \quad (1.14)$$

where $\boldsymbol{\tau}$ is the strength of the point torque.

A peritrichous bacterium can be thought as being composed of two separate components: the cell body and the flagellar bundle, which both exert point forces and torques on the surrounding fluid. A flagellar bundle that is pushing the cell body forward exerts a force on the fluid, away from the cell body. Given Newton's third law, the cell body, then, experiences a forward force. The flow field of the combined system can, therefore, be mathematically modeled as a force dipole, with the body and flagellar bundle having Stokeslet solutions of equal and opposite magnitude [75]. Similarly, the system can also be modeled as a torque dipole, with the body and flagellar bundle having rotlet solutions

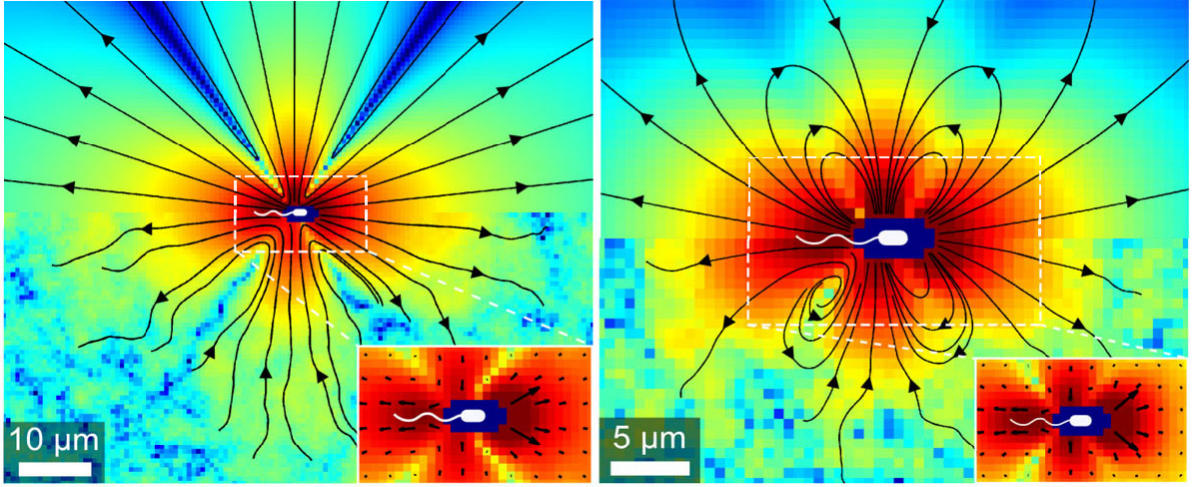


Figure 1.8: The flow field around *E. coli* as measured experimentally (Bottom) and modeled theoretically (Top). The flow field for bulk swimmers (Left) has unbound flow lines whereas the field for swimmers next to a surface (Right) wrap back into the swimmer. Adapted from the original figure in reference [75].

of equal and opposite magnitude. The equation describing the flow field of a bacterium is then a superposition of Stokeslet (Equation 1.13) and/or rotlet (Equation 1.14) solutions.

The flow generated by a bacterium is closely modeled by a force dipole. In fact, Goldstein et al. showed experimentally that swimming cells of *E. coli* exhibit a force dipole flow field [75]. The flow pattern shown in the left panel of Figure 1.8 is characteristic of pushing-type bacteria; the flow lines come in at the sides of the cell body and go out towards the head and tail. The theoretical flow field, shown in the top half of Figure 1.8, is in agreement with a superposition of Stokeslet solutions. For bacteria that swim with the flagellum leading the cell body, so-called pullers, the flow pattern is exactly the opposite of that for pushers. A knowledge of the flow patterns for individual microswimmers is important for researchers that model the behavior of bacteria in complex environments [76].

1.4.6 Near-Surface Swimming

The presence of a wall has many interesting effects on swimming. Hydrodynamically, the wall introduces a no-slip boundary condition, such that the magnitude of the flow

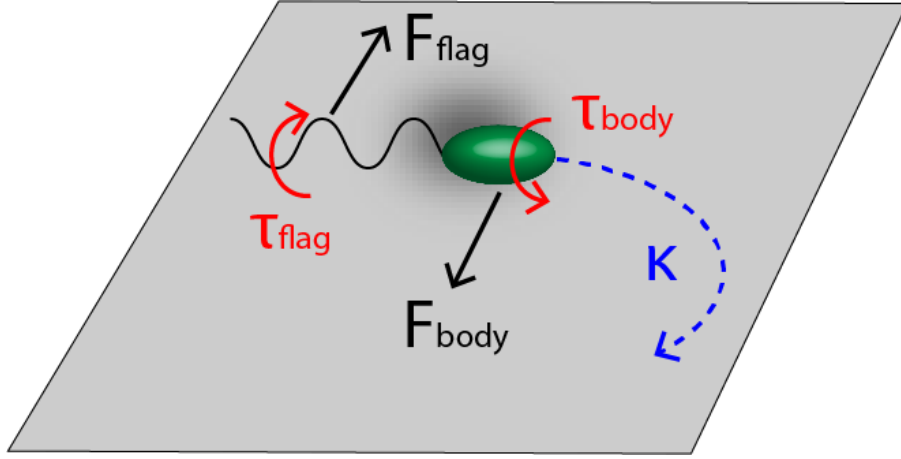


Figure 1.9: A bacterium close to the surface swimming with a curved trajectory. The opposing torques of the cell body and flagellum (black) exerts opposing forces on each part (red), resulting in a trajectory with a curvature, κ (blue).

field along the wall must be zero. Therefore, the flow field of a bacterium next to a wall must change to satisfy this boundary condition. The modified flow field for a Stokeslet next to a planar wall was first solved by Blake (1971) by introducing an image Stokelet on the other side of the surface, an approach similar to the method of images in electrostatics [77]. The equation for the modified flow field is not shown here for brevity's sake. An important feature of the modified flow field is that the flow lines “wrap” back into the cell body, and this is seen experimentally (Figure 1.8, Bottom Right). Therefore, bacteria swim differently when next to a surface, as compared to when in bulk liquid.

As a bacterium swims close to a surface, it experiences a viscous drag force from the underlying fluid layer. The flagellar bundle, which rotates counter to the cell body, experiences a drag force that is opposite in direction to the drag force on the cell body. Therefore, the bacterium experiences a net torque and trajectories become curved and circular (Figure 1.9) [78, 79]. Left-handed bacteria trace out CW trajectories when the flagellar bundle rotates CCW. Additionally, as bacteria swim in close proximity to the surface, the angle between the cell body and the surface oscillates, with an average angle around 10° [80]. Bacteria can leave the surface; however, bacteria are generally attracted to the surface because of their image [81, 82] and are pinned to it by the propulsion of

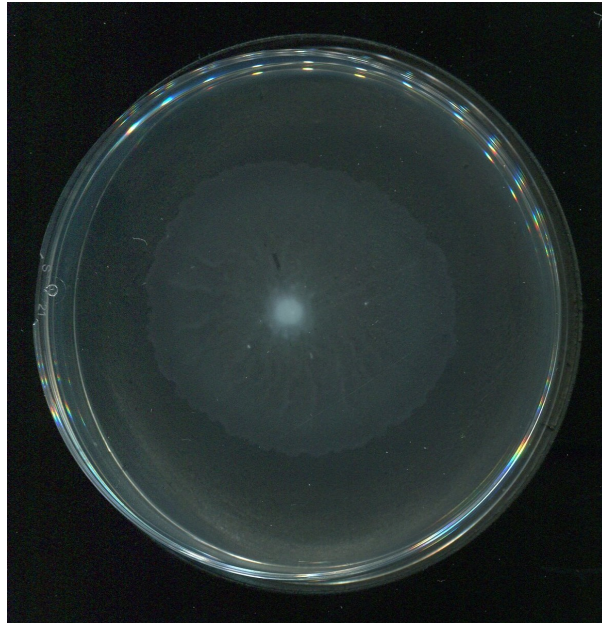


Figure 1.10: A typical swarm of *Enterobacter* SM3. Bacteria spread radially to fill one-half to three-quarters of the Petri dish six hours after the point of inoculation. The result is a circularly symmetric growth with the initial footprint of inoculation appearing as an opaque, white disk in the center. See the Methods for an explanation of the swarming assay procedure. Image acquired using the method described in reference [85].

their flagella [83]. Thus, bacteria can swim at the surface with fairly long residence times, increasing the probability of permanent adhesion [84].

1.5 Other Modes of Motility

So far, planktonic swimming—that is, individual bacteria swimming freely with no interactions with other bacteria—has been primarily discussed. However, nature is more complicated and, often, bacteria live in complex environments while strongly interacting with one another. The most common of these environments is the solid-liquid interface.

1.5.1 Swarming

Bacteria are not required to swim alone. In fact, bacteria are often found in nature in dense packs moving together on semi-solid surfaces, such as the lining of our intestines. The rapid movement of a dense collection of bacteria across a surface, powered by flagella,

is called swarming—and the collection of bacteria is called a swarm [86]. The presence of flagella seems to be critical for proficient swarming motility, as bacteria lacking functional flagella [87] or chemotaxis pathways [88] are often poor swimmers and do not swarm easily or at all. In fact, peritrichous bacteria tend to be the most proficient at swarming due to the increased stiffness and force generation of the flagellar bundle [86]. Swarming bacteria are often studied atop of agar, a gelatin-like substrate composed of an interwoven matrix of polysaccharide macromolecules. The surface of agar is porous, and bacteria can extract water and nutrients from the agar by osmosis [89]. The swarm itself consists of a thin layer of fluid atop of the agar with bacteria, biosurfactants, metabolites, and nutrients suspended within it. As the swarm grows, it spreads across the surface assisted by hydrodynamic effects (e.g., volume exclusion, pressure-driven flow, and Marangoni flow) [90] and secreted biosurfactants that make the gas-liquid interface easier to spread [91]. Close to the edge of the swarm, bacteria can be seen moving quickly as a monolayer directly behind the interface [67]. For proficient swimmers, such as SM3, a standard Petri dish can be covered within a matter of hours (Figure 1.10).

Swarming bacteria (swimmers) are quite different from swimming ones (swimmers)². Biologically, swimmers differentiate into swimmers once on a surface, which is thought to be initiated by the presence of secreted signaling chemicals (auto inducers) [92] and by signals from the flagellum that may act as a mechanosensor [93]. Swimmers tend to be longer [94], have more flagella [95], multinucleate [96], and secrete biosurfactants at a higher rate [97], although this is not the case for every species of swarming bacteria. The fact that swimmers change in length make them useful for experiments that require bacteria of different length. Several emergent behaviors arise in swarms including the formation of dynamic rafts [98], jets [99], and vortices [47]. However, the extent to which particular intercellular interactions affect these patterns remains unclear.

²This nomenclature should not be confused with a growth phase of *Caulobacter Crescentus*.

1.5.2 Living on the Edge: Biofilms

Bacteria have many reasons to live on surfaces. Surfaces exposed to aqueous solutions spontaneously attract dissolved molecules, some of which have nutritional value, that form a conditioning film that is beneficial for bacteria [100]. Once at the surface, bacteria can “settle down,” so to speak, and form robust colonies called biofilms [101]. An individual bacterium can initially attach to the surface by either the cell body or a flagellum; however, they are more likely to attach by the cell body, which has a large surface area and many “sticky” macromolecules, unlike the thread-like flagellar filament [102]. Over time, bacteria aggregate and form colonies of individual cells stuck to the surface and to one another by secreted extracellular polymeric substances (EPS), which acts as mortar that solidifies the integrity of the biofilm [103]. Once established, biofilms can be quite hard to remove and serve as a way for bacteria to reap the benefits of increased nutritional content at the interface while also protecting them from environmental stressors such as physical disturbances, predation, and toxins. Bacteria can leave the biofilm and, aided by flagella, explore other areas of the surface and establish more colonies [104]. Biofilms are a major line of research in microbiology, as they are responsible for many issues that negatively impact humans such as recurrent infections [105], dental plaque [106], and biofouling of medical equipment [107], to name a few. Therefore, understanding how bacteria swim at a solid interface is of primary interest to researchers hoping to combat biofilm formation.

1.6 In Summary

That concludes a brief introduction to bacteria: their inner workings, the world in which they live, and the strategies that they employ to meet the challenges of everyday existence. The research cataloged in this thesis concerns SM3, which is a Gram-negative, rod-shaped, peritrichously flagellated bacterium. SM3 is a novel bacterium that exhibits proficient swarming behavior, making it an ideal species for studies of swarming phenomena. Additionally, it has been shown to have potential probiotic properties, which can be

leveraged to treat inflammatory bowel disease (IBD) [108]. The projects cataloged in this thesis include my work: 1) characterizing the run-and-tumble motility of SM3 swimmers and swarms, 2) studying the effects of cell length on the near surface swimming trajectories of SM3, and 3) beginning to characterize SM3 in three-dimensions using digital holographic microscopy. I hope that this introduction has excited an interest in the work that I have conducted over the past three years.

References

- [1] R. Stocker and J. R. Seymour. Ecology and physics of bacterial chemotaxis in the ocean. *MMBR*, 76:792–812, 2012.
- [2] R. Hayat, S. Ali, U. Amara, R. Khalid, and I. Ahmed. Soil beneficial bacteria and their role in plant growth promotion: a review. *Ann. Microbiol.*, 60:579–598, 2010.
- [3] A. B. Shreiner, J. Y. Kao, and V. B. Young. The gut microbiome in health and in disease. *Curr. Opin. Gastroenterol.*, 31:69–75, 2015.
- [4] G. Horneck, D. M. Klaus, and R. L. Mancinelli. Space microbiology. *MMBR*, 74:121–156, 2010.
- [5] n.a. Microbiology by numbers. *Nat. Rev. Microbiol.*, 9:628, 2011.
- [6] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. *Molecular Biology of the Cell*. Garland Science, New York, 4 edition, 2002.
- [7] J. W. Bartholomew and T. M. The gram stain. *Bacteriol. Rev.*, 16:1–29, 1952.
- [8] T. J. Beveridge and J. A. Davies. Cellular responses of *Bacillus subtilis* and *escherichia coli* to the gram stain. *J. Bacteriol.*, 156:846–858, 1983.
- [9] D. F. Blair and H. C. Berg. The MotA protein of *E. coli* is a proton-conducting component of the flagellar motor. *Cell*, 60:439–449, 1990.
- [10] I. R. Booth, M. D. Edwards, and S. Miller. Bacterial ion channels. *Biochemistry*, 42:10045–10053, 2003.
- [11] D. Valverde-Mendez, A. M. Sunol, B. P. Bratton, M. Delarue, J. L. Hofmann, J. P. Sheehan, Z. Gitai, L. J. Holt, J. W. Shaevitz, and R. N. Zia. Macromolecular interactions and geometrical confinement determine the 3D diffusion of ribosome-sized particles in live *escherichia coli* cells. *PNAS*, 122:e2406340121, 2025.

- [12] A. Einstein. *Investigations on the Theory of the Brownian Movement*. Courier Corporation, 1956.
- [13] P. Nelson. *Biological physics*. WH Freeman New York, 2004.
- [14] E. M. Purcell. Life at low reynolds number. In *Physics and our world: reissue of the proceedings of a symposium in honor of Victor F Weisskopf*, pages 47–67. World Scientific, 2014.
- [15] J. Happel and H. Brenner. *Low Reynolds number hydrodynamics: with special applications to particulate media*. Springer Science and Business Media, 2012.
- [16] V. A. Bloomfield. Survey of biomolecular hydrodynamics. *On-Line Biophysics Textbook: Separations and Hydrodynamics*, 2000.
- [17] F. Perrin. Mouvement brownien d’un ellipsoide-i. dispersion diélectrique pour des molécules ellipsoïdales. *J. Phys. Radium*, 5:497–511, 1934.
- [18] V. A. Varma, I. Malhotra, and S. B. Babu. Enhancement in the diffusivity of brownian spheroids in the presence of spheres. *Phys. Rev. E*, 106:014602, 2022.
- [19] H. C. Berg. The rotary motor of bacterial flagella. *Annu. Rev. Biochem.*, 72:19–54, 2003.
- [20] D. H. Bergey. *Bergey’s Manual of Determinative Bacteriology*. Williams & Wilkins, 1925.
- [21] R. M. Macnab. Examination of bacterial flagellation by dark-field microscopy. *J. Clin. Microbiol.*, 4:258–265, 1976.
- [22] J. Adler. Chemotaxis in Escherichia coli. In *Cold Spring Harbor Symp. Quant. Biol.*, volume 30, pages 289–292, 1965.
- [23] L. Turner, W. S. Ryu, and H. C. Berg. Real-time imaging of fluorescent flagellar filaments. *J. Bacteriol.*, 182:2793–2801, 2000.

- [24] C. G. Ehrenberg. *Die Infusionstierchen als vollkommene Organismen*. Leopold Voss, 1838.
- [25] H. C. Berg. *E. coli in Motion*. Springer, 2004.
- [26] K. B. Reichert. *Über die Sichtbarmachung der Geisseln und die Geisselbewegung der Bakterien*. Gustav Fischer, 1909.
- [27] R. M. Macnab. How bacteria assemble flagella. *Annu. Rev. Microbiol.*, 57:77–100, 2003.
- [28] T. T. Renault, A. O. Abraham, T. Bergmiller, G. Paradis, S. Rainville, E. Charpentier, C. C. Guet, Y. Tu, K. Namba, J. P. Keener, et al. Bacterial flagella grow through an injection-diffusion mechanism. *Elife*, 6:e23136, 2017.
- [29] S. M. Block, D. F. Blair, and H. C. Berg. Compliance of bacterial polyhooks measured with optical tweezers. *Cytometry*, 12:492, 1991.
- [30] E. Lauga and T. R. Powers. The hydrodynamics of swimming microorganisms. *Rep. Prog. Phys.*, 72:096601, 2009.
- [31] J. A. Nirody, Y. Sun, and C. Lo. The biophysicist’s guide to the bacterial flagellar motor. *Adv. Phys. X*, 2:324–343, 2017.
- [32] P. K. Singh, P. Sharma, O. Afanзар, M. H. Goldfarb, E. Maklashina, M. Eisenbach, G. Cecchini, and T. M. Iverson. CryoEM structures reveal how the bacterial flagellum rotates and switches direction. *Nat. Microbiol.*, pages 1–11, 2024.
- [33] M. Silverman and M. Simon. Flagellar rotation and the mechanism of bacterial motility. *Nature*, 249:73–74, 1974.
- [34] H. C. Berg and R. A. Anderson. Bacteria swim by rotating their flagellar filaments. *Nature*, 245:380–382, 1973.
- [35] N. R. Francis, G. E. Sosinsky, D. Thomas, and D. J. DeRosier. Isolation, characteri-

- zation and structure of bacterial flagellar motors containing the switch complex. *J. Mol. Biol.*, 235:1261–1270, 1994.
- [36] J. Liu, T. Lin, D. J. Botkin, E. McCrum, H. Winkler, and S. J. Norris. Intact flagellar motor of *Borrelia burgdorferi* revealed by cryo-electron tomography: evidence for stator ring curvature and rotor/c-ring assembly flexion. *J. Bacteriol.*, 191:5026–5036, 2009.
 - [37] A. N. Glagolev and V. P. Skulachev. The proton pump is a molecular engine of motile bacteria. *Nature*, 272(5650):280–282, 1978.
 - [38] C. V. Gabel and H. C. Berg. The speed of the flagellar rotary motor of *Escherichia coli* varies linearly with protonmotive force. *PNAS*, 100:8748–8751, 2003.
 - [39] T. F. Braun, S. Poulson, J. B. Gully, J. C. Empey, S. VanWay, A. Putnam, and D. F. Blair. Function of proline residues of MotA in torque generation by the flagellar motor of *Escherichia coli*. *J. Bacteriol.*, 181:3542–3551, 1999.
 - [40] S. A. Lloyd, H. Tang, X. Wang, S. Billings, and D. F. Blair. Torque generation in the flagellar motor of *Escherichia coli*: evidence of a direct role for FliG but not for FliM or FliN. *J. Bacteriol.*, 178:223–231, 1996.
 - [41] W. S. Ryu, R. M. Berry, and H. C. Berg. Torque-generating units of the flagellar motor of *Escherichia coli* have a high duty ratio. *Nature*, 403:444–447, 2000.
 - [42] J. Howard, A. J. Hudspeth, and R. D. Vale. Movement of microtubules by single kinesin molecules. *Nature*, 342:154–158, 1989.
 - [43] A. Yildiz, M. Tomishige, R. D. Vale, and P. R. Selvin. Kinesin walks hand-over-hand. *Science*, 303:676–678, 2004.
 - [44] N. Wadhwa, R. Phillips, and H. C. Berg. Torque-dependent remodeling of the bacterial flagellar motor. *PNAS*, 116:11764–11769, 2019.
 - [45] S. W. Reid, M. C. Leake, J. H. Chandler, C. Lo, J. P. Armitage, and R. M. Berry.

- The maximum number of torque-generating units in the flagellar motor of *Escherichia coli* is at least 11. *PNAS*, 103:8066–8071, 2006.
- [46] Y. Sowa and R. M. Berry. Bacterial flagellar motor. *Q. Rev. Biophys.*, 41:103–132, 2008.
 - [47] X. Chen and H. C. Berg. Torque-speed relationship of the flagellar rotary motor of *Escherichia coli*. *Biophys. J.*, 78:1036–1041, 2000.
 - [48] Y. Sowa, H. Hotta, M. Homma, and A. Ishijima. Torque-speed relationship of the na⁺-driven flagellar motor of *Vibrio alginolyticus*. *J. Mol. Biol.*, 327:1043–1051, 2003.
 - [49] D. F. Blair and H. C. Berg. Restoration of torque in defective flagellar motors. *Science*, 242:1678–1681, 1988.
 - [50] S. M. Block and H. C. Berg. Successive incorporation of force-generating units in the bacterial rotary motor. *Nature*, 309:470–472, 1984.
 - [51] R. M. Berry, L. Turner, and H. C. Berg. Mechanical limits of bacterial flagellar motors probed by electrorotation. *Biophys. J.*, 69:280–286, 1995.
 - [52] H. C. Berg and L. Turner. Torque generated by the flagellar motor of *Escherichia coli*. *Biophys. J.*, 65:2201–2216, 1993.
 - [53] C. Qian, C. C. Wong, S. Swarup, and K. Chiam. Bacterial tethering analysis reveals a “run-reverse-turn” mechanism for *Pseudomonas* species motility. *Appl. Environ. Microbiol.*, 79:4734–4743, 2013.
 - [54] H. C. Berg. Chemotaxis in bacteria. *Annu. Rev. Biophys.*, 4:119–136, 1975.
 - [55] G. H. Wadhams and J. P. Armitage. Making sense of it all: bacterial chemotaxis. *Nat. Rev. Mol. Cell Biol.*, 5:1024–1037, 2004.
 - [56] K. A. Borkovich, N. Kaplan, J. F. Hess, and M. I. Simon. Transmembrane sig-

- nal transduction in bacterial chemotaxis involves ligand-dependent activation of phosphate group transfer. *PNAS*, 86:1208–1212, 1989.
- [57] J. J. Falke, R. B. Bass, S. L. Butler, S. A. Chervitz, and M. A. Danielson. The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes. *Annu. Rev. Cell Dev. Biol.*, 13:457–512, 1997.
 - [58] J. F. Hess, K. Oosawa, N. Kaplan, and M. I. Simon. Phosphorylation of three proteins in the signaling pathway of bacterial chemotaxis. *Cell*, 53:79–87, 1988.
 - [59] T. Minamino, M. Kinoshita, and K. Namba. Directional switching mechanism of the bacterial flagellar motor. *Comp. Struct. Biotech. J.*, 17:1075–1081, 2019.
 - [60] M. K. Sarkar, K. Paul, and D. Blair. Chemotaxis signaling protein CheY binds to the rotor protein FliN to control the direction of flagellar rotation in *Escherichia coli*. *PNAS*, 107:9370–9375, 2010.
 - [61] F. M. Rossmann, I. Hug, M. Sangermani, U. Jenal, and M. Beeby. In situ structure of the *Caulobacter crescentus* flagellar motor and visualization of binding of a CheY-homolog. *Mol. Microbiol.*, 114:443–453, 2020.
 - [62] F. Bai, R. W. Branch, D. V. Nicolau Jr, T. Pilizota, B. C. Steel, P. K. Maini, and R. M. Berry. Conformational spread as a mechanism for cooperativity in the bacterial flagellar switch. *science*, 327:685–689, 2010.
 - [63] M. Morse, J. Bell, G. Li, and J. X. Tang. Flagellar motor switching in *Caulobacter crescentus* obeys first passage time statistics. *Phys. Rev. Lett.*, 115:198103, 2015.
 - [64] J. Bell. *The tethered motion of uni-flagellated bacteria*. PhD thesis, Brown University, 2019.
 - [65] S. Johnson, B. Freedman, and J. X. Tang. Run-and-tumble kinematics of *Enterobacter* sp. SM3. *Phys. Rev. E*, 109:064402, 2024.

- [66] M. Kim, J. C. Bird, A. J. Van Parys, K. S. Breuer, and T. R. Powers. A macroscopic scale model of bacterial flagellar bundling. *PNAS*, 100:15481–15485, 2003.
- [67] N. C. Darnton, L. Turner, S. Rojevsky, and H. C. Berg. On torque and tumbling in swimming *Escherichia coli*. *J. Bacteriol.*, 189:1756–1764, 2007.
- [68] T. Nakai, T. Ando, and T. Goto. Biased reorientation in the chemotaxis of peritrichous bacteria *Salmonella enterica* serovar typhimurium. *Biophys. J.*, 120:2623–2630, 2021.
- [69] H. Huo, R. He, R. Zhang, and J. Yuan. Swimming *Escherichia coli* cells explore the environment by levy walk. *Environ. Microbiol.*, 87:e02429–20, 2021.
- [70] G. Ariel, A. Rabani, S. Benisty, and J. D. Partridge. Swarming bacteria migrate by levy walk. *Nat. Comm.*, 6:1–6, 2015.
- [71] A. Be’er and G. Ariel. A statistical physics view of swarming bacteria. *Mov. Ecol.*, 7:1–17, 2019.
- [72] H. Barbosa, M. Barthelemy, G. Ghoshal, C.R. James, M. Lenormand, T. Louail, R. Menezes, J.J. Ramasco, F. Simini, and M. Tomasini. Human mobility: Models and applications. *Phys. Rep.*, 734:1–74, 2018.
- [73] J. R. Blake and A. T. Chwang. Fundamental singularities of viscous flow: part i: the image systems in the vicinity of a stationary no-slip boundary. *J. Eng. Math.*, 8: 23–29, 1974.
- [74] A. Chamolly and E. Lauga. Stokes flow due to point torques and sources in a spherical geometry. *Phys. Rev. Fluids*, 5:074202, 2020.
- [75] K. Drescher, J. Dunkel, L. H. Cisneros, S. Ganguly, and R. E. Goldstein. Fluid dynamics and noise in bacterial cell–cell and cell–surface scattering. *PNAS*, 108: 10940–10945, 2011.

- [76] E. Lushi, H. Wioland, and R. E. Goldstein. Fluid flows created by swimming bacteria drive self-organization in confined suspensions. *PNAS*, 111:9733–9738, 2014.
- [77] J. R. Blake. A note on the image system for a stokeslet in a no-slip boundary. *Math. Proc. Camb. Philos. Soc.*, 70:303–310, 1971.
- [78] P. D. Frymier, R. M. Ford, H. C. Berg, and P. T. Cummings. Three-dimensional tracking of motile bacteria near a solid planar surface. *PNAS*, 92:6195–6199, 1995.
- [79] E. Lauga, W. R. DiLuzio, G. M. Whitesides, and H. A. Stone. Swimming in circles: motion of bacteria near solid boundaries. *Biophys. J.*, 90:400–412, 2006.
- [80] S. Bianchi, F. Saglimbeni, and R. Di Leonardo. Holographic imaging reveals the mechanism of wall entrapment in swimming bacteria. *Phys. Rev. X*, 7:011010, 2017.
- [81] A. P. Berke, L. Turner, H. C. Berg, and E. Lauga. Hydrodynamic attraction of swimming microorganisms by surfaces. *Phys. Rev. Lett.*, 101:038102, 2008.
- [82] J. P. Hernandez-Ortiz, C. G. Stoltz, and M. D. Graham. Transport and collective dynamics in suspensions of confined swimming particles. *Phys. Rev. Lett.*, 95:204501, 2005.
- [83] G. Li and J. X. Tang. Accumulation of microswimmers near a surface mediated by collision and rotational brownian motion. *Phys. Rev. Lett.*, 103:078101, 2009.
- [84] K. Schaar, A. Zöttl, and H. Stark. Detention times of microswimmers close to surfaces: Influence of hydrodynamic interactions and noise. *Phys. Rev. Lett.*, 115:038101, 2015.
- [85] J. Bru, A. Siryaporn, and N. M. Høyland-Kroghsbo. Time-lapse imaging of bacterial swarms and the collective stress response. *JoVE*, 159:e60915, 2020.
- [86] D. B. Kearns. A field guide to bacterial swarming motility. *Nat. Rev. Microbiol.*, 8:634–644, 2010.

- [87] F. D. Williams and R. H. Schwarzhoff. Nature of the swarming phenomenon in *Proteus*. *Annu. Rev. Microbiol.*, 32:101–138, 1978.
- [88] M. Burkart, A. Toguchi, and R. M. Harshey. The chemotaxis system, but not chemotaxis, is essential for swarming motility in *Escherichia coli*. *PNAS*, 95:2568–2573, 1998.
- [89] L. Ping, Y. Wu, B. G. Hosu, J. X. Tang, and H. C. Berg. Osmotic pressure in a bacterial swarm. *Biophys. J.*, 107:871–878, 2014.
- [90] J. Bru, S. J. Kasallis, N. M. Høyland-Kroghsbo, and A. Siryaporn. Swarming of *P. aeruginosa*: through the lens of biophysics. *Biophys. Rev.*, 4, 2023.
- [91] C. Pawul, T. T. Dutta, S. G. Johnson, and J. X. Tang. Mucin promotes bacterial swarming by making the agar surface more slippery. *Langmuir*, 40:27307–27313, 2024.
- [92] R. Daniels, J. Vanderleyden, and J. Michiels. Quorum sensing and swarming migration in bacteria. *FEMS Microbiol. Rev.*, 28:261–289, 2004.
- [93] P. P. Lele, B. G. Hosu, and H. C. Berg. Dynamics of mechanosensing in the bacterial flagellar motor. *PNAS*, 110:11839–11844, 2013.
- [94] P. N. Rather. Swarmer cell differentiation in *Proteus mirabilis*. *Environ. Microbiol.*, 7:1065–1073, 2005.
- [95] J. Henrichsen. Bacterial surface translocation: a survey and a classification. *Bacteriol. Rev.*, 36:478–503, 1972.
- [96] C. Allison and C. Hughes. Bacterial swarming: an example of prokaryotic differentiation and multicellular behaviour. *Sci. Prog.*, pages 403–422, 1991.
- [97] S. Kasallis, J. Bru, R. Chang, and A. Siryaporn. Understanding how bacterial collectives organize on surfaces by tracking surfactant flow. *Curr. Opin. Solid State Mater. Sci.*, 27:101080, 2023.

- [98] M. F. Copeland and D. B. Weibel. Bacterial swarming: a model system for studying dynamic self-assembly. *Soft matter*, 5(6):1174–1187, 2009.
- [99] N. H. Mendelson, A. Bourque, K. Wilkening, K. R. Anderson, and J. C. Watkins. Organized cell swimming motions in *Bacillus subtilis* colonies: patterns of short-lived whirls and jets. *J. Bacteriol.*, 181:600–609, 1999.
- [100] G. S. Lorite, C. M. Rodrigues, Alessandra A. De S., C. Kranz, B. Mizaikoff, and M. Cotta. The role of conditioning film formation and surface chemical changes on *Xylella fastidiosa* adhesion and biofilm evolution. *J. Colloid Interface Sci.*, 359: 289–295, 2011.
- [101] P. Watnick and R. Kolter. Biofilm, city of microbes. *J. Bacteriol.*, 182:2675–2679, 2000.
- [102] J. Bell, S. Johnson, B. Pugnet, and J. X. Tang. Bacteria can rotate while body tethered to a solid surface. *Biophys. J.*, 2025.
- [103] C. Berne, C. K. Ellison, A. Ducret, and Y. V. Brun. Bacterial adhesion at the single-cell level. *Nat. Rev. Microbiol.*, 16:616–627, 2018.
- [104] A. Penesyan, I. T. Paulsen, S. Kjelleberg, and M. R. Gillings. Three faces of biofilms: a microbial lifestyle, a nascent multicellular organism, and an incubator for diversity. *NPJ Biofilms Microbiomes*, 7:80, 2021.
- [105] J. W. Costerton, P. S. Stewart, and E. P. Greenberg. Bacterial biofilms: a common cause of persistent infections. *science*, 284:1318–1322, 1999.
- [106] P. D. Marsh. Dental plaque as a microbial biofilm. *Caries Res.*, 38:204–211, 2004.
- [107] I. Banerjee, R. C. Pangule, and R. S. Kane. Antifouling coatings: recent developments in the design of surfaces that prevent fouling by proteins, bacteria, and marine organisms. *Adv. Mater.*, 23:690–718, 2011.
- [108] A. De, W. Chen, H. Li, J. R. Wright, R. Lamendella, D. J. Lukin, W. A. Szymczak,

K. Sun, L. Kelly, S. Ghosh, et al. Bacterial swarms enriched during intestinal stress ameliorate damage. *Gastroenterology*, 161:211–224, 2021.

CHAPTER 2

METHODS

“Tracking is fun!”

—Howard C. Berg

2.1 Bacterial Culture Preparations

2.1.1 Overnight Growth

A single bacterial species, *Enterobacter* sp. SM3, was used for all experiments. Bacteria were stored at -80°C in a cryotube containing an aqueous mixture of bacteria and 15-30% glycerin. The addition of glycerin prevents the crystallization of water during the freezing and thawing of the bacterial culture, which causes damage to the cell walls of the bacteria [1].

Bacteria were taken from the -80°C stock by gently scratching the surface of the frozen culture using a sterilized inoculation loop and dipping it in 10 mL of lysogeny broth (LB) medium (10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl, dissolved in de-ionized water). The bacteria were then grown at 37°C , which is the human body temperature and one degree higher than the body temperature of mice, at approximately 160 rotations per

minute (rpm) in a shaking incubator overnight. Bacteria stop growing after approximately 16 hours and reach a saturation population with a well known concentration. Thus, an overnight growth serves as an easily reproducible equilibrium population.

2.1.2 Hard Agar Plate Colonies

Bacteria from an overnight growth can be preserved for future use on Petri dishes containing agar. For the preservation of bacterial colonies over two to four weeks, it is generally recommended to inoculate them on “hard” agar containing >1% (w/v) agar. The pore size of hard agar, which is determined by the concentration of agar, is small enough such that bacteria cannot penetrate the substrate. Additionally, they can grow as a localized colony, but due to the severe restriction in the flow of liquid from the hard agar, they cannot spread across the surface.

Hard agar plates with bacterial colonies were created using the following procedure. Polystyrene Petri dishes (100 mm x 15 mm) were poured with 20 mL of autoclaved 1.5% LB agar solution (10 g/L tryptone, 5 g/L yeast extract, 1.5 g/L agar, and 5 g/L NaCl, dissolved in de-ionized water). Bacteria from an overnight growth were diluted 1:10 in deionized water to reduce cell density. Using a sterilized inoculation loop, an unmeasured portion of this dilution was streaked on the surface of the agar in one quadrant of the Petri dish. The loop was sterilized again and then used to streak a second quadrant of the Petri dish, taking a few passes through the previous streak. This was repeated for the four quadrants. With this method, a smaller number of bacteria was relocated with each successive streak, effectively reducing the cell density and creating individual clone colonies. After streaking the plates, they were covered, sealed with parafilm, and stored in an incubator maintained at 34°C and 60-80% relative humidity (RH) to grow overnight. The resulting plates had nicely separated single colonies of bacteria. These plates were stored in a refrigerator at 4-10°C to use for up to two weeks. The bacteria from either an overnight growth or hard agar plates were used to make all subsequent growths.

2.1.3 Fresh Growths

All experiments required fresh bacterial growth. Bacteria in an overnight growth are inactive and do not swim and are reactivated when displaced to fresh growing media. To make a fresh growth from an overnight growth, 100 μ L of overnight growth was moved to 10 mL of sterile LB. The culture was then placed in a shaking incubator at 37°C and 160 rpm and grown for four hours until reaching an optical density ~ 1 . We found that 4 hours of growth was optimal for observing a significant fraction of swimming versus non-swimming bacteria.

A fresh growth can also be made using colonies from refrigerated hard agar plates. To do so, a sterilized inoculation loop was used to scrape bacteria from an individual colony on the surface of the agar and to move them to 10 mL of sterile LB, which was then grown at 37°C and 160 rpm to an OD ~ 1 . The advantage of this approach is that one does not have to constantly make a growth overnight, which saves time and reduces the chance of contaminating the original frozen stock due to frequent access. However, the disadvantage is that one cannot precisely control the amount of bacteria used to create the fresh growth, as one can when making a fresh growth from an overnight equilibrium population. This results in variability in the time when the most swimming bacteria can be observed. Therefore, OD measurements are necessary to determine the best time for subsequent experiments.

2.1.4 Swarm Assays

For swarm assays, 10 mL of autoclaved $<1\%$ (w/v) “soft” agar solution (10 g/L tryptone, 5 g/L yeast extract, 5 g/L agar, and 5 g/L NaCl, dissolved in de-ionized water) was poured into Petri dishes. Prior to pouring, the autoclaved agar solution was allowed to reach thermal equilibrium with a water bath at 55°C. After pouring, the dishes were set to gel uncovered for 10 minutes at room temperature in a biological safety cabinet. Each plate was inoculated with 5-10 μ L of 4 hour fresh growth, which was deposited on the

surface of the agar and allowed to absorb for 5 minutes. Following absorption, the dishes were covered and placed upright in an incubator at a temperature of approximately 34°C and a RH of 60-80%. Swarm plates were incubated for 6 to 7 hours, at which time bacteria had swarmed to cover one-half to three-fourths of the plate. The swarm front was circular and symmetric with a clear boundary between the swarm front and the surrounding agar.

2.2 Other Chemical Preparations

2.2.1 Motility Buffer

When observing bacteria, it is important to provide ideal physiological conditions while not influencing motility. Motility buffer (MB) is a commonly used solution to preserve the pH of the environment, reduce osmotic pressure on the cell wall, and reduce the influence of metabolism on the observed motility. The simplest recipe consists of a phosphate compound to preserve pH, salt to control osmotic pressure, and EDTA to preserve cell membranes and support flagella function [2]. MB does not have nutritional value for bacteria; therefore, it serves as a control for the effects of metabolism on motility.

A simple MB was used for most experiments consisting of 67 mM NaCl, 0.1 mM EDTA, and 10 mM sodium phosphate at pH 7.0, dissolved in deionized water.

2.2.2 Chemoattractant Solutions

For chemoattractant exposure studies, a 0.1 M stock solution of either l-serine or l-aspartic acid monosodium salt dissolved in de-ionized water was made. All subsequent concentrations of each chemoattractant were made by diluting the stock solution into MB. For exposure experiments, 10 μ L of dilute chemoattractant solutions at varying concentrations were added to 1 mL of solutions each consisting of 990 μ L of MB and 10 μ L of bacterial solution. For select 3D tracking studies, glucose was added to MB at a concentration of 10 mM.

2.3 Microscopy

2.3.1 Sample Preparations

Prior to performing microscopy, samples were prepared depending on the experiment type. To image planktonic bacteria using traditional optical microscopy, 10 μL of 4 hour fresh growth was diluted 1:100 in 990 μL of MB at room temperature.

Swarmers were also prepared by diluting in MB. This was done after 6 hours of growth, when the bacterial colony covered a substantial fraction of the plate. For run-and-tumble studies, a small portion of bacteria from the swarm front were extracted using a sterilized inoculation loop and mixed into 1 mL of MB at room temperature. The exact cell density was unknown but was found to be adequate for imaging. Swarmers for near-surface swimming studies were similarly diluted in 1 mL of MB at room temperature. However, 250 μL of the resulting solution was then further diluted into an additional 750 μL of MB. Microscopy of dilute swarmers was performed within 30 minutes after they were taken from the swarm front to limit the effects of genetic changes on the observed behavior [3]. All sample preparations were mixed to ensure a uniform cell density.

For holographic microscopy, bacteria were diluted 3:500 in MB by combining 600 μL of a 1:100 stock dilution of bacteria in MB with 400 μL of fresh MB. This is equivalent to transferring 6 μL of fresh growth to 994 μL of MB. We found that a 3:500 dilution maximized the number of swimming bacteria and minimized the number of bacteria stuck to the wall of the cuvette. 475 μL of this 3:500 solution was then deposited into a 1 mm cuvette—which filled it completely—that was then sealed with a piece of parafilm to isolate the sample from the atmosphere. Atmospheric air flow, if not controlled for, can induce downward flow, which can affect the measurement. The surface of the cuvette was carefully cleaned with lens cleaning fluid and paper to prevent laser light diffraction from dust and other contaminants.

2.3.2 Phase Contrast and Dark-Field Microscopy

The perimeter of a glass coverslip (22 mm x 22 mm) was coated with a thin layer of vacuum grease. In the center of the coverslip, 5-10 μL of bacterial solution was deposited and a glass microscope slide (3" x 1" x 1 mm) was gently pressed on top until the fluid formed a thin, uniform layer with an estimated thickness in the range of 10-20 μm . This created a pseudo-2D environment in which bacteria could move, reducing out-of-focal-plane motion and minimizing the ambiguities associated with traditional projection microscopy [4]. For imaging bacteria near the surface, which required knowing the exact chamber thickness, short (~ 1 cm) segments of 50 μm diameter fiberglass wires were placed around the perimeter of the coverslip as spacers to fix the thickness of the chamber. Slides were transferred to the stage of an inverted optical microscope with the coverslip facing down.

A Nikon Eclipse TE2000-U inverted optical microscope was used for all optical microscopy measurements. Movies were captured using a digital scientific complementary metal-oxide semiconductor (CMOS) camera (Point Grey GS3-U3-51S5M-C Grasshopper3) with a pixel conversion factor which was determined using a calibration slide. The Fly-Capture software development kit (SDK) (Flir Teledyne) was used to record movie frames in .tiff format.

For run-and-tumble experiments that required high spatial and temporal resolution, a 40x Ph3 objective (Nikon S Plan Fluor; numerical aperture 0.60; working distance 3.60-2.80 mm) was used to produce phase-contrast images at 100 frames per second (FPS) (pixel conversion factor 1 px = 0.0863 μm). To obtain long, continuous trajectories for use with the tracking algorithm, a 10x Ph1 objective (Nikon Plan Fluor; numerical aperture 0.30; working distance 15.2 mm) was used with a mismatched phase ring to produce dark-field images at 25 FPS (pixel conversion factor 1 px = 0.342 μm).

When imaging bacteria near the surface, movies were taken at the bottom surface of the chamber, in the laboratory frame. For movies of shorter bacteria (2-4 μm), which

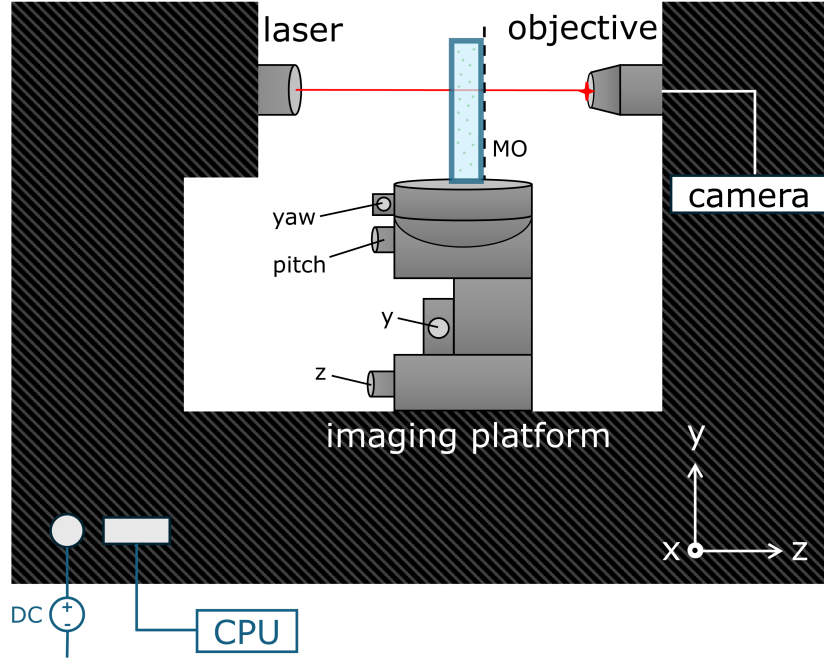


Figure 2.1: Schematic of the digital holographic microscopy system. A laser illuminates a cuvette from the left, which is fixed to an adjustable imaging platform. The laser light scatters from the bacteria in the sample and interferes with the unscattered background light, creating an interference. The light is gathered by a microscope objective to the right of the cuvette and recorded digitally with a CMOS camera concealed within the chassis. Note the coordinate axes, with the positive y-direction being against gravity and the positive z-direction perpendicular to the cuvette and pointing along the laser direction.

required higher spatial resolution, a $40\times$ Ph3 objective (Nikon S Plan Fluor; numerical aperture 0.60; working distance 3.60-2.80 mm) was used to produce phase-contrast images at 25 FPS (pixel conversion factor $1 \text{ px} = 0.0863 \text{ }\mu\text{m}$). For experiments of longer bacteria ($5 \text{ }\mu\text{m}$ or longer), which often required a larger field of view for trajectories of larger diameter, a $10\times$ Ph1 objective (Nikon Plan Fluor; numerical aperture 0.30; working distance 15.2 mm) was used to produce phase-contrast images at 25 FPS (pixel conversion factor $1 \text{ px} = 0.342 \text{ }\mu\text{m}$).

2.3.3 Digital Holographic Microscopy

The digital holographic microscopy (DHM) system has four main components: a laser ($\lambda = 659 \text{ nm}$), an adjustable imaging platform, an objective, and a CMOS camera (see

Figure 2.1). A 1 mm path length cuvette containing bacterial solution was placed upright and fixed on the imaging platform. Then, using the yaw and pitch knobs, the cuvette was adjusted until the laser was normally incident on the glass surface, which was noted as occurring when the laser reflected back to the source. Bacteria on the cuvette wall closest to the objective (inner right surface of the cuvette) were brought into focus and observed by adjusting the z-position of the cuvette. The focal plane of the objective (MO in Figure 2.1) was set 20 μm to the right in the cuvette glass (see MO in Figure 2.1). The system was allowed to rest for 30 minutes prior to recording to allow the fluid in the cuvette to settle. Movies were recorded from this initial configuration.

A 20x objective (Olympus UPlanFL N; numerical aperture 0.50; working distance 2.1 mm) was used to produce bright-field images of bacteria and their fringes (pixel conversion factor 1 px = 0.217362 μm). Movies were captured at 25 FPS using a CMOS camera (Teledyne, Blackfly S) for a duration of 1 minute. The camera exposure was set to 200 μs and the gain to 0 dB. The Spinnaker SDK (Flir Teledyne) was used to record movie frames in .tiff format.

2.4 Bacteria Detection and Tracking

2.4.1 Image Processing and Bacteria Tracking Using Fiji

For all two-dimensional studies using traditional optical microscopy, including run-and-tumble and near-surface swimming experiments, the open-source software, *Fiji*, was used to process movie frames and perform tracking.

Image Processing

Images were loaded into *Fiji* and the software was calibrated using the known pixel conversion factors for the 10x and 40x objectives. The images were then converted to an 8-bit format and thresholded using a maximum entropy filter. By this process, contrasted

images with bacteria appearing black on a white background were produced for effective bacteria tracking.

Bacteria Detection

Bacteria were automatically detected and tracked using *Trackmate*. *Trackmate* is a free plugin for *Fiji* that allows for easy and efficient semi-automatic tracking of bacteria over time. The program implements tracking in two main steps: detection of bacteria and tracking [5, 6].

In the detection step, the user can choose from a selection of different detectors. For our tracking experiments, a difference-of-Gaussian (DoG) detector was implemented to detect bacteria. Prior to spot detection, the detector applies a DoG filter to the images to approximate a Laplacian of Gaussian (LoG) filter. This serves two purposes: to blur the image, which reduces high-frequency spatial noise, and to enhance the edges of the bacteria, making them easier to detect by the program [7]. After filtering, the program detects bacteria subject to two main criteria: the approximate bacterium diameter and the quality threshold. For SM3, the average length of planktonic bacteria was found to be $1.4 \pm 0.3 \mu\text{m}$. This was determined by measuring the long axis of 200 separate bacteria, using the length tool in *Fiji*, and taking the average. Thus, the program was provided with a reasonable size around which an object could be identified as a bacterium. The quality threshold is a measure of the quality of the spots detected. Spots below the designated quality threshold are filtered out by the program to reduce the detection of spurious spots. The threshold is determined by trial and error. Once the detection parameters are optimized and bacteria have been detected, tracking can begin.

Bacteria Tracking

Trackmate has multiple trackers that can be used depending on the system of interest. For our experiment, a simple linear assignment problem (LAP) tracker (also known as the

Hungarian method) was used to link detected bacteria into cohesive tracks. The simple LAP tracker works by assigning a cost to linking two detected bacteria at a time t and $t + \Delta t$ (i.e. between two consecutive frames), which is proportional to the squared distance between the objects. The tracker assigns links in such a way as to minimize the total linking cost [8, 9]. Simply put, the tracker links bacteria between frames that are close to one another in position. There are, however, factors that can disrupt the linking process [5]. For instance, bacteria can move in and out of focus, causing gaps, and can also collide with other bacteria or anomalous spots, causing confusion for the tracker.

To circumvent these issues, three key parameters can be optimized. The first parameter, called the max linking distance, is the minimum distance over which the tracker looks for candidate bacteria to link. If the max linking distance is set to x μm , then spots detected in frame $t + \Delta t$ must be within x μm of a spot in frame t to be linked. For bacteria with an average speed of 30 $\mu\text{m/s}$, and for a time interval between frames of 0.04 s, a max linking distance of 1-2 μm is reasonable. We chose to give the tracker more freedom and set the max linking distance to 5 μm . The second parameter of interest, the gap-closing max distance, is the maximum distance the tracker can bridge a gap between two track segments. This preserves the track in the event of a bacterium disappearing from the plane of focus for multiple frames. We set this parameter to 5 μm , or slightly over three cell body lengths. However, it should be noted that, in selecting bacteria to track, we went out of our way to avoid trajectories which exhibited noticeable out-of-plane motion. The last parameter is called the gap-closing max frame gap. It is the maximum frame interval between which two track segments will be linked. We chose this value to be two frame intervals or 0.08 s. With these parameters for detection and linking, long reliable trajectories of SM3 were produced.

All data were exported from *Fiji* in .csv format to be analyzed by a custom algorithm. The exported data included the positions and frame numbers of the tracked bacteria.

2.4.2 Image Processing and Bacteria Tracking Using Using HoloPro

The process of recording holograms is relatively simple. However, extracting the 3D information encapsulated in the interference fringes of the imaged bacteria is nontrivial. We used the commercial software *HoloPro* (MetroLaser, Inc.) [10] to process our holograms and perform tracking. Similar to *Trackmate*, the procedure used by *Holopro* to process holograms follows the steps of image processing, detection, and tracking.

Image Processing

The interference fringes of bacteria are weak in contrast and, therefore, are difficult to detect. This is because bacteria only weakly scatter light [10]. To improve the signal-to-noise ratio of the detected fringes, the background noise must be subtracted from each raw hologram image.

One strategy for subtracting the background noise is to average over all of the raw holograms and assign the resulting image as the background for all other images. Mathematically, this operation is written as [10]:

$$\bar{I}_{all}(x, y) = \frac{1}{N} \sum_{i=0}^{N-1} I_{raw}^{(i)}(x, y), \quad (2.1)$$

where $\bar{I}_{all}(x, y)$ is the greyscale map of the background, N is the total number of holograms, and $I_{raw}^{(i)}(x, y)$ is the greyscale map of the i^{th} hologram. This approach, however, does a poor job accounting for variance in the background between holograms [10]. For example, the variance introduced by vibrations in the laboratory environment.

One way to improve this approach is to assume that such variances are local and, for a given hologram, calculate the background as an average over its nearest-neighbors. This can be written as [10]:

$$\bar{I}_{local}^{(j)}(x, y) = \frac{1}{2N_l + 1} \sum_{i=j-N_l}^{j+N_l} I_{raw}^{(i)}(x, y), \quad (2.2)$$

where $\bar{I}_{local}^{(j)}(x, y)$ is the background hologram for the j^{th} hologram and N_l defines the window size to be averaged over. This approach is a much improved version of the previous method, which averages over all holograms to determine a single background hologram. However, there is a more sophisticated approach that can be used.

The method employed in our analysis involves determining the set of holograms with backgrounds that are most similar to a given hologram and using them to calculate the background. Similarity is quantified by the correlation coefficient between the j^{th} and i^{th} hologram [10]:

$$C_{ij} = \frac{\frac{1}{N_x N_y} \sum_{m=0}^{N_x-1} \sum_{n=0}^{N_y-1} \left[I_{raw}^{(j)}(m, n) - \mu_j \right] \left[I_{raw}^{(i)}(m, n) - \mu_i \right]}{\sigma_j \sigma_i}, \quad (2.3)$$

where (m, n) are the pixel coordinates, (N_x, N_y) are the number of pixels in the horizontal and vertical direction, respectively, and μ_i and σ_i are the mean and standard deviation of the hologram array for the i^{th} hologram. The correlation coefficient between each hologram in the sequence is a value between negative one and positive one: one indicating a perfect direct relationship and negative one indicating a perfect inverse relationship; a value of zero shows no relationship. These coefficients are used to construct a correlation matrix with dimensions $N \times N$ and diagonal elements of 1.

For a given hologram $I_{raw}^{(j)}$, the j^{th} row of the correlation matrix lists the correlation coefficients between it and all other holograms (e.g. $C_{j0}, C_{j1}, C_{j2}, C_{j3}, \dots$). Ordering these coefficients in descending order—with one being the highest possible value—gives a sorted list of those holograms which are most similar to $I_{raw}^{(j)}$. The background hologram to be subtracted from $I_{raw}^{(j)}$ is then just a summation of those holograms that most closely correlate with $I_{raw}^{(j)}$; the number of which is decided by the user. The number of similar

holograms to average over that we chose was 51.

Bacteria Detection

Detection consists of three steps: numerical reconstruction, thresholding, and detection. Raw holograms are taken at the focal plane of the microscope objective (MO) (see Figure 2.1). To obtain the 3D positions of each bacterium in the sample volume, the hologram must be reconstructed on a series of planes parallel to the focal plane, along the z-axis, by an increment set by the user. The reconstructed complex amplitude at a given distance z from the focal plane is given by [10],

$$E(p, q, z) = FFT^{-1} \left\{ FFT\{I(m, n)\} \exp \left\{ \frac{i2\pi z}{\lambda n_1} \left[1 - \left(\frac{\lambda m'}{N_x \Delta \xi} \right)^2 - \left(\frac{\lambda n'}{N_y \Delta \eta} \right)^2 \right]^{\frac{1}{2}} \right\} \right\}, \quad (2.4)$$

where:

- (p, q) are the reconstructed spatial coordinates,
- (m', n') are the coordinates in the spatial frequency domain,
- FFT and FFT^{-1} are the forward and inverse fast Fourier transform, respectively,
- $I(m, n)$ is the image array of the processed hologram,
- λ is the wavelength of the laser light used to record the hologram,
- n_1 is the refractive index of the medium,
- $\Delta \xi$ and $\Delta \eta$ are the effective pixel size in the horizontal and vertical direction, respectively, and
- N_x and N_y are the number of pixels in the horizontal and vertical direction, respectively.

The reconstructed amplitude at a given plane is then $A = |E|$. For our reconstructions, $\lambda = 659$ nm, $N_x = 3208$, $N_y = 2200$, $\Delta\xi = \Delta\eta = 0.217362$ μm , and $n_1 = 1.3333$. Holograms were reconstructed in 1 μm increments from $z = 0$ μm to $z = 800$ μm , corresponding to 800 reconstruction planes.

Once the holograms have been reconstructed, bacteria can be detected in each plane by the voxel (the 3D counterpart to a pixel) brightness. A brightness threshold is imposed to reduce spurious detections of noise. We found that a threshold value of 4.5 worked best for our reconstructions. The plane on which the bacterium is brightest corresponds to the z -coordinate at which the bacterium is in focus. The centroid position is determined by averaging the coordinates of all the voxels associated with a given bacterium weighted by the amplitude; essentially, the centroid position corresponds to the brightest voxel [10].

Bacteria Tracking

Once bacteria have been detected in each hologram and at each time step (e.g., every 40 ms for a frame rate of 25 FPS), they are linked by *HoloPro* using a tracking algorithm based on the Hungarian method similar to that described in Section 2.4.1. Bacteria are linked to one another in sequential holograms based on their distance to one another and the cost associated with linking them. The algorithm is assisted by imposing a maximum bacterium displacement between consecutive frames, depending on the expected average speed of the bacteria. Furthermore, tracks shorter than a given time span are excluded to account for tracks produced by random noise.

2.5 Analysis of Trajectories

Trajectories were analyzed using a custom algorithm (github.com/Tang-BioPhys/SM3-Motility) based on the work of Masson *et al.* [11] and modified by Theves *et al.* [12]. Trajectories were chosen not to include tethered bacteria, dead bacteria, and other external interactions that could influence bacterial movement. The algorithm processes

position data inputted as a .csv file and outputs several key run-and-tumble parameters such as the tumble time, run time, tumble angle, and average run speed.

2.5.1 Calculation of Kinematic Variables

The kinematic equations,

$$\mathbf{v}(t) = \frac{\mathbf{r}(t) - \mathbf{r}(t - \Delta t)}{\Delta t} \quad (2.5)$$

and

$$\omega(t) = \frac{\phi(t) - \phi(t - \Delta t)}{\Delta t}, \quad (2.6)$$

were used to calculate the semi-instantaneous linear and angular velocity as functions of time, respectively. Here, $\mathbf{r}(t)$ is the position vector of the bacteria, $\phi(t)$ is the angle defining the orientation of the linear velocity, and $\Delta t = 1/\text{framerate}$.

The calculation of the orientation, $\phi(t)$, depended on whether the track was 2D or 3D. For two dimensions, $\phi(t)$ was calculated from the x-component of $\mathbf{v}(t)$ as:

$$\phi(t) = \cos^{-1} \left[\frac{v_x(t)}{v(t)} \right]. \quad (2.7)$$

Since the inverse cosine function in Python returns ϕ from 0 to π and only with respect to the positive x-axis, care was taken to keep track of the orientation of $\mathbf{v}(t)$. In 3D, $\phi(t)$ was calculated as the angle between $\mathbf{v}(t)$ and $\mathbf{v}(t + \Delta t)$ at every time instant:

$$\mathbf{v}(t) \cdot \mathbf{v}(t + \Delta t) = v(t)v(t + \Delta t)\cos[\phi(t)]. \quad (2.8)$$

From $\phi(t)$, the angular speed, $\omega(t)$, was calculated using Equation 2.6.

The linear and angular speed raw data were passed through a Savitsky-Golay filter for smoothing. Briefly, the filter works by replacing a given subset of data—determined by the window size—with a data point calculated with a polynomial function approximating the subset. It was found empirically that a window size of five and a polynomial order of two yielded the best smoothing while minimizing the amount of lost information. Smoothing was not performed when analyzing the 3D tracking data.

For 2D circular trajectories, the instantaneous curvature, average radius of curvature, and the average angular velocity were calculated as follows. The instantaneous curvature is defined as:

$$\kappa(t) = \frac{|x'(t)y''(t) - y'(t)x''(t)|}{[x'(t)^2 + y'(t)^2]^{3/2}}, \quad (2.9)$$

where $[x(t), y(t)]$ are the time-dependent position coordinates and the primes represent the number of derivatives with respect to time (i.e., d/dt). The first and second derivatives of the coordinates with respect to time correspond to the velocity and acceleration, respectively; the former given by Equation 2.5 and the later by:

$$\mathbf{a}(t) = \frac{\mathbf{v}(t) - \mathbf{v}(t - \Delta t)}{\Delta t}. \quad (2.10)$$

The curvature as a function of time can then be calculated using the components of Equation 2.5 and Equation 2.10.

The average curvature of a single circular trajectory was determined by averaging over every ten values of the curvature along the trajectory. This was done to reduce the noise associated with local curvature fluctuations. The average radius of curvature is then given by:

$$R_c = \frac{1}{\kappa}. \quad (2.11)$$

Assuming a nearly constant linear velocity over a symmetrical, single loop or segment of a circular trajectory, the average angular velocity of the trajectory can be calculated, assuming uniform circular motion, as:

$$\omega_c = \frac{v}{r}. \quad (2.12)$$

It was found that the values calculated for R_c and ω_c closely matched the measured trajectories.

2.5.2 Detection of Parameters in 2D

The algorithm automatically detected local minima in the smoothed linear speed data and, of those minima, selected for tumbles. If the depth of the minima satisfied the requirement,

$$\frac{\Delta v}{v_{min}} > 0.7, \quad (2.13)$$

where Δv is the depth and v_{min} is the minimum speed, then the minimum was recorded by the algorithm as a possible tumble. The value of 0.7 corresponds to a speed decreases of approximately 40% and was empirically found to detect tumbles consistent with those noted by the naked eye. Once potential tumbles were detected based on this speed criterion, the algorithm calculated the tumble time corresponding to each minimum by counting the frames of the neighboring speeds around v_{min} as part of the tumble if and only if:

$$v(t) \leq v(t_{min}) + 0.3\Delta v. \quad (2.14)$$

Where, again, the choice of threshold was empirical and 0.3 returned results most consistent

with observation.

In addition to the speed criterion (Equation 2.13), a sufficient increase in the bacterium’s angular speed, occurring at the same frame (plus or minus one) as a decrease in linear speed, was required for an event to be considered a tumble. If a dip in the linear speed corresponded with a peak in the angular speed that was greater than 20% above the baseline, then the event was considered a tumble.

Runs are observed as long periods of uninterrupted motion in which a bacterium moves at nearly a constant speed along its long axis between two tumbles. The algorithm determines run times by summing the frames between adjacent tumbles and multiplying by the time interval. Tumbles that do not meet both criteria above are ignored and considered to be part of a larger run. Once the tumble and run times have been determined, other relevant parameters are readily determined by the algorithm. The average speed is calculated by identifying the long runs in a given trajectory—defined as any run lasting six frames or more—assigning an average speed to each run segment, and then averaging those values to assign an average speed to a given trajectory. In this way, the average speed is not affected by the speed decrease during tumbles. The average tumble angle was determined by using the average incident and outgoing angle of the trajectories. The incident and outgoing trajectories were taken over three frames before and after the tumbling event, respectively. Since the tumble angle is defined by a directional change in the trajectory and not the cell body orientation, it is unaffected by rotations of the cell body into the third dimension during a tumble, as long as the trajectory itself remains pseudo-2D. Using these criteria, a large data set of tumble times, run times, tumble angles, and average speeds were obtained.

The advantage of this algorithm is two-fold. First, it selects against the detection of false positives by requiring that both a linear and angular speed criterion be met. However, because of the requirement that there be a sufficient decrease in the bacterium’s linear speed, the algorithm is prone to missing certain tumbles. For example, the algorithm may

not detect events in which the bacterium rapidly changes its angle but does not show a significant change in linear speed. This manifests itself primarily when the timescale of the tumble is less than the frame time interval or when the linear speed data is noisy around the minimum. However, these instances are rare. Thus, requiring that both the linear and angular speed criteria be met ensures that all detected tumbles are valid at the expense of missing short tumbles on rare occasions.

In addition, this approach filters out anomalies that may have passed the criterion given by Equation 2.13. For instance, events in which the bacterium slows down or even stops without showing a significant change in angular speed. These events should not be confused with the form of bacterial motility known as pausing, which has been reported for other species of bacteria [13, 14, 15]. In short, we found that the algorithm effectively and reliably identifies tumbles.

2.5.3 Detection of Parameters in 3D

The linear speed and orientation criteria were modified when analyzing 3D tracking data. The linear speed was required to satisfy,

$$\frac{\Delta v}{v_{min}} > 1, \quad (2.15)$$

which corresponds to a speed decrease of 50%. We found that requiring bacteria to decrease their linear speed by half resulted in more accurate tumble detection, as determined by eye.

To better automate the 3D analysis, we adopted the orientation criterion as described by Berg et al. [16]. The bacterium orientation was quantified as the angle between two successive linear velocity vectors, given by Equation 2.8. If $\phi > 35^\circ$ for two successive instants, then the bacterium was taken to be in a state of tumbling. If $\phi > 35^\circ$ for only one instant, then the angle between the vector sum of the two velocity vectors before

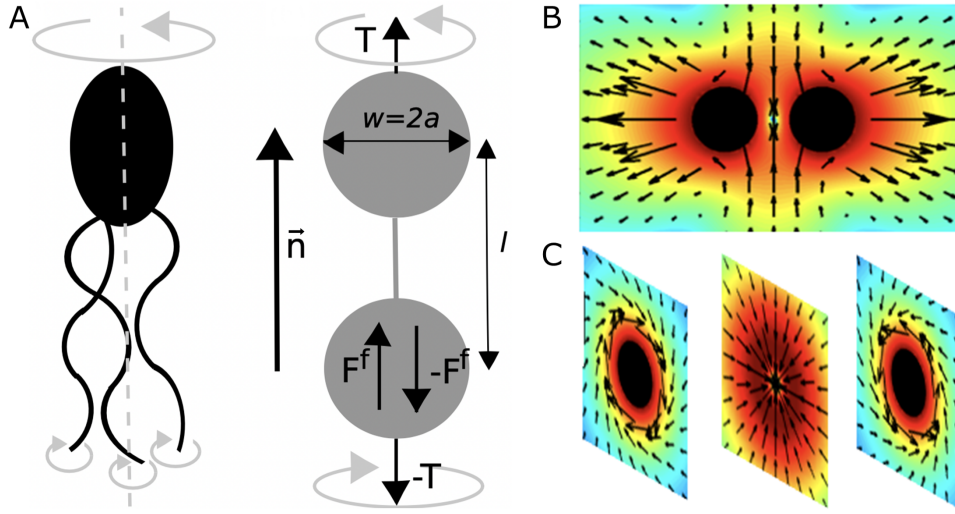


Figure 2.2: (a) Schematic of the two-bead micro-swimmer model. The cell body and flagellar bundle are modeled as two beads of diameter $2a$ connected by a spring, and counter-rotating with equal and opposite torques T and $-T$. The flagellar force F^f on the cell body is prescribed on the bead tail. The swimming direction, which is along the tail-to-head vector, is $\vec{n}$. (b-c) The fluid flow velocity field $\mathbf{u}$ produced by the locomotion of the two-bead micro-swimmer. The color plots $\log(|\mathbf{u}|)$. Whereas in (b) we can only see the effect of the force-dipole, cross-sections along the tail, center, and head beads reveal the effect of the torque-dipole.

and after the instant was calculated and, if that angle exceeded 35° , then the bacterium was also taken to be in a state of tumbling. This algorithm allowed the analysis to be automated across many different trajectories.

The calculation of the tumble times, run times, tumble angles, and average speed was computed in the same way as for 2D data.

2.6 Hydrodynamic Simulations

Bacteria were modeled hydrodynamically near the surface and the results of the simulation were compared to experimental measurements. The following description of the simulation was written in collaboration with Dr. Enkeleida Lushi, who worked with us on this project and contributed her computational and theoretical knowledge.

2.6.1 Mathematical Model for a Swimmer in Free Space

Extending the dumbbell microswimmer model by Hernandez-Ortiz et al. [17, 18], we assume that our single swimmer is composed of two equal-sized beads: one for the head with center $\mathbf{x}_2$ and the other for the tail with center $\mathbf{x}_1$, connected by a finite extensible nonlinear elastic (FENE) spring. A flagellum force $\mathbf{F}^f$ is applied to the tail bead in the tail-to-head direction, $\mathbf{n} = \mathbf{x}_2 - \mathbf{x}_1$, with strength F^f . The swimmer length and width are $\ell + 2a$ and $2a$, respectively, where $\ell = |\mathbf{n}|$ (Figure 2.2(a)).

Moreover, we apply torques $\mathbf{T} = T\mathbf{n}/\ell$ and $-\mathbf{T}$ at the head and tail beads, respectively. Note that these torques have equal magnitude T but are in opposite directions, along the swimmer's main axis (Figure 2.2(a)).

In free space, at each bead $k = 1, 2$ (T, H), the force balance is:

$$\mathbf{F}_k^h + \mathbf{F}_k^c + \delta_{k1}\mathbf{F}_k^f = 0. \quad (2.16)$$

$\mathbf{F}^h$ is the hydrodynamic drag force, $\mathbf{F}^c$ is the spring force, and $\mathbf{F}^f$ is the flagellum force.

For the spring force, we assume a spring constant $h = 25$ and that the springs are finitely-extensible with $L_{max}/L = 1.01$ and $L_{min}/L = 0.99$, where L is the relaxed length and L_{max} and L_{min} are the maximum and minimum stretched lengths, respectively.

The hydrodynamic drag force on a bead $k = 1, 2$ is given by

$$\mathbf{F}_k^h = \zeta(\mathbf{v}_k - \mathbf{u}_k), \quad (2.17)$$

where $\zeta = 6\pi\mu a$ is the Stokes drag and μ is the fluid viscosity.

Since $\mathbf{v}_k = d\mathbf{x}_k/dt$ is the velocity of the bead and $\mathbf{u}_k$ is the fluid velocity at each beads' position, we can derive the equation of motion for each bead by substituting Equation

2.17 into Equation 2.16:

$$\frac{d\mathbf{x}_k}{dt} = \mathbf{u}_k + \frac{1}{\zeta}(\mathbf{F}_k^c + \delta_{1k}\mathbf{F}_k^f). \quad (2.18)$$

The fluid velocity at some point $\mathbf{x}$ in the free-space domain is

$$\mathbf{u}(\mathbf{x}) = \sum_{l=1}^2 \mathcal{S}(\mathbf{x}, \mathbf{x}_l)(-\mathbf{F}_l^h - \delta_{1l}\mathbf{F}_l^f) + \sum_{l=1}^2 \mathcal{R}(\mathbf{x}, \mathbf{x}_l)\mathbf{T}_l = \sum_{l=1}^2 [\mathcal{S}(\mathbf{x}, \mathbf{x}_l)\mathbf{F}_l^c + \mathcal{R}(\mathbf{x}, \mathbf{x}_l)\mathbf{T}_l],$$

where $\mathcal{S}$ and $\mathcal{R}$ refer to the Stokeslet and Rotlet Green's functions for Stokes flow.

Note that the fluid flow due to a point force $\mathbf{F}$ applied at a point $\mathbf{x}_0$ is given by $\mathbf{u}(\mathbf{x}) = \mathcal{S}(\mathbf{x}, \mathbf{x}_0)\mathbf{F}$ for $\mathcal{S}(\mathbf{x}, \mathbf{x}_0) = 1/(8\pi\mu r)(\mathbf{I} + \hat{\mathbf{r}}\hat{\mathbf{r}}/r^2)$ (Equation 1.13) with $\mathbf{r} = (\mathbf{x} - \mathbf{x}_0)$, $r = |\mathbf{r}|$, and $\hat{\mathbf{r}} = \mathbf{r}/r$. The flow resulting from a point torque $\mathbf{T}$ applied at a point $\mathbf{x}_0$ is given by $\mathbf{u}(\mathbf{x}) = \mathcal{R}(\mathbf{x}, \mathbf{x}_0)\mathbf{T} = 1/(8\pi\mu r^3)[\mathbf{T} \times (\mathbf{x} - \mathbf{x}_0)]$ (Equation 1.14) [19].

Because $\mathbf{F}_2^c = -\mathbf{F}_1^c$, the fluid flow induced by the swimmer in free space is

$$\mathbf{u}(\mathbf{x}) = [\mathcal{S}(\mathbf{x}, \mathbf{x}_1) - \mathcal{S}(\mathbf{x}, \mathbf{x}_2)]\mathbf{F}_1^c + [\mathcal{R}(\mathbf{x}, \mathbf{x}_1) - \mathcal{R}(\mathbf{x}, \mathbf{x}_2)]\mathbf{T}. \quad (2.19)$$

Note that this fluid flow is the result of a force dipole (two oppositely applied forces) and a torque dipole (two oppositely applied torques) (Figure 2.2(b) and 2.2(c), respectively).

The dynamics of each bead is then:

$$\begin{aligned} d\mathbf{x}_1/dt &= (\mathbf{F}^f + \mathbf{F}_1^c)/\zeta + \mathcal{S}(\mathbf{x}_1, \mathbf{x}_2)\mathbf{F}_2^c \\ d\mathbf{x}_2/dt &= \mathbf{F}_2^c/\zeta + \mathcal{S}(\mathbf{x}_2, \mathbf{x}_1)\mathbf{F}_1^c. \end{aligned} \quad (2.20)$$

The torques do not appear in the beads' dynamics in free space because the Rotlet is an axisymmetric operator and the torques $\mathbf{T}$ are parallel to the swimmer direction.

As $\mathcal{S}(\mathbf{x}_1, \mathbf{x}_2) = \mathcal{S}(\mathbf{x}_2, \mathbf{x}_1)$ and $\mathbf{F}_1^c = -\mathbf{F}_2^c$, the swimmer's center of mass $\mathbf{x}_c = (\mathbf{x}_1 + \mathbf{x}_2)/2$

has the free-space dynamics

$$d\mathbf{x}_c/dt = \mathbf{F}^f/(2\zeta). \quad (2.21)$$

Note that this gives the *exact swimmer speed* of $1/(12\pi\mu a)F^f$ in free space in the absence of other swimmers or surfaces. Thus we just need to specify the propulsive or flagellum force $\mathbf{F}^f$, the torques $\mathbf{T}$, and the FENE spring constants h, L_{min}, L_{max} .

Figure 2.2(b)-(c) shows the fluid flow in free-space, i.e. not near a boundary, that is generated by the locomotion of the micro-swimmer following Equation 2.19. It is axisymmetric with respect to the swimmer's main axis $\vec{n}$, and in the far field—far from the swimmer—it can be regarded as the sum of a force-dipole with strength $F^f\ell$ and a torque dipole with strength T . As the fluid flow due to the torques has no component along the torque or main-swimmer axis direction $\vec{n}$, in Figure 2.2(b) we do not see its effect but only that of the force-dipole. The effect of the torque dipole is seen when we take slice-cuts perpendicular to $\vec{n}$ at the head, center, and tail, as seen in Figure 2.2(c).

2.6.2 Swimmer Above a Planar No-Slip Wall

When the swimmer is near a no-slip solid plane wall, we must add the image systems to the dynamics [20]. The fluid flow at a point $\mathbf{x}$ now changes from Equation 2.19 to:

$$\begin{aligned} \mathbf{u}(\mathbf{x}) = & [\mathcal{S}(\mathbf{x}, \mathbf{x}_1) - \mathcal{S}(\mathbf{x}, \mathbf{x}_2)]\mathbf{F}_1^c + [\mathcal{R}(\mathbf{x}, \mathbf{x}_1) - \mathcal{R}(\mathbf{x}, \mathbf{x}_2)]\mathbf{T} \\ & + [\mathcal{S}^*(\mathbf{x}, \mathbf{x}_1) - \mathcal{S}^*(\mathbf{x}, \mathbf{x}_2)]\mathbf{F}_1^c + [\mathcal{R}^*(\mathbf{x}, \mathbf{x}_1) - \mathcal{R}^*(\mathbf{x}, \mathbf{x}_2)]\mathbf{T} \end{aligned} \quad (2.22)$$

where $\mathcal{S}^*$ and $\mathcal{R}^*$ refer to the image systems for the Stokeslet and Rotlet respectively [21].

The dynamics of each bead is then

$$d\mathbf{x}_1/dt = (\mathbf{F}^f + \mathbf{F}_1^c + \mathbf{F}_1^x)/\zeta + \mathcal{S}(\mathbf{x}_1, \mathbf{x}_2)[\mathbf{F}_2^c + \mathbf{F}_2^x] \\ + \mathcal{S}^*(\mathbf{x}_1, \mathbf{x}_1)(\mathbf{F}_1^c + \mathbf{F}_1^x) + \mathcal{S}^*(\mathbf{x}_1, \mathbf{x}_2)\mathbf{F}_2^c + \mathcal{R}^*(\mathbf{x}_1, \mathbf{x}_1)\mathbf{T} + \mathcal{R}^*(\mathbf{x}_1, \mathbf{x}_2)(-\mathbf{T}) \quad (2.23)$$

$$d\mathbf{x}_2/dt = (\mathbf{F}_2^c + \mathbf{F}_2^x)/\zeta + \mathcal{S}(\mathbf{x}_1, \mathbf{x}_2)[\mathbf{F}_1^c + \mathbf{F}_1^x] \\ + \mathcal{S}^*(\mathbf{x}_2, \mathbf{x}_1)\mathbf{F}_1^c + \mathcal{S}^*(\mathbf{x}_2, \mathbf{x}_2)(\mathbf{F}_2^c + \mathbf{F}_2^x) + \mathcal{R}^*(\mathbf{x}_2, \mathbf{x}_1)\mathbf{T} + \mathcal{R}^*(\mathbf{x}_2, \mathbf{x}_2)(-\mathbf{T}). \quad (2.24)$$

We have added short-ranged purely repulsive steric forces $\mathbf{F}^x$ that are calculated with a Weeks-Chandler-Anderson repulsive potential activated at a distance $2^{1/6}a \approx 1.12a$ from the bead's center. These steric collision forces prevent the bead's overlap with the wall.

Equation 2.23 and Equation 2.24 are numerically integrated in time with a second order method (Adams-Bashforth). The time-step is chosen sufficiently small to avoid numerical stability issues that may arise due to the stiff spring forces and the steric forces, with the latter smoothed-out at the activation boundary.

2.6.3 Parameter Values for the Model

We set the parameter values for the swimmer based on the experimental measurements for SM3 and similar flagellated bacteria. The characteristic swimmer length will be set to $\bar{L} = \ell + 2a = 2.5 \mu\text{m}$ and the characteristic swimming speed in free space to $\bar{V} = 33.4 \mu\text{m/s}$ [22], which gives us the characteristic flagellum force magnitude $\bar{F}^f = 0.315 \text{ pN}$, according to our model (Equation 2.21). In the table below we show experimental values for the parameters and the non-dimensional values used for the simulations.

Parameter	Experimental Value	Non-dimensional
L	$1 - 10 \text{ }\mu\text{m}$	1
a	$0.25 \text{ }\mu\text{m}$	0.1
V	$31.2 - 44.5 \text{ }\mu\text{m s}^{-1}$	1
$ \mathbf{F}^f $	0.315 pN	1
$ \mathbf{T} $	$0.355 \text{ pN }\mu\text{m}$	1.1
μ	$10^{-3} \text{ pN s }\mu\text{m}^{-2}$	1

References

- [1] S. Au. The use of glycerol for preservation of living cells at low temperatures. *PNAS*, 47:57–60, 1954.
- [2] J. Adler. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. *Microbiology*, 74:77–91, 1973.
- [3] J. D. Partridge, N. T. Q. Nhu, Y. S. Dufour, and R. M. Harshey. *Escherichia coli* remodels the chemotaxis pathway for swarming. *MBio*, 10:10–1128, 2019.
- [4] K. M. Taute, S. Gude, S. J. Tans, and T. S. Shimizu. High-throughput 3D tracking of bacteria on a standard phase contrast microscope. *Nat. Comm.*, 6:8776, 2015.
- [5] A. Klemm. Trackmate: My favorite image analysis tool, by Neubias members. *Imaging & Microscopy*, 2020.
- [6] D. Ershov, M. Phan, J. W. Pylvänäinen, S. U. Rigaud, L. Le Blanc, A. Charles-Orszag, J. R. W. Conway, R. F. Laine, N. H. Roy, and D. Bonazzi. Trackmate 7: integrating state-of-the-art segmentation algorithms into tracking pipelines. *Nat. Methods*, 19:829–832, 2022.
- [7] R. C. Gonzalez and R. E. Woods. *Digital Image Processing*. Pearson, 2018.
- [8] J. Tinevez, N. Perry, J. Schindelin, G. M. Hoopes, G. D. Reynolds, E. Laplantine, S. Y. Bednarek, S. L. Shorte, and K.W. Eliceiri. Trackmate: An open and extensible platform for single-particle tracking. *Methods*, 115:80–90, 2017.
- [9] M. M. Dubay, J. Acres, M. Riekes, and J. L. Nadeau. Recent advances in experimental design and data analysis to characterize prokaryotic motility. *J. Microbiol. Methods.*, 204:106658, 2022.
- [10] J. Gao, J. Carnahan, G. Alexandre, and J. Trolinger. Digital holographic microscopy

- for volumetric, three-dimensional characterization of bacterial motility. *Interferometry XXI*, 12223:100–110, 2022.
- [11] J. Masson, G. Voisinne, J. Wong-Ng, A. Celani, and M. Vergassola. Noninvasive inference of the molecular chemotactic response using bacterial trajectories. *PNAS*, 109:1802–1807, 2012.
 - [12] M. Theves, J. Taktikos, V. Zaburdaev, H. Stark, and C. Beta. A bacterial swimmer with two alternating speeds of propagation. *Biophys. J.*, 105:1915–1924, 2013.
 - [13] I. Lapidus, M. Welch, and M. Eisenbach. Pausing of flagellar rotation is a component of bacterial motility and chemotaxis. *J. Bacteriol.*, 170:3627–3632, 1988.
 - [14] C. Qian, C. C. Wong, S. Swarup, and K. Chiam. Bacterial tethering analysis reveals a “run-reverse-turn” mechanism for pseudomonas species motility. *Appl. Environ. Microbiol.*, 79:4734–4743, 2013.
 - [15] K. M. Thormann, C. Beta, and M. J. Kühn. Wrapped up: The motility of polarly flagellated bacteria. *Annu. Rev. Microbiol.*, 76:349–367, 2022.
 - [16] L. Turner, L. Ping, M. Neubauer, and H.C. Berg. Visualizing flagella while tracking bacteria. *Biophys. J.*, 111:630–639, 2016.
 - [17] J. P. Hernandez-Ortiz, C.G. Stoltz, and M. D. Graham. Transport and collective dynamics in suspensions of confined swimming particles. *Phys. Rev. Lett.*, 95:204501, 2005.
 - [18] J. Hernandez-Ortiz, C. Stoltz, and M. Graham. Dynamics of confined suspensions of swimming particles. *J. Phys. Condens. Matter.*, 21:204107, 2009.
 - [19] J. R. Blake and A. T. Chwang. Fundamental singularities of viscous flow: part i: the image systems in the vicinity of a stationary no-slip boundary. *J. Eng. Math.*, 8: 23–29, 1974.

- [20] J. R. Blake. A note on the image system for a stokeslet in a no-slip boundary. *Math. Proc. Camb. Philos. Soc.*, 70:303–310, 1971.
- [21] S. E. Spagnolie and E. Lauga. Hydrodynamics of self-propulsion near a boundary: predictions and accuracy of far-field approximations. *J. Fluid Mech.*, 700:105–147, 2012.
- [22] S. Johnson, B. Freedman, and J. X. Tang. Run-and-tumble kinematics of *Enterobacter* sp. SM3. *Phys. Rev. E*, 109:064402, 2024.

CHAPTER 3

RUN-AND-TUMBLE KINEMATICS OF *ENTEROBACTER* SP. SM3

“Not all those who wander are lost.”

—J. R. R. Tolkien

3.1 Introduction

Many species of bacteria swim by rotating an organized bundle of helical, independently motor-driven filaments called flagella [1, 2, 3, 4]. One mode of swimming, known as run-and-tumble, is characterized by periods of smooth translation at near-constant velocity, or runs, punctuated by short intervals of cell body rotation with little spatial translation, tumbles. Specifically, runs occur during periods of CCW rotation of all of the flagella, resulting in a helical bundle that pushes the cell body forward. A tumble occurs when one or more flagella switch to the CW rotation state and leave the bundle [5, 6, 7]. The repetition of runs and tumbles results in trajectories resembling that of a random walk. In the presence of chemical stimuli, run-and-tumble motion can appear as a biased random walk with runs being prolonged towards favorable locations. This phenomenon results in

chemotaxis, which is an important microbial behavior [8, 9, 10, 11]. Bacterial motility has inspired physicists to create and study run-and-tumble in abiotic systems [12, 13].

Run-and-tumble has been identified in many peritrichous bacterial species [14, 15, 16, 17]. Of these, *E. coli* has been the most extensively studied. In the absence of stimuli, *E. coli* wild type AW405 runs for 1.0 s, tumbles for 0.1 s, and changes direction between successive runs by 68° , on average [16, 18, 19, 20]. In regard to chemoattractants, *E. coli* has been shown to be highly responsive to the amino acid serine in the steady state, suppressing tumbles and increasing run times when compared with a control sample. However, it adapts faster to the amino acid aspartate, showing no significant response in the steady state [18, 20].

A more complex mode of motility known as swarming is defined as the rapid movement of dense, flagellated bacteria over the surface of a solid substrate [21]. In a typical swarming experiment, motile bacteria are inoculated onto nutrient-rich agar from which they draw water and nutrients [22]. As bacteria grow and replicate they rapidly expand across the surface, moving in a thin layer of liquid, with those directly behind the swarm front being the most motile [23, 24]. The properties of bacterial swarms are influenced by numerous factors, including biological [25, 26, 27, 28, 29] and hydrodynamic [30, 31, 32, 33] ones. Recently, there has been an increased interest in the relationship between swimming and swarming [34, 35, 14, 36, 37]. What links the two modes of motility are the flagella that support motility and the biological machinery that govern their physical behavior. It is known that the presence of flagella is necessary for swarming, with bacteria lacking flagella being unable to swarm [23, 38]. Bacteria that lack a functional chemotaxis system, and thereby lack the ability to modulate the flagellum motor rotation direction, also show impaired swarming motility [39, 40]. Flagella intertwining between neighboring bacteria, as evidenced by electron microscopy, have been speculated, although not confirmed, to be relevant to swarming [41, 42]. These interactions have been further suggested to contribute to swarming-related phenomena such as the formation of dynamic rafts, jets, and vortices

[38, 43, 44]. Therefore, it is clear that flagella-driven swimming plays an important role in swarming.

SM3 is a strongly swarming bacterium that has been recently isolated from mouse feces and has been found to reduce intestinal inflammation in mice suffering from symptoms associated with IBD. The swarming motility of SM3 is directly related to its ability to ameliorate IBD symptoms, whereas non-swarming strains of a nearly identical genome provide no physiological benefits [45]. It is therefore essential to study the swimming and swarming motility of SM3 to gain insight into the effects of swarming on gut physiology. In this work, we determine the run-and-tumble parameters of SM3 as both planktonic swimmers and swarmers. The findings of this study offer new insights on the relationship between swimming and swarming motility.

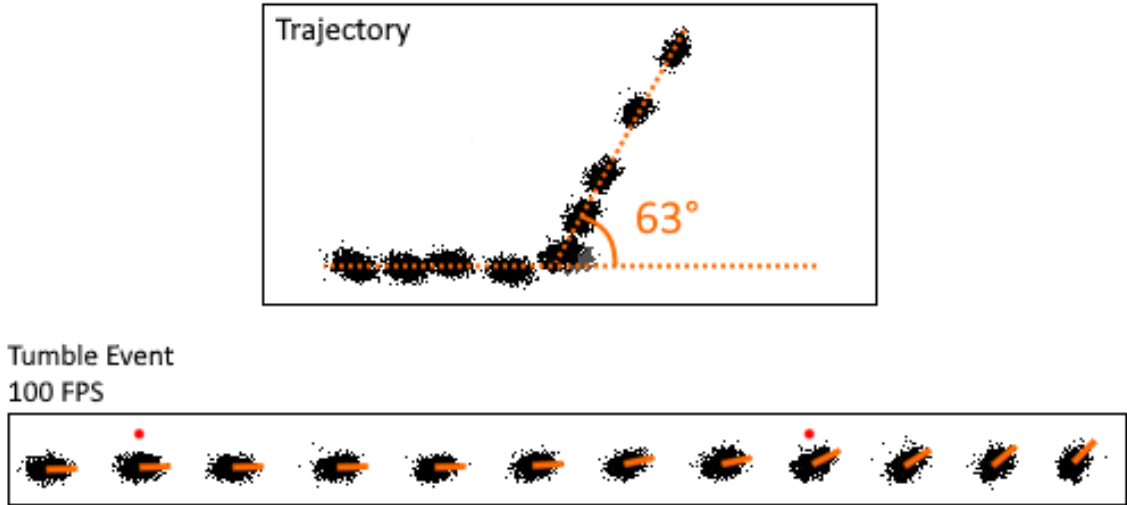


Figure 3.1: (Top) A segment of an SM3 trajectory. The segment was taken at 100 FPS for 0.7 s, resulting in 70 consecutive frames. Images were selected seven frames apart and combined into an overlay image, showing the trajectory with visual clarity. (Bottom) Frame-by-frame sequence of the tumbling event from the above trajectory. The event lasts 12 frames, corresponding to a tumble time of 0.12 s. The tumble angle of this event is 63° . Red dots denote the two frames that are included in the image overlay above, which overlap at the position of the tumble. A movie of this tumbling event is available at [46].

3.2 Results

3.2.1 SM3 Exhibits Run-and-Tumble Behavior

The swimming motility of planktonic SM3 was observed in motility buffer using phase contrast microscopy. Bacteria were observed to be rod-shaped with a short and long axis, similar to other genera of gram-negative gut bacteria such as *Escherichia*. Bacteria ran in relatively straight segments separated by brief moments of decreased speed of movement and erratic changes in the cell body orientation. To better understand the kinematics of these direction-changing events, movies were taken at a frame rate of 100 FPS. The top panel of Figure 3.1 shows a representative direction-changing event as an overlay of

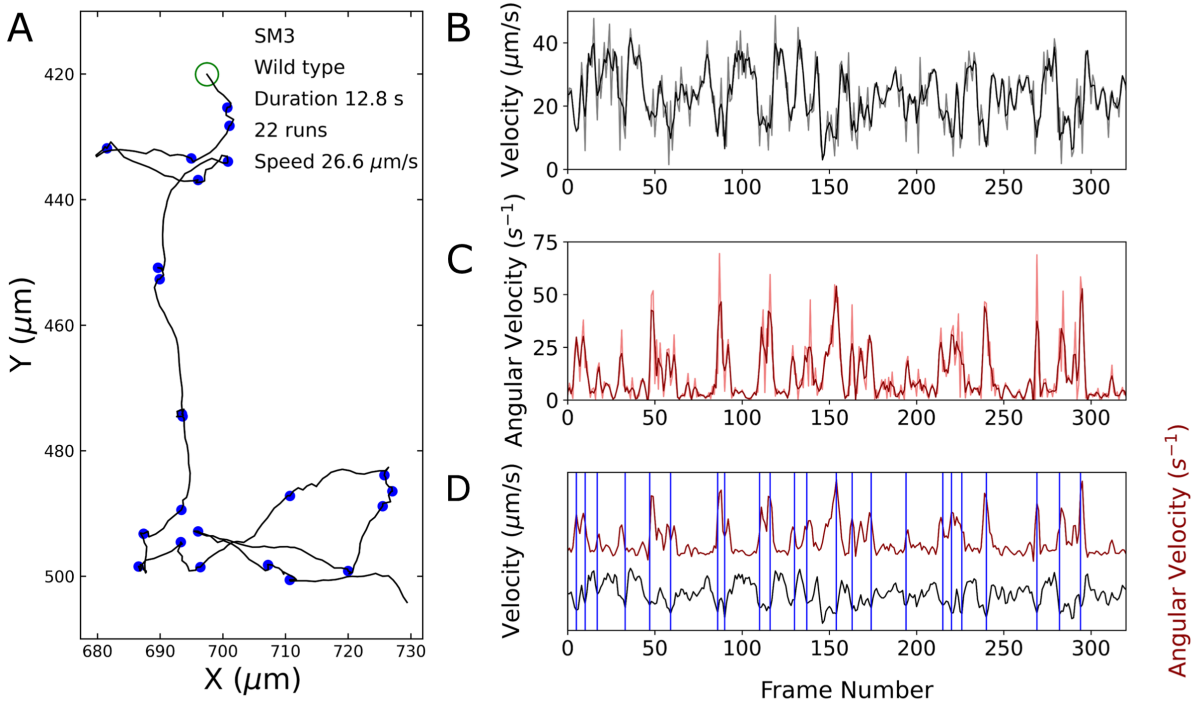


Figure 3.2: Analysis of SM3 trajectories performed using a tracking algorithm. (a) A representative trajectory. The green circle marks the beginning of the trajectory. The blue dots indicate tumbles detected by the algorithm. A movie of this trajectory is available at [47]. (b) Linear speed versus frame number. (c) Angular speed versus frame number. Opaque curves in (b) and (c) show the unsmoothed data, with the solid lines after smoothing. (d) Overlay of the linear and angular speeds on the same plot. Each blue line notes the alignment of a dip in the smoothed linear speed with a peak in the smoothed angular speed, indicating a tumble.

images with every seventh frame selected for visual clarity. In this event the bacterium moved horizontally, from left to right, with its long body axis parallel to the direction of motion. Then, during a sequence of 12 frames corresponding to a time interval of 0.12 s (shown in the bottom panel), the bacterium ceased to translate and began to rotate in the counterclockwise direction. Following rotation, the bacterium resumed its motion in a new direction at an angle of approximately 63° off its initial direction of travel, with its long body axis parallel to the new direction of motion. However, it was observed in separate instances that bacteria could also alternate directions of rotation. These features of the observed motion are consistent with run-and-tumble behavior, as previously observed for other peritrichous bacteria such as *E. coli* [6, 20, 48] and *Bacillus subtilis* (*B. subtilis*) [15, 19, 16].

To get a quantitative measure of the run-and-tumble motility of SM3, we adopted a custom tracking algorithm (see the Methods) to analyze trajectories of swimmers imaged using dark-field microscopy at 25 FPS. Figure 3.2(a) shows a representative trajectory, which consists of relatively straight segments of runs flanked by adjacent tumbling events. The algorithm detected tumbles as instances of decreased linear speed occurring at the same instance of increased angular speed. Figures 3.2(b) and 3.2(c) plot the linear and angular speed, respectively, as functions of time for the bacterium whose trajectory is shown in Figure 3.2(a). The average run speed is approximately 30 $\mu\text{m/s}$. Figure 3.2(d) shows the tumbles detected by the algorithm denoted as blue lines marking the coincidence of dips in linear speed with peaks in angular speed. The translational speed during most of the detected tumbles decreases by more than 50% as seen in Figure 3.2(b). The detected tumbles are also indicated on the trajectory in Figure 3.2(a) as blue dots.

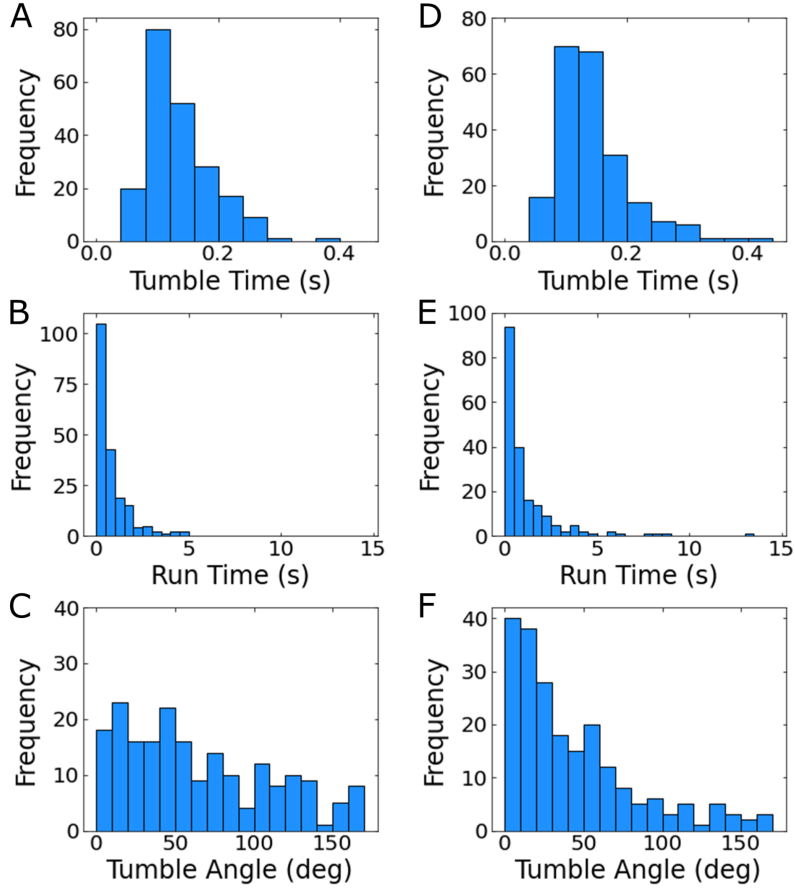


Figure 3.3: Histograms of the measured tumble times, run times, and tumble angles for SM3 swimmers (a)-(c) and swarmers (d)-(f). The total counts and average values are listed in Table 3.1.

	Concentration (μM)	Tumble Time (s)	Run Time (s)	Tumble Angle (deg)	Run Speed ($\mu\text{m/s}$)
Motility Buffer		0.12 ± 0.05 [209]	0.77 ± 0.86 [199]	69 ± 50 [209]	30.4 ± 7.5 [10]
Serine	10	0.12 ± 0.06 [216]	0.83 ± 0.82 [207]	69 ± 50 [216]	28.4 ± 5.2 [9]
	100	0.10 ± 0.05 [210]	0.99 ± 1.09 [198]	61 ± 48 [210]	31.6 ± 5.3 [20]
	200	0.11 ± 0.05 [213]	1.23 ± 1.61 [199]	52 ± 41 [213]	29.4 ± 6.5 [13]
	500	0.10 ± 0.04 [229]	1.41 ± 1.69 [198]	47 ± 42 [229]	31.8 ± 6.7 [20]
Aspartate	100	0.12 ± 0.05 [216]	0.84 ± 0.91 [202]	69 ± 51 [216]	29.6 ± 5.0 [9]
	1000	0.12 ± 0.06 [211]	0.94 ± 1.18 [198]	65 ± 50 [211]	30.2 ± 6.3 [11]
Swarmers		0.12 ± 0.06 [216]	1.15 ± 1.69 [196]	45 ± 42 [216]	33.4 ± 4.0 [17]

Table 3.1: Run-and-tumble parameters of SM3. The results include measurements of swimmers in motility buffer, swimmers with added serine or aspartate, and swarmers in motility buffer. Tabulated data includes the average tumble time, run time, tumble angle, and run speed based on the number of data points shown in brackets. Reported errors are standard deviations, showing a large range of values for each measured parameter.

Analysis of the trajectories produced histograms of the measured tumble times, run times, and tumble angles (Figures 3.3(a)-3.3(c)). The histogram of tumble times is bell-shaped and peaks around an average of 0.12 ± 0.05 s, with a longer tail on the right than on the left. The histogram of run times resembles an exponential distribution that drops off for longer run times, with 52% of runs being less than or equal to 0.44 s. The average run time is 0.77 ± 0.86 s. The tumble angle histogram for swimming bacteria also resembles an exponential distribution, with the average tumble angle being 69° . The run-and-tumble parameters of SM3 in motility buffer, tabulated in Table 3.1, are similar to those reported for *E. coli* in previous studies [16, 18, 19, 49].

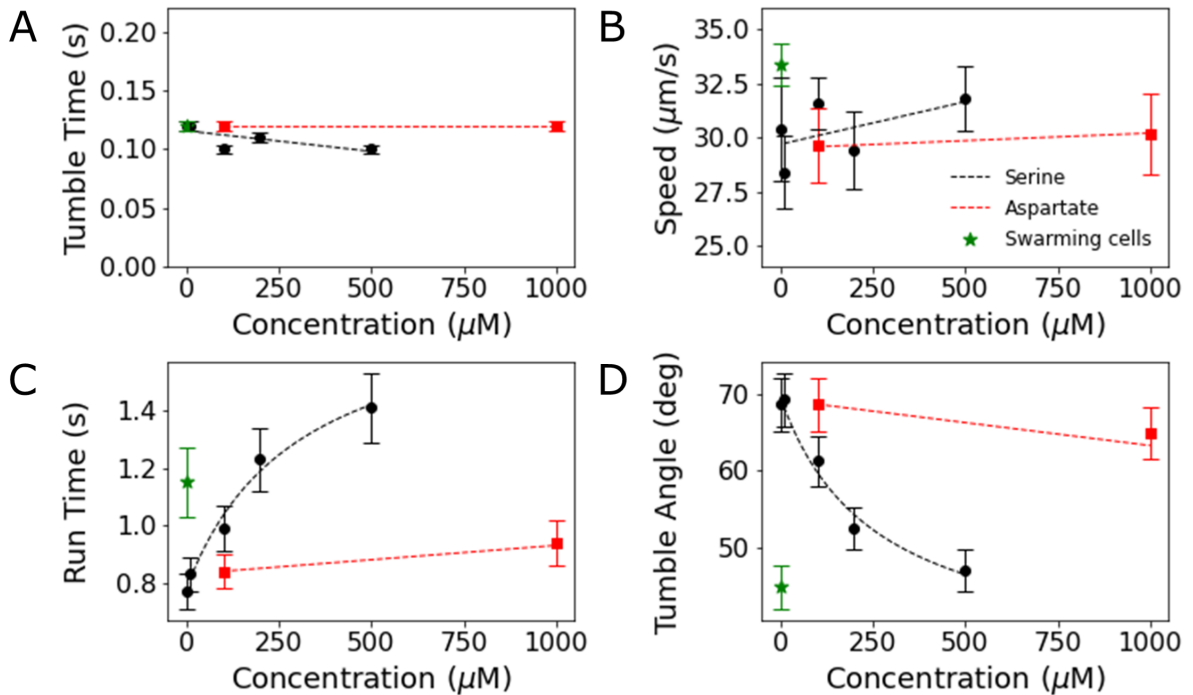


Figure 3.4: The effect of serine (black) and aspartate (red) on the run-and-tumble behavior of SM3. The data for dilute swimmers (green) is included for comparison. Plots include the (a) average tumble time, (b) average run time, (c) average tumble angle, and (d) average bacterium speed as functions of concentration. Dotted lines serve to guide the eye to apparent trends. Error bars are standard errors to the mean.

3.2.2 SM3 Alters Its Behavior in Response to Chemoattractants

We exposed planktonic swimmers of SM3 to bulk concentrations of two chemoattractants, the amino acids serine and aspartate, and measured the run-and-tumble response in the steady state. SM3 shows a response to serine in the steady state, as shown in Figure 3.4. The average run time of SM3 increases with serine concentration (Figure 3.4(c)) whereas the average tumble angle decreases (Figure 3.4(d)). The average tumble time and speed, however, remain mostly constant over the entire range of serine concentrations (Figures 3.4(a) and 3.4(b)).

To determine if SM3 has an adaptation preference for the type of chemoattractant, we also exposed it to aspartate. We chose to study the response to aspartate at concentrations of 100 μM and 1 mM, which for serine led to large changes. Aspartate, however, was found to not have a measurable effect on run-and-tumble parameters at both concentrations in the steady state (Figure 3.4). The data used to produce Figure 3.4 for both chemoattractants are tabulated in Table 3.1. The measured response of SM3 upon exposure to serine and aspartate is similar to *E. coli* [18, 20], suggesting that SM3 adapts faster to aspartate than to serine when measured in the steady state.

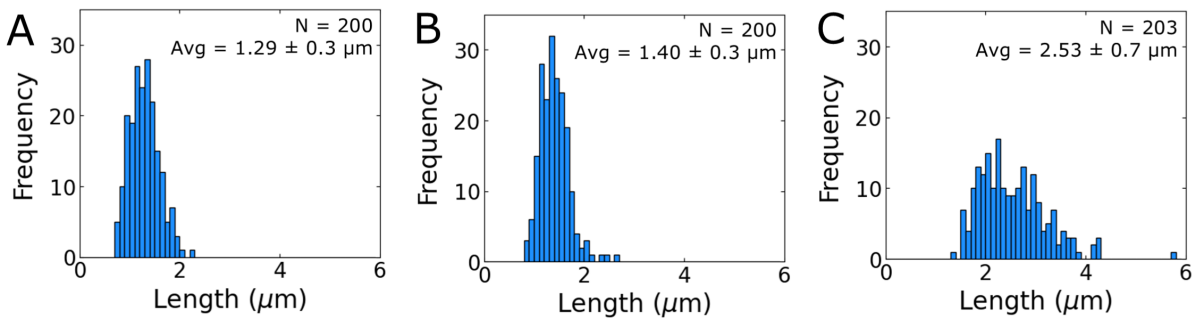


Figure 3.5: Histograms of the measured bacterium length. Bacteria were measured for (a) growth incubated overnight for 16 hours, (b) fresh growth incubated from overnight growth for 4 hours, and (c) bacteria extracted from the swarm front after 6 hours of growth on a Petri dish (refer to Figure 1.10 for an example swarm). Histograms represent 200, 200, and 203 data points, respectively. Average values for each data set are reported in the upper right of each panel with the reported error being the standard deviation.

3.2.3 SM3 Swimmers Have Different Run-and-Tumble Parameters

In a similar fashion to the planktonic swimmers, the run-and-tumble parameters of dilute swimmers were measured. Bacteria from the swarm front were found to be $2.5 \pm 0.7 \mu\text{m}$ on average measured along the long body axis, almost twice as long as their planktonic counterparts, which were measured to be $1.4 \pm 0.3 \mu\text{m}$, on average, during the exponential phase of growth (Figures 3.5(b) and 3.5(c)). The measured tumble times, run times, and tumble angles, plotted as histograms in Figures 3.3(d)-3.3(f), show notable differences between swimmers and swimmers. The swimmers have a longer run time on average, $1.15 \pm 1.69 \text{ s}$, as evidenced by the frequency of longer run times in the tail of the

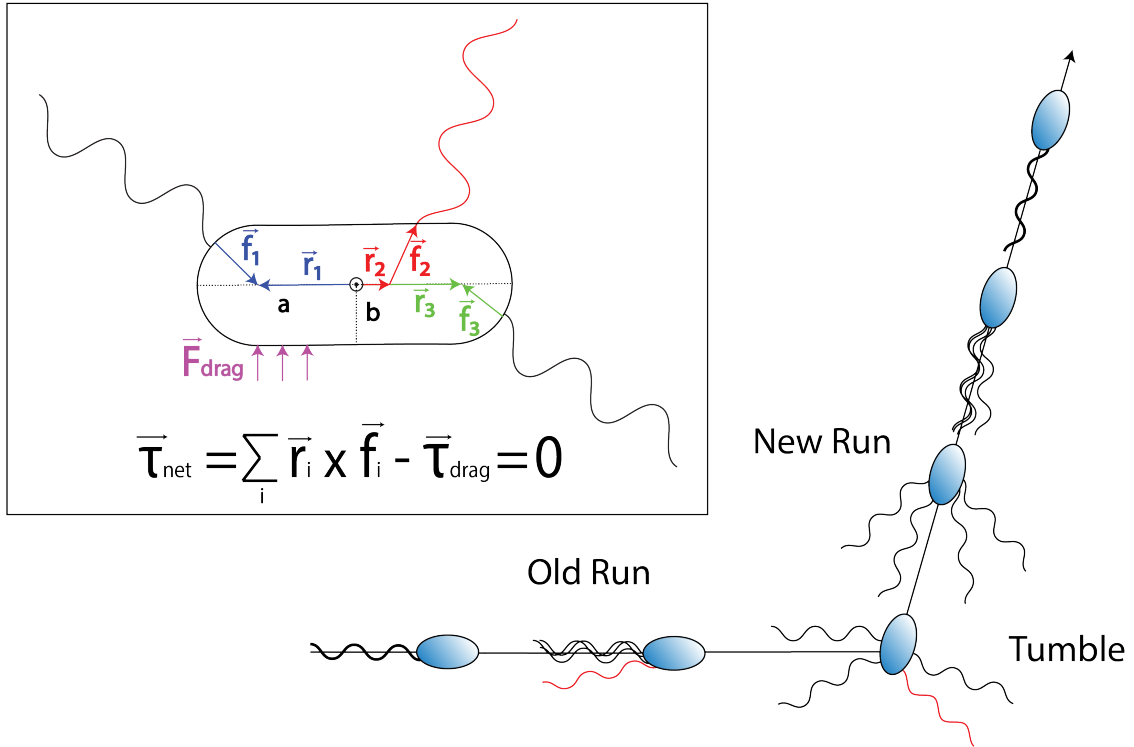


Figure 3.6: Schematic illustration of a tumble event in SM3. When one flagellum switches rotation direction from CCW to CW (colored red), it leaves the bundle and causes the bundle to become uncoordinated, initiating a tumble. During the tumble, swimming motility is reduced as the bacterium reorients itself. This diagram can be directly compared with the observed behavior in Figure 3.1. (Inset) The net torque exerted by the flagella drives the rotation of the cell body with viscous drag opposing the rotation direction. Flagella were not observed in this study.

run histogram. The swimmers tumble with a smaller angle of 45° on average, as seen in the increased weight towards the left in the tumble angle histogram. Interestingly, the average tumble time for the swimmers, 0.12 ± 0.06 s, is the same as that for the swimmers. The run-and-tumble data for both swimmers and swimmers are tabulated in Table 3.1 for comparison. The average values for swimmers are also indicated as green data points in Figure 3.4 for visual comparison with those of swimmers. Suppressed tumbles, longer run times, and shorter tumble angles have been observed for swarming strains of *E. coli*, such as HCB1737 and MG1655 [16, 14]. These results show that SM3 swimmers have different run-and-tumble parameters than swimmers.

3.2.4 Flagellum-Driven Tumble Model

We propose a simple physical mechanism for flagellum-driven tumbling in rod-like bacteria, such as SM3, based on rotational dynamics in Newtonian fluids. We model the cell body during a tumble as a prolate ellipsoid rotating about a semi-minor axis without translation (see inset of Figure 3.6). This assumption is justified by the observation that SM3 does not change its position drastically during a tumble (see Figure 3.1). During the tumble, each flagellum exerts a force on the cell body, with CW rotating flagella exerting a force opposite in sign compared with CCW rotating flagella (see red vectors in the inset of Figure 3.6). The sum of the torques, $\vec{T}$, generated by the flagellum forces over the short duration of a tumble is given by:

$$\vec{T} = \sum_i \vec{r}_i \times \vec{f}_i, \quad (3.1)$$

where $\vec{r}_i$ is the position vector from the bacterium's center of mass to the base of the i^{th} flagellum and $\vec{f}_i$ is the force vector of that flagellum.

The viscous drag torque for a prolate ellipsoid rotating about a semi-minor axis opposes the rotation and obeys an adjusted Stokes Law given by Equation 1.9:

$$\vec{\tau}_{drag} = -8\pi\eta ab^2\vec{\omega}F_r, \quad (3.2)$$

with F_r given by Equation 1.11.

In a low Reynolds number environment, the net torque on a prolate ellipsoid and the viscous drag torque balance, as shown in the inset of Figure 3.6. The functional form for $\vec{T}$ therefore follows that of $\vec{\tau}_{drag}$ given in Equation 3.2:

$$\vec{T} = 8\pi\eta ab^2\vec{\omega}F_r. \quad (3.3)$$

We see from Equation 3.3 that the net torque on the cell body is proportional to its length. For an applied net torque, the angular velocity of the bacterium, and therefore the tumble angle, is inversely proportional to a . Thus, the tumble angle is expected to be smaller for longer bacteria, which experience an increased rotational viscous drag.

Here is an order of magnitude estimate for the tumble angle of a planktonic swimmer based on this theory. Taking $T = 10^{-19}$ N·m, $\eta = 1$ mPa·s, $a = 1$ μm , and $b = 0.5$ μm , we get $\omega = 11$ rad/s, which for a tumble time of 0.1 s corresponds to a tumble angle of 61° . This estimate agrees with the observed behavior of the bacterium in Figure 3.1, which is 1.7 μm in length ($a = 0.85$ μm) and has a tumble angle of 63° , corresponding to 9 rad/s over a duration of 0.12 s.

Extending this model quantitatively to swimmers is complicated by phenotypical changes. It is known that many species of bacteria increase their average flagella number and motor torque as they differentiate into the swarming phenotype [14, 26, 27, 28]. Referring to Equation 3.3, in addition to the bacterium length a , the total net torque T also changes for swimmers when applying this model to calculate rotation speed. If we assume that the number of flagella doubles and the motor torque of each flagellum increases by 10%, a finding reported recently [14], we can rewrite Equation 3.3 with

changes to factors on both sides of the equation, as the following:

$$\left(2 \cdot 1.1 \cdot \vec{T}\right) \approx 8\pi(2a)b^2\vec{\omega} (2.26 \cdot F_r), \quad (3.4)$$

where the factor 2.26 is the calculated increase in the Perrin rotational friction factor due to doubling the bacterium length. The approximate cancellation of the factor 2.2 on the left-hand side with 2.26 on the right-hand side yields the relationship:

$$\vec{T} \approx 2 \times 8\pi ab^2\vec{\omega} F_r. \quad (3.5)$$

Note that, despite accounting for an increase in the flagella number and motor torque, the increase in rotational friction due to an increase in the bacterium length is still more significant. Performing the same calculation as before for swimmers with $T = 2.2 \times 10^{-19}$ N·m and $a = 2 \mu\text{m}$, we get $\omega = 5$ rad/s, which for a tumble time of 0.1 s corresponds to a tumble angle of 30° . The fact that this estimate does not agree completely with the measured value of 45° as tabulated in Table 3.1 is not surprising, owing to the complexity of the system this simple model attempts to describe. The simple model does not account for factors such as inter-flagellum interactions during a tumble and incomplete dissociation of the bundle, to name a few. Still, this model provides a good understanding of tumbling mechanics.

3.3 Discussion

Tumbles were observed to be characterized by reduced swimming speed and a noticeable reorientation of the cell body (Figure 3.1). It has been shown in previous works on run-and-tumble that one flagellum leaving the bundle is sufficient to initiate a tumble [5, 6, 16]. When one flagellum switches rotation direction it leaves the bundle and may also cause others to leave the bundle due to the hydrodynamic coupling between the flagella and the

high rotation rate of each motor (on the order of 100 Hz). The flagella can then splay apart and become uncoordinated, causing the bacterium to stop swimming and start to tumble, as illustrated in Figure 3.6. During the tumble, the cell body reorientation is driven by the net torque on the cell body due to forces exerted by both the CCW- and CW-rotating flagella (see inset of Figure 3.6). In some instances, the cell body continuously reorients over an angle less than 180° , such as shown in Figure 3.1. When the stray flagellum switches back to the CCW state, the tumble ends and a new run begins as the bundle reforms. This picture of flagellum-driven tumble behavior is consistent with what we have observed for SM3. However, further experimental confirmation is needed, by fluorescence imaging of live flagella, as done previously for other peritrichous bacteria such as *E. coli* [5, 6, 16].

The data acquired in this study demonstrates that SM3 performs run-and-tumble motility similar to *E. coli*. SM3 swims in trajectories that consist of straight runs separated by short tumbles (Figure 3.2) during which the cell body stops translating and reorients itself in the direction of a new run (Figure 3.1). During the tumble, the translational speed decreases by more than 50% (Figure 3.2(b)). The average tumble time of SM3 in LB medium, 0.12 seconds, is nearly identical to that of *E. coli* wild type AW405, which has an average tumble time of 0.14 seconds. The average tumble angle of SM3, 69° , is also consistent with *E. coli*, which has an average tumble angle of 68° . The average run time of SM3, 0.77 s, is slightly shorter than the 0.86 s average run time reported for *E. coli*. The histograms of tumble time and run time also closely resemble those of *E. coli*, with the tumble time distribution being bell-shaped and the run time distribution resembling an exponential distribution [18, 20, 49]. It should be taken into account, however, that the original data for *E. coli* were obtained with three-dimensional tracking techniques. We may be slightly underestimating the average tumble angle when taking measurements in a pseudo-2D environment. This should be taken into account when comparing our values to *E. coli*. Despite this technical difference, our results support the conclusion that

SM3 performs run-and-tumble motion with parameters similar to *E. coli* [16, 18].

The response of SM3 to common chemoattractants is also similar to *E. coli*. When exposed to serine, SM3 suppresses tumbles in the steady state, resulting in longer run times and shorter tumble angles, on average (Figure 3.4). The reduced tumble angle results in a swimming bias in the forward direction [48, 50, 17]. In response to aspartate, however, SM3 seems to adapt faster to local bulk concentrations, as evidenced by both the run time and tumble angle remaining unchanged in the steady state. The steady state response of SM3 to serine and aspartate is similar to the behavior that has been previously observed for *E. coli* [18, 20]. Whether SM3 exhibits true chemotaxis in addition to a chemoattractant response, however, will require gradient experiments in the future.

Swarms of SM3 show suppressed tumble behavior when compared to swimmers. The suppressed occurrence of tumbles for swarms results in longer runs and shorter tumble angles on average (Figure 3.4 and Table 3.1). The decrease in the average tumble angle gives rise to smoother trajectories, which can be seen when comparing the tracks of swimmers and swarms (compare the movies at [47] and [51]). This altered behavior of swarms has been identified for multiple species of swarming bacteria, including *E. coli*, *B. subtilis*, and *Proteus mirabilis* [34, 16]. These results add to the growing evidence that changes occur in run-and-tumble motility when motile, planktonic bacteria attain swarming motility by growing and spreading over an agar surface for several hours.

In the case of chemoattractant exposure, a possible explanation for the decrease in the average tumble angle could be a reduction of the average tumble time [48]. For a constant applied net torque, Equation 3.3 states that the cell body rotates with a constant angular velocity. With a decrease in the tumble duration, the cell body would sweep across a decreased range of tumble angles, reducing the average. Although the measured data show that the average tumble time only decreases slightly over a large range of serine concentrations (Figure 3.4(a)), the results may be limited by the time resolution of the data acquired. Since the movies acquired for the run-and-tumble analysis were taken at

25 FPS, corresponding to a time interval of 0.04 s between frames, measuring a difference on the order of one-hundredth of a second in average tumble time would not be reliable. Such a difference, however, could cause a significant change in the average tumble angle due to the rapid rotation of the cell body during a tumble. Another possible explanation for the observed change could be a modification of the flagella motor behavior due to chemoattractant exposure, resulting in a smaller applied net torque on the cell body and a decreased tumble angle. We have not identified a report on such an effect in the literature for any species. For SM3, in particular, further studies on its motor behavior, including its response to chemoattractants, are clearly required.

For swimmers, we believe the reduction in tumble angle can be attributed to their longer length in comparison to swimmers. Since $\omega \propto a^{-1}$, an increase in the bacterium length can result in a significant decrease in the angular velocity and tumble angle (see Section 3.2.4 for more details and a sample calculation). Since swimmers are significantly longer than swimmers (Figure 3.5), we would expect swimmers to rotate less on average. A future study measuring the tumble angle as a function of cell length would further explore this mechanical model.

References

- [1] H. C. Berg. The rotary motor of bacterial flagella. *Annu. Rev. Biochem.*, 72:19–54, 2003.
- [2] E. M. Purcell. Life at low reynolds number. In *Physics and our world: reissue of the proceedings of a symposium in honor of Victor F Weisskopf*, pages 47–67. World Scientific, 2014.
- [3] E. Lauga and T. R. Powers. The hydrodynamics of swimming microorganisms. *Rep. Prog. Phys.*, 72:096601, 2009.
- [4] M. Silverman and M. Simon. Flagellar rotation and the mechanism of bacterial motility. *Nature*, 249:73–74, 1974.
- [5] N. C. Darnton, L. Turner, S. Rojevsky, and H. C. Berg. On torque and tumbling in swimming *Escherichia coli*. *J. Bacteriol.*, 189:1756–1764, 2007.
- [6] L. Turner, W. S. Ryu, and H. C. Berg. Real-time imaging of fluorescent flagellar filaments. *J. Bacteriol.*, 182:2793–2801, 2000.
- [7] R. Xue, Q. Ma, M. A. B. Baker, and F. Bai. A delicate nanoscale motor made by nature—the bacterial flagellar motor. *Adv. Sci.*, 2:1500129, 2015.
- [8] S. H. Larsen, R. W. Reader, E. N. Kort, W. Tso, and J. Adler. Change in direction of flagellar rotation is the basis of the chemotactic response in *Escherichia coli*. *Nature*, 249:74–77, 1974.
- [9] R. R. Vuppula, M. S. Tirumkudulu, and K. V. Venkatesh. Chemotaxis of *Escherichia coli* to l-serine. *Phys. Biol.*, 7:026007, 2010.
- [10] E. Tamar, M. Koler, and A. Vaknin. The role of motility and chemotaxis in the bacterial colonization of protected surfaces. *Sci. Rep.*, 6:1–11, 2016.

- [11] Z. Alirezaeizanjani, R. Großmann, V. Pfeifer, M. Hintsche, and C. Beta. Chemotaxis strategies of bacteria with multiple run modes. *Sci. Adv.*, 6:eaaz6153, 2020.
- [12] T. Mano, J. Delfau, J. Iwasawa, and M. Sano. Optimal run-and-tumble-based transportation of a Janus particle with active steering. *PNAS*, 114:E2580–E2589, 2017.
- [13] H. Karani, G. E. Pradillo, and P. M. Vlahovska. Tuning the random walk of active colloids: From individual run-and-tumble to dynamic clustering. *Phys. Rev. Lett.*, 123:208002, 2019.
- [14] J. D. Partridge, N. T. Q. Nhu, Y. S. Dufour, and R. M. Harshey. Escherichia coli remodels the chemotaxis pathway for swarming. *MBio*, 10:10–1128, 2019.
- [15] J. Najafi, M. R. Shaebani, T. John, F. Altegoer, G. Bange, and C. Wagner. Flagellar number governs bacterial spreading and transport efficiency. *Sci. Adv.*, 4:eaar6425, 2018.
- [16] L. Turner, L. Ping, M. Neubauer, and H.C. Berg. Visualizing flagella while tracking bacteria. *Biophys. J.*, 111:630–639, 2016.
- [17] T. Nakai, T. Ando, and T. Goto. Biased reorientation in the chemotaxis of peritrichous bacteria Salmonella enterica serovar typhimurium. *Biophys. J.*, 120:2623–2630, 2021.
- [18] H.C. Berg and D.A. Brown. Chemotaxis in Escherichia-Coli Analyzed by 3-Dimensional Tracking. *Nature*, 239:500–504, 1972.
- [19] K. M. Taute, S. Gude, S. J. Tans, and T. S. Shimizu. High-throughput 3D tracking of bacteria on a standard phase contrast microscope. *Nat. Comm.*, 6:8776, 2015.
- [20] H. C. Berg. *E. coli in Motion*. Springer, 2004.
- [21] D. B. Kearns. A field guide to bacterial swarming motility. *Nat. Rev. Microbiol.*, 8: 634–644, 2010.

- [22] R. M. Harshey. Bacterial motility on a surface: many ways to a common goal. *Annu. Rev. Microbiol.*, 57:249–273, 2003.
- [23] A. Be’er and G. Ariel. A statistical physics view of swarming bacteria. *Mov. Ecol.*, 7: 1–17, 2019.
- [24] N. C. Darnton, L. Turner, S. Rojevsky, and H. C. Berg. Dynamics of bacterial swarming. *Biophys. J.*, 98:2082–2090, 2010.
- [25] R. Daniels, J. Vanderleyden, and J. Michiels. Quorum sensing and swarming migration in bacteria. *FEMS Microbiol. Rev.*, 28:261–289, 2004.
- [26] Judith F. M. Hoeniger. Development of flagella by *Proteus mirabilis*. *Microbiology*, 40:29–42, 1965.
- [27] F. D. Williams and R. H. Schwarzhoff. Nature of the swarming phenomenon in *Proteus*. *Annu. Rev. Microbiol.*, 32:101–138, 1978.
- [28] R. M. Harshey and T. Matsuyama. Dimorphic transition in *Escherichia coli* and *Salmonella typhimurium*: surface-induced differentiation into hyperflagellate swarmer cells. *PNAS*, 91:8631–8635, 1994.
- [29] A. M. Abdel-Mawgoud, F. Lépine, and E. Déziel. Rhamnolipids: diversity of structures, microbial origins and roles. *Appl. Microbiol. Biotech.*, 86:1323–1336, 2010.
- [30] C. Giverso, M. Verani, and P. Ciarletta. Emerging morphologies in round bacterial colonies: comparing volumetric versus chemotactic expansion. *BMMB*, 15:643–661, 2016.
- [31] L. Ping, Y. Wu, B. G. Hosu, J. X. Tang, and H. C. Berg. Osmotic pressure in a bacterial swarm. *Biophys. J.*, 107:871–878, 2014.
- [32] M. Fauvart, P. Phillips, D. Bachaspatimayum, N. Verstraeten, J. Fransaer, J. Michiels, and J. Vermant. Surface tension gradient control of bacterial swarming in colonies of *Pseudomonas aeruginosa*. *Soft Matter*, 8:70–76, 2012.

- [33] H. Ma, J. Bell, W. Chen, S. Mani, and J. X. Tang. An expanding bacterial colony forms a depletion zone with growing droplets. *Soft Matter*, 17:2315–2326, 2021.
- [34] J. D. Partridge, N. T. Q. Nhu, Y. S. Dufour, and R. M. Harshey. Tumble suppression is a conserved feature of swarming motility. *Mbio*, 11:e01189–20, 2020.
- [35] W. Chen, N. Mani, H. Karani, H. Li, S. Mani, and J. X. Tang. Confinement discerns swarmers from planktonic bacteria. *Elife*, 10:e64176, 2021.
- [36] L. Turner, R. Zhang, N. C. Darnton, and H. C. Berg. Visualization of flagella during bacterial swarming. *J. Bacteriol.*, 192:3259–3267, 2010.
- [37] M. Sidortsov, Y. Morgenstern, and A. Be’Er. Role of tumbling in bacterial swarming. *Phys. Rev. E*, 96:022407, 2017.
- [38] M. F. Copeland and D. B. Weibel. Bacterial swarming: a model system for studying dynamic self-assembly. *Soft matter*, 5(6):1174–1187, 2009.
- [39] M. Burkart, A. Toguchi, and R. M. Harshey. The chemotaxis system, but not chemotaxis, is essential for swarming motility in *Escherichia coli*. *PNAS*, 95:2568–2573, 1998.
- [40] S. Mariconda, Q. Wang, and R. M. Harshey. A mechanical role for the chemotaxis system in swarming motility. *Mol. Microbiol.*, 60:1590–1602, 2006.
- [41] S. J. Stahl, K. R. Stewart, and F. D. Williams. Extracellular slime associated with *Proteus mirabilis* during swarming. *J. Bacteriol.*, 154:930–937, 1983.
- [42] B. V. Jones, R. Young, E. Mahenthiralingam, and D. J. Stickler. Ultrastructure of *Proteus mirabilis* swarmer cell rafts and role of swarming in catheter-associated urinary tract infection. *Infect. Immun.*, 72:3941–3950, 2004.
- [43] N. H. Mendelson, A. Bourque, K. Wilkening, K. R. Anderson, and J. C. Watkins. Organized cell swimming motions in *Bacillus subtilis* colonies: patterns of short-lived whirls and jets. *J. Bacteriol.*, 181:600–609, 1999.

- [44] E. Lushi, H. Wioland, and R. E. Goldstein. Fluid flows created by swimming bacteria drive self-organization in confined suspensions. *PNAS*, 111:9733–9738, 2014.
- [45] A. De, W. Chen, H. Li, J. R. Wright, R. Lamendella, D. J. Lukin, W. A. Szymczak, K. Sun, L. Kelly, S. Ghosh, et al. Bacterial swimmers enriched during intestinal stress ameliorate damage. *Gastroenterology*, 161:211–224, 2021.
- [46] S. Johnson, B. Freedman, and J. X. Tang. Video segment of SM3 performing a tumble. *Brown Digital Repository*, 2025. doi: <https://doi.org/10.26300/3wdm-jp33>.
- [47] S. Johnson, B. Freedman, and J. X. Tang. Animated trajectory of SM3 performing run-and-tumble. *Brown Digital Repository*, 2025. doi: <https://doi.org/10.26300/151b-ff02>.
- [48] J. Saragosti, P. Silberzan, and A. Buguin. Modeling E. coli tumbles by rotational diffusion. Implications for chemotaxis. *PloS One*, 7:e35412, 2012.
- [49] L. Lemelle, T. Cajgfinger, C. C. Nguyen, A. Dominjon, C. Place, E. Chatre, R. Barbier, J. Palierne, and C. Vaillant. Tumble kinematics of Escherichia coli near a solid surface. *Biophys. J.*, 118:2400–2410, 2020.
- [50] N. Vladimirov, D. Lebedz, and V. Sourjik. Predicted auxiliary navigation mechanism of peritrichously flagellated chemotactic bacteria. *PLoS Comp. Biol.*, 6:e1000717, 2010.
- [51] S. Johnson, B. Freedman, and J. X. Tang. Animated trajectory of swarming SM3 performing run-and-tumble motility. *Brown Digital Repository*, 2025. doi: <https://doi.org/10.26300/9t40-0288>.

CHAPTER 4

CELL LENGTH AFFECTS THE SWIMMING TRAJECTORY OF FLAGELLATED BACTERIA NEAR A SOLID SURFACE

“Everything is a manifestation of nature. There is nothing that happens in the world that doesn’t involve its purpose.”

—Marcus Aurelius

4.1 Introduction

Bacteria swim in circular trajectories when in close proximity to a surface. Flagellated bacteria initially come into contact with the surface by swimming into it, which results in a collision-mediated alignment of the cell body with the surface [1]. Flagellum-powered swimming [2], hydrodynamic coupling to the surface [3, 4], sedimentation under gravity [5, 6], van der Waals forces, and Brownian motion [7] are all known to facilitate close

contact with the surface, with electrostatic forces less influential [8, 9]. As a result, bacteria are found in higher concentrations near surfaces rather than in free space [3, 1, 10]. Once at the surface, bacteria swim in circles tens of nanometers from the surface [9]—with their cell body pointing downward at an average angle of 10° [11]—for several seconds or minutes, depending on whether tumbling is suppressed [12]. The curvature of the trajectory is caused by the counter-rotation of the flagellar bundle and the cell body, which establishes forces in opposing directions, resulting in a net torque on the system [2, 7]. The radii of these trajectories are on the order of 20-50 μm long [2, 13, 14] and have been experimentally shown to vary with temperature [13], ionic concentration [8], and with distance from the surface [15]. Despite the amount of work on circular trajectories, little has been done to experimentally probe the effects of cell length on them.

An early study on circular swimming trajectories by Lauga et al. found experimentally that the radius of curvature depended on cell length for a strain of *E. coli* [14]. The dependency was described analytically using resistive force theory for the flagellum and by considering the hydrodynamic mobilities of the cell body. Experimentally, however, the length range they studied was limited, only spanning from 0.8 to 1.2 μm . Furthermore, their theoretical approach neglected the aspect ratio of the cell—modeling it as a sphere—which is important for near-surface swimming hydrodynamics. In this study, we use cells taken from a swarm, which are variable in length and can grow abundantly up to 10 μm , to study the effect of cell length on circular trajectories. Additionally, we use a two-sphere model to simulate the hydrodynamics of bacteria swimming next to the surface, which takes into consideration both the hydrodynamics and shape of the swimmer [16].

SM3 is a rod-shaped, peritrichously flagellated bacterium that performs run-and-tumble [17]. It is also a strongly swarming species that can collectively cover agar substrates in a matter of hours [18, 17, 19]. Like other swarming species [20, 21], SM3 elongates when differentiating into the swarming phenotype [17]. This fact, in addition to the finding that

swarming bacteria (swarmers¹) suppress the rate of tumbling compared to those grown as individuals (swimmers) [22], makes SM3 the ideal species for studying the effect of cell length on circular trajectories for prolonged lengths of time.

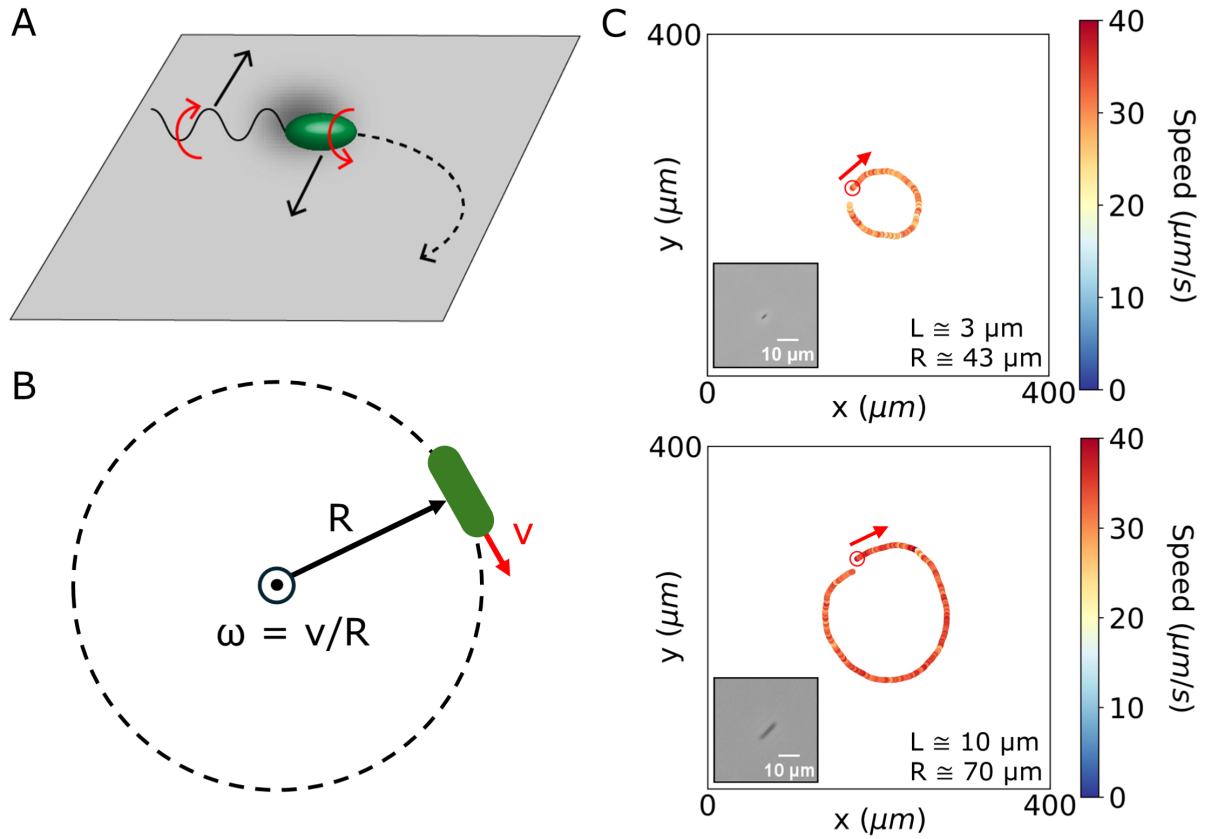


Figure 4.1: (a) Illustration of the forces and torques acting on a bacterium above a surface. The counter-rotation of the cell body and flagellar bundle (red arrows) produce forces in opposite directions (black arrows), causing a net torque that rotates the system. (b) Schematic of a circular trajectory with labeled kinematic parameters. (c) Representative circular trajectories of SM3 near the surface with length 3 and 10 μm . The colormap indicates the instantaneous linear speed. The average radius of curvature and linear speed of the trajectories are noted on each plot. Movies of these two trajectories are available at [23] and [24].

¹This nomenclature should not be confused with the swarmer phase of the life cycle of *Caulobacter crescentus*.

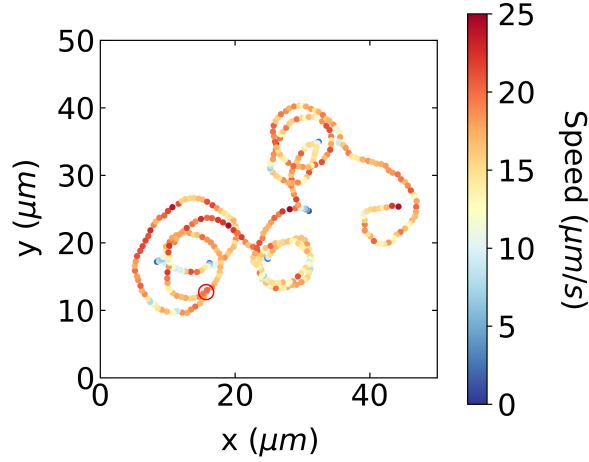


Figure 4.2: An example of SM3 swimming at the surface with multiple circular loops. A tumble occurs where the trajectory is kinked and the linear speed decreases sharply, as shown by the colormap. The start of the trajectory is indicated by a red circle. A movie of this trajectory is available at [25].

4.2 Results

4.2.1 SM3 Swims in Circular Trajectories Near the Surface

We observed the swimming behavior of SM3 swimmers above a glass surface. When viewed from the top, SM3 traces circular trajectories in a clockwise direction while propelling itself forward (Figures 4.1(a)-4.1(c)), indicating that the cell body rotates clockwise, while the flagellar bundle rotates counterclockwise, when viewed from behind (Figure 4.1(a)). This is consistent with the fact that the flagella of SM3 rotate counterclockwise during a run [17]. Additionally, it is known that other peritrichous bacteria with left-handed flagellar helices, such as *E. coli* [26], swim in clockwise trajectories near a surface when viewed from above [2]. Therefore, we can conclude that SM3 is also a left-handed bacterial species.

Near the surface, we observed that SM3 swam in circles at a nearly constant linear velocity. This is seen in the uniformity of the speed color map for the trajectories shown in Figure 4.1(c). The trajectories shown in Figure 4.1(c), however, are only a

single representative loop of longer trajectories, and the nontruncated trajectories often consisted of multiple loops (Figure 4.2). Occasionally, we observed tumbles, which were marked by a decrease in linear speed and a reorientation of the cell body. A tumble event occurs as a “kink” in the trajectory. However, we observed that tumbling events occurred at a lower rate in swimmers than is typical for swimmers, i.e., they were largely suppressed. Most often, swimmers swam in smooth, continuous clockwise trajectories near the surface at nearly constant speed, long enough to trace out multiple loops over the course of tens of seconds.

We noticed that longer swimmers tended to swim in circular trajectories of larger radii than shorter ones. Figure 4.1(c) shows representative trajectories for two swimmers of length 3 and 10 μm . The longer bacterium follows a circular trajectory with a radius ($R \approx 70 \mu\text{m}$) that is almost twice the length of the shorter one ($R \approx 43 \mu\text{m}$). So, we noted that the radius of curvature is a function of the cell length.

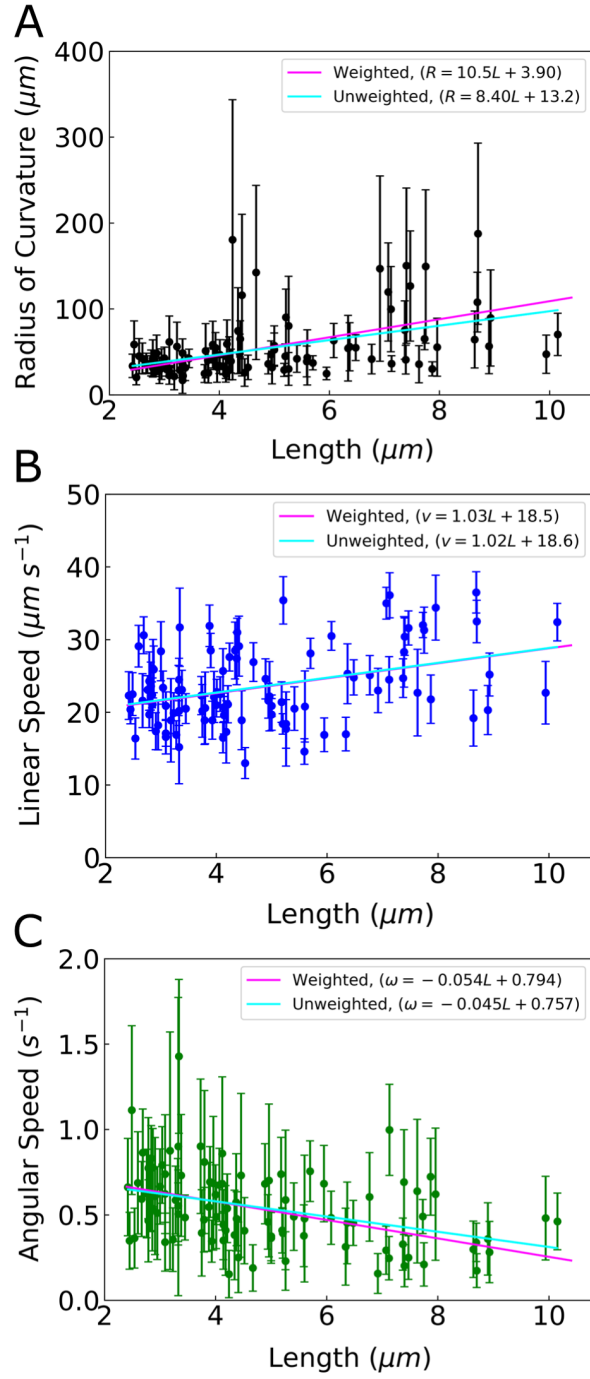


Figure 4.3: Average (a) radius of curvature, (b) linear speed, and (c) angular speed as functions of cell length for 97 different bacteria. Two fit lines are plotted for each data set: a weighted best fit (magenta), that accounts for the greater number of data points at shorter lengths, and an unweighted fit (cyan). The weighted fit is the linear fit to the average values of data points within each $1 \mu\text{m}$ length bin. Error bars are based on a sample standard deviation of the data used to produce a single data point.

4.2.2 The Radii of Circular Trajectories Depend on the Cell Length

We were interested in how the average radius of curvature, linear speed, and angular speed of circular trajectories would change as a function of cell length. To probe this, we collected 97 different circular trajectories for bacteria ranging from 2-10 μm in length. The average radius of curvature, linear speed, and angular speed as functions of cell length are plotted in Figure 4.3(a)-4.3(c).

For shorter swimmers (2-3 μm), the average radius of curvature and linear speed is 36.9 μm and 22.6 $\mu\text{m/s}$, respectively. These values are in agreement with previous measurements for *E. coli*, another rod-like, peritrichous bacterium, which typically measure appropriately 2 μm in length in the planktonic state. For *E. coli*, the average radius of curvature at the surface ranges from 10-50 μm and the swimming speed is on the order of 20 $\mu\text{m/s}$ [2, 13, 14]. SM3 swimmers, at a comparable length, have an average radius of curvature and linear speed at the surface that is similar to *E. coli*.

The average linear speed of swimmers near the surface is significantly decreased compared to that in free space. When free to swim without confinement, swimmers swim at a speed of 33.4 $\mu\text{m/s}$, on average [17]. This is approximately 40% faster than the average speed of swimmers near the surface, which is 23.6 $\mu\text{m/s}$, over all lengths. The measured reduction in speed near the surface can be attributed to the stronger hydrodynamic drag that bacteria experience close to a confining surface.

The radius of curvature for circular trajectories increases with cell length over a large sample size (Figure 4.3(a)), as seen in Figure 4.1(c) for representative trajectories. The longest of the radii belonged to a cell 8.7 μm in length and measured 188 μm in diameter. In fact, the circular diameter was so large that only half of it remained in the field of view during the imaging, resulting in a circular arc segment. Furthermore, the average linear speed increases slightly with cell length, increasing from roughly 21 $\mu\text{m/s}$ to 29

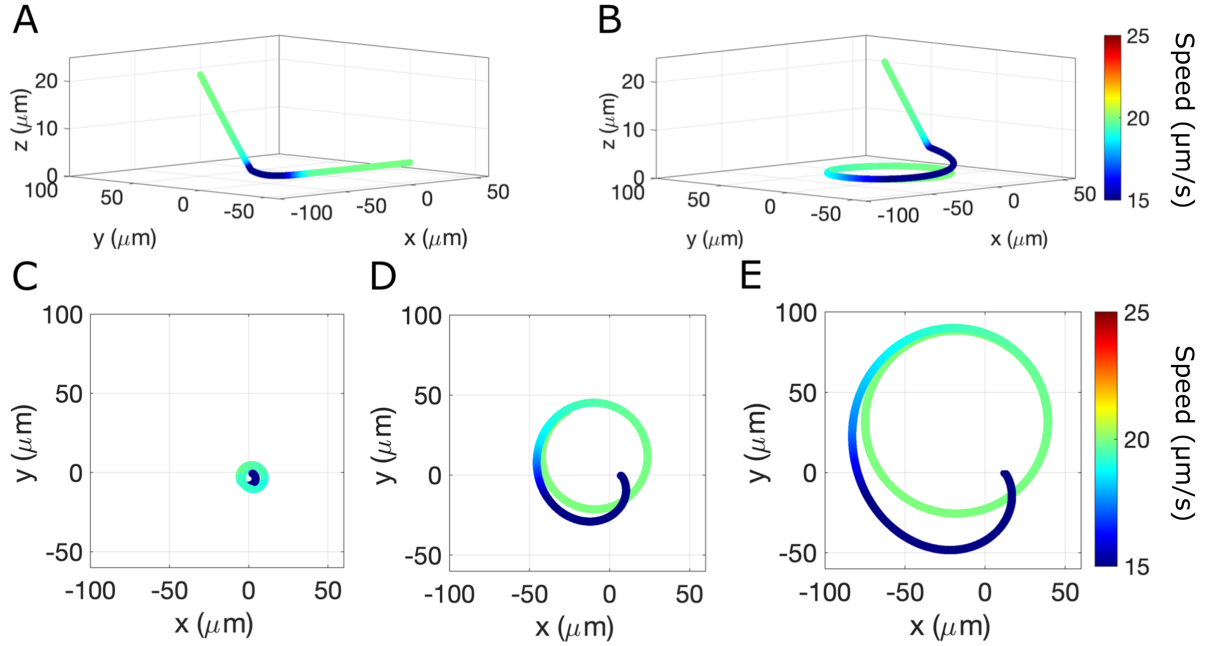


Figure 4.4: (a) A simulated microswimmer with only force-dipole hydrodynamics. Once at the surface, it swims in a straight trajectory. (b) A simulated microswimmer with force- and torque-dipole hydrodynamics. It swims in a circle at the surface. (c)-(e) Top-down view of three circular trajectories for simulated microswimmers 1, 3, and 10 μm in length.

$\mu\text{m/s}$ over the length range 2-10 μm (Figure 4.3(b)). The average angular velocity, which is the ratio between the average linear speed and average radius of curvature, decreases with increased cell length, since the average radius of curvature increases at a faster rate than the average linear speed (Figure 4.3(c)). The kinematics of bacteria swimming near the surface is significantly affected by cell length.

4.2.3 Simulations Show that Circular Trajectories Emerge From the Head-Tail Counter-Rotation

To probe the mechanism that gives rise to the circular swimming trajectories observed in experiment, we simulated bacteria as microswimmers constructed from two counter-rotating beads (see Section 2.6 of the Methods), one for the cell body and the other for the flagellar bundle, connected by a spring (Figure 2.2(a)). The inclusion of two beads separated by a distance, l , accounts for the shape (length) of the bacterium. The

simulated swimmers approached the surface from above along a quasi-straight trajectory (Figures 4.4(a) and 4.4(b)). Once near the surface, the speed of the swimmers initially decreased (dark blue segments of Figures 4.4(a)-4.4(e)) before gradually returning to a steady-state value, at which point they began to move along circular trajectories (Figures 4.4(b)-4.4(e)). The simulations show that the mechanism that gives rise to the clockwise circular trajectories at the no-slip surface is the head-tail counter-rotation—specifically, the two same-magnitude, opposite-direction torques applied at the head and tail. Without the head-tail counter-rotation (torque-dipole), the swimmer hits the no-slip wall and swims in a straight trajectory (Figure 4.4(a)). Without noise, it remains on the surface because of its elongated shape and hydrodynamic attraction to its image. Simulations that do not account for the swimmer shape (e.g., bacteria modeled as a single sphere) or torque dipole hydrodynamics do not manifest circular trajectories.

We varied the length of the simulated microswimmer to observe the effects of cell length on the radius of curvature. While keeping the force and torque of the flagellar bundle constant, the separation between the beads, l , was increased in $0.5 \mu\text{m}$ increments from 2.5 to $10 \mu\text{m}$. Similar to what was observed in experiment, the simulation predicts that the radius of curvature increases with swimmer length (Figures 4.4(c)-4.4(e)). The

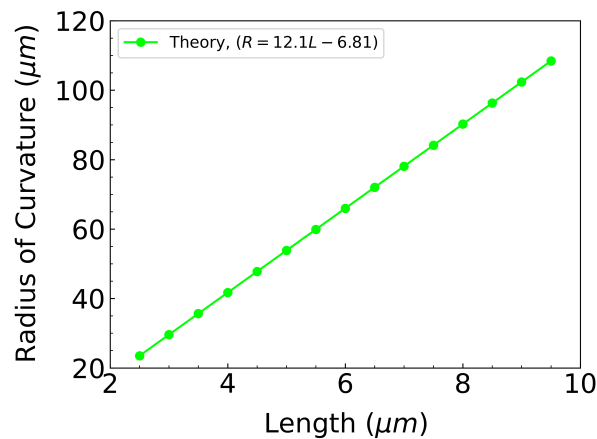


Figure 4.5: Numerical results for the radius of curvature as a function of length for constant force and torque. Numerical data points are shown with the corresponding trend line based on a linear fit.

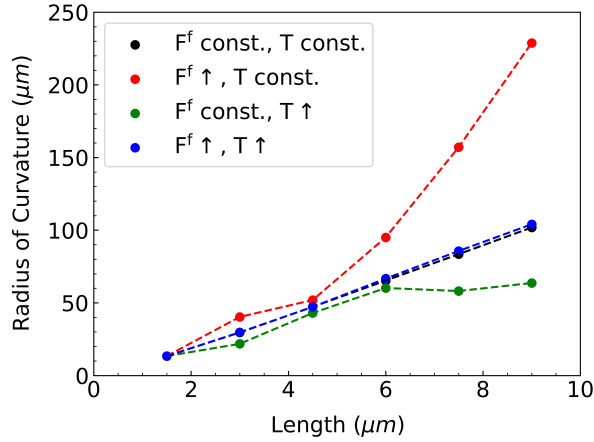


Figure 4.6: Simulated results for the radius of curvature as a function of length for four combinations of increasing force and torque.

numerical data generated by our simulation (Figure 4.5) also shows a linear dependence of radius of curvature on cell length. These findings demonstrate that simulations accounting for the hydrodynamics of an elongated swimmer near the surface successfully produce length-dependent circular radii.

4.3 Discussion

The forces that primarily affect the radius of curvature of the trajectory are the propulsion force of the flagellar bundle, F^f , the torque of the flagellar bundle, T , and the hydrodynamic drag. If F^f increases as a function of cell, so would the linear speed and the average radius of curvature. However, there is little evidence in the literature for a length-dependent increase in run speed. It has been experimentally reported for freely swimming *Enterobacter* sp. SM1_HS2B—a closely related genetic cousin of SM3 that can hyper-elongate—that the average speed remains nearly constant over a large length range of 2 to 180 μm [27]. Furthermore, the speed of freely swimming *E. coli* is not size-dependent [28]. Therefore, we believe that the slight increase in linear speed that we measured (Figure 4.3(b)) is most likely negligible and we can safely assume that F^f is constant across swarmers of different length. Since T is directly proportional to F^f , we

can then also assume that it remains constant as a function of cell length.

We wanted to convince ourselves that F^f and T could be held constant for our simulations, so we ran our simulation to obtain numerical data for the radius of curvature across all combinations of increasing F^f and T (Figure 4.6). Physically, if F^f increases, so should T in direct proportion. If T increases, the cell body and flagellar bundle rotate with a larger angular velocity, causing the combined system to turn more sharply with respect to the surface. Therefore, an increase in T reduces the average radius of curvature, which opposes the effect of increasing F^f . Thus, the effects of increasing F^f and T should cancel one another out. Indeed, we found that increasing both F^f and T returned similar data as when holding both constant (black and blue lines in Figure 4.6). Therefore, based on the experimental literature and our numerical results, we justify holding both F^f and T constant.

The observation that longer bacteria trace out circles of larger radii than shorter ones can be understood by considering the physics of the system. In addition to considering F^f and T , one must also consider the effects of drag. As bacteria elongate, they become harder to turn for a given F^f and T due to increased hydrodynamic drag on the cell body. Furthermore, as bacteria elongate, they require a greater T to turn the same amount about a given axis as compared to shorter bacteria [17]. Thus, swimmers manifest circles with longer radii, on average.

The large variation in the data in Figure 4.3 can be attributed to natural cell-to-cell variation in the number, length, and rotation frequency of flagella, all of which may vary with the cell length [14]. Additionally, we did not control the distance between the bacteria and the surface, which may also be a function of cell length. Bacteria closer to the surface experience more viscous drag from the underlying fluid layer, which could affect the measured kinematic parameters.

These uncontrolled variables, however, did not seem to affect the trends observed in

Figure 4.3. For each length, there are data both above and below the trend line and, on average, the data increase or decrease accordingly with increasing length. This is expected as, for a given length, bacteria may follow a normal distribution for flagella number, cell length, and rotation frequency. So, on average, the effects of natural cell-to-cell variation cancel out over a large sample size.

Brownian motion randomizes the displacement and swimming direction of bacteria, causing them to swim closer towards or away from the surface [7]. One would expect longer bacteria, which are more massive and therefore experience more sedimentation under gravity, to accumulate closer to the surface, on average, skewing the distribution towards a Boltzmann distribution. However, because of the amplified effect of Brownian motion near the surface, the effects of sedimentation may only be secondary. Therefore, although we did not control for the distance from the surface, the variation in the distance from the surface introduced by Brownian motion allowed the dependency of the kinematic parameters on cell length to emerge over a large sample size.

We have showed experimentally that SM3, like other peritrichous bacteria, follow circular trajectories when in close proximity of a confining surface (Figures 4.1 and 4.2). Furthermore, we observed that the radius of the trajectories depends on the length of the elongated swimmers, with longer bacteria tracing out circles of larger radii than shorter ones, on average (Figure 4.3). Hydrodynamic simulations, which include both a force- and a torque-dipole flow field, reproduce circular swimming behavior (Figure 4.4), as well as produce numerical data that are in agreement with observation (Figure 4.5), verifying the dependence of the radius of curvature on cell length. Since every parameter other than length was held constant in our simulation, we conclude that the radius of curvature increases due primarily due to an increase in cell length.

References

- [1] G. Li and J. X. Tang. Accumulation of microswimmers near a surface mediated by collision and rotational brownian motion. *Phys. Rev. Lett.*, 103:078101, 2009.
- [2] H. C. Berg and L. Turner. Chemotaxis of bacteria in glass capillary arrays. *Escherichia coli*, motility, microchannel plate, and light scattering. *Biophys. J.*, 58:919–930, 1990.
- [3] A. P. Berke, L. Turner, H. C. Berg, and E. Lauga. Hydrodynamic attraction of swimming microorganisms by surfaces. *Phys. Rev. Lett.*, 101:038102, 2008.
- [4] J. R. Blake. A note on the image system for a stokeslet in a no-slip boundary. *Math. Proc. Camb. Philos. Soc.*, 70:303–310, 1971.
- [5] J. Wan, T. K. Tokunaga, and C. Tsang. Bacterial sedimentation through a porous medium. *Water Resour. Res.*, 31:1627, 1995.
- [6] P. Nelson. *Biological Physics: Energy, Information, Life*. W. H. Freeman and Company, New York, 2014.
- [7] G. Li, L. Tam, and J. X. Tang. Amplified effect of brownian motion in bacterial near-surface swimming. *PNAS*, 105:18355–18359, 2008.
- [8] M. A. Vigeant and R. M. Ford. Interactions between motile *Escherichia coli* and glass in media with various ionic strengths, as observed with a three-dimensional-tracking microscope. *Appl. Environ. Microbiol.*, 63:3474–3479, 1997.
- [9] M. A. S. Vigeant, R. M. Ford, M. Wagner, and L. K. Tamm. Reversible and irreversible adhesion of motile *Escherichia coli* cells analyzed by total internal reflection aqueous fluorescence microscopy. *Appl. Environ. Microbiol.*, 68:2794–2801, 2002.
- [10] G. Li, J. Bensson, L. Nisimova, D. Munger, P. Mahautmr, J. X. Tang, M. R. Maxey, and Y. V. Brun. Accumulation of swimming bacteria near a solid surface. *Phys. Rev. E*, 84:041932, 2011.

- [11] S. Bianchi, F. Saglimbeni, and R. Di Leonardo. Holographic imaging reveals the mechanism of wall entrapment in swimming bacteria. *Phys. Rev. X*, 7:011010, 2017.
- [12] P. D. Frymier, R. M. Ford, H. C. Berg, and P. T. Cummings. Three-dimensional tracking of motile bacteria near a solid planar surface. *PNAS*, 92:6195–6199, 1995.
- [13] K. Maeda, Y. Imae, J. Shioi, and F. Oosawa. Effect of temperature on motility and chemotaxis of *Escherichia coli*. *J. Bacteriol.*, 127:1039–1046, 1976.
- [14] E. Lauga, W. R. DiLuzio, G. M. Whitesides, and H. A. Stone. Swimming in circles: motion of bacteria near solid boundaries. *Biophys. J.*, 90:400–412, 2006.
- [15] Q. Liu, C. Zhang, R. Zhang, and J. Yuan. Speed-dependent bacterial surface swimming. *Appl. Environ. Microbiol.*, 90:e00508–24, 2024.
- [16] J. Dunstan, G. Mino, E. Clement, and R. Soto. A two-sphere model for bacteria swimming near solid surfaces. *Phys. Fluids.*, 24, 2012.
- [17] S. Johnson, B. Freedman, and J. X. Tang. Run-and-tumble kinematics of *Enterobacter* sp. SM3. *Phys. Rev. E*, 109:064402, 2024.
- [18] A. De, W. Chen, H. Li, J. R. Wright, R. Lamendella, D. J. Lukin, W. A. Szymczak, K. Sun, L. Kelly, S. Ghosh, et al. Bacterial swimmers enriched during intestinal stress ameliorate damage. *Gastroenterology*, 161:211–224, 2021.
- [19] C. Pawul, T. T. Dutta, S. G. Johnson, and J. X. Tang. Mucin promotes bacterial swarming by making the agar surface more slippery. *Langmuir*, 40:27307–27313, 2024.
- [20] P. N. Rather. Swarmer cell differentiation in *Proteus mirabilis*. *Environ. Microbiol.*, 7:1065–1073, 2005.
- [21] C. Allison and C. Hughes. Bacterial swarming: an example of prokaryotic differentiation and multicellular behaviour. *Sci. Prog.*, pages 403–422, 1991.

- [22] J. D. Partridge, N. T. Q. Nhu, Y. S. Dufour, and R. M. Harshey. Tumble suppression is a conserved feature of swarming motility. *Mbio*, 11:e01189–20, 2020.
- [23] S. Johnson, J. Hall, E. Lushi, and J. X. Tang. SM3 swimming in a circular trajectory near the surface. *Brown Digital Repository*, 2025. doi: <https://doi.org/10.26300/xrrm-7t56>.
- [24] S. Johnson, J. Hall, E. Lushi, and J. X. Tang. SM3 swimming in a circular trajectory near the surface. *Brown Digital Repository*, 2025. doi: <https://doi.org/10.26300/27p5-xs25>.
- [25] S. Johnson, J. Hall, E. Lushi, and J. X. Tang. SM3 swimming in multiple circular loops near the surface. *Brown Digital Repository*, 2025. doi: <https://doi.org/10.26300/aw5y-9408>.
- [26] L. Turner, W. S. Ryu, and H. C. Berg. Real-time imaging of fluorescent flagellar filaments. *J. Bacteriol.*, 182:2793–2801, 2000.
- [27] Z. Zhang, H. Liu, H. Karani, J. Mallen, W. Chen, A. De, S. Mani, and J. X. Tang. *Enterobacter* sp. strain SM1_HS2B manifests transient elongation and swimming motility in liquid medium. *Microbiol. Spectr.*, 10:e02078–21, 2022.
- [28] S. Kamdar, D. Ghosh, W. Lee, M. Tătulea-Codrean, Y. Kim, S. Ghosh, Y. Kim, T. Cheepuru, E. Lauga, S. Lim, et al. Multiflagellarity leads to the size-independent swimming speed of peritrichous bacteria. *PNAS*, 120:e2310952120, 2023.

CHAPTER 5

THREE-DIMENSIONAL DIGITAL HOLOGRAPHIC TRACKING OF BACTERIA

“Every piece of a hologram contains the whole. It’s a striking metaphor for interconnectedness in nature.”

—David Bohm

5.1 Introduction

In nature, bacteria inhabit complex three-dimensional (3D) environments, such as in ponds, lakes, soil, and our stomachs. The physiology and motility patterns of bacteria have evolved to meet the challenges of living in a 3D space. A species of *Desulfobacterota*, a multicellular magnetotactic bacteria, for instance, uses internal compasses to swim along the local magnetic field lines of the earth, guiding it up through the cramped labyrinths of underwater sediment [1]. Therefore, to gain a more accurate picture of bacterial motility, 3D tracking methods are often preferable.

3D imaging has certain advantages over conventional two-dimensional (2D) imaging. In phase-contrast microscopy, for example, bacteria are confined to a thin layer of fluid—tens of micrometers in thickness—and are imaged within a fixed focal plane for a period of time. This 2D slicing approach has the drawback of limiting the number of trajectories obtained for analysis. When a bacterium tumbles, it can leave the focal plane; an event that is more likely to occur for tumbles around 90° . So, on average, bacteria are unlikely to remain in focus long enough to produce long, robust trajectories, reducing the sampling efficiency and requiring more effort to acquire enough data for statistical analysis [2]. In a similar vein, run lengths of considerable spatial length and random orientation within a 2D slice are biased towards shorter instances [2]. Additionally, 2D slicing introduces error in the calculation of certain run-and-tumble parameters. The error introduced for the average run speed is small, $< 2\%$, but measurable; whereas the average run duration has been stated to be off by more than a factor of two [2]. Tumble angle measurements in 2D slicing, however, are quite accurate, as most tumbles occur within the plane of focus, although they are still limited by poor sampling efficiency. Finally, imaging bacteria within a thin fluid layer and between two glass surfaces can introduce unwanted surface effects, such as circling within the proximity of a surface, that must be controlled for, which can also affect sampling efficiency. When imaging bacteria in a 3D bulk sample, surface interactions are negligible. Therefore, 3D imaging offers many improvements over conventional 2D imaging techniques.

The run-and-tumble motility of peritrichous bacteria, such as *E. coli*, has been well studied [3, 4, 5, 6]. Most of the early work on run-and-tumble has been conducted using conventional 2D imaging techniques, with a few notable exceptions [6, 7]. Recently, however, novel imaging techniques have been developed to track cells and particles, which have revealed the limitations of 2D imaging in terms of statistical power and measurement error [2]. Among these developments, digital holographic microscopy (DHM) is currently at the forefront [8, 9, 10, 11, 12]. DHM offers a volumetric, high-throughput method

for tracking the motion of hundreds of bacteria. It works by digitally reconstructing the spatial position of cells within a sample, using the intensity and phase of the light that scatters from bacteria and interferes with unscattered background light [12]. The in-line construction, which consists of a red laser light source, a cuvette, an objective, and a commercial CMOS camera, is simple in design and easy to use by researchers across many disciplines. With the availability of commercial software to perform the nontrivial post-processing of holograms [12], DHM is rapidly becoming an effective means for researchers to track bacteria in 3D.

In this chapter, we report on the run-and-tumble motility of SM3 as measured using 3D DHM. SM3, a newly isolated strain of gut bacteria, lives within the complex 3D environment within the mucus-lined walls of the gut and intestines. SM3 has been shown to reduce symptoms associated with inflammatory bowel disease in mice due to its strong swarming motility, making its behavior of primary interest [13]. Although SM3 has been imaged and tracked extensively in 2D, both as swimmers and swarmers [3], it is yet to be studied how the swimming motility of SM3 manifests in 3D, and the extent to which the increased dimensionality affects motility parameters. We demonstrate that SM3 performs run-and-tumble in 3D with parameters similar to those measured in 2D—such as tumble time, run time, tumble angle, and average speed. We also observe that SM3 demonstrates a steady state chemotactic response to the chemoattractant glucose in 3D. This study aims to be a preliminary characterization of the motility of SM3 in 3D.

5.2 Results

5.2.1 SM3 Performs Run-and-Tumble in Three Dimensions

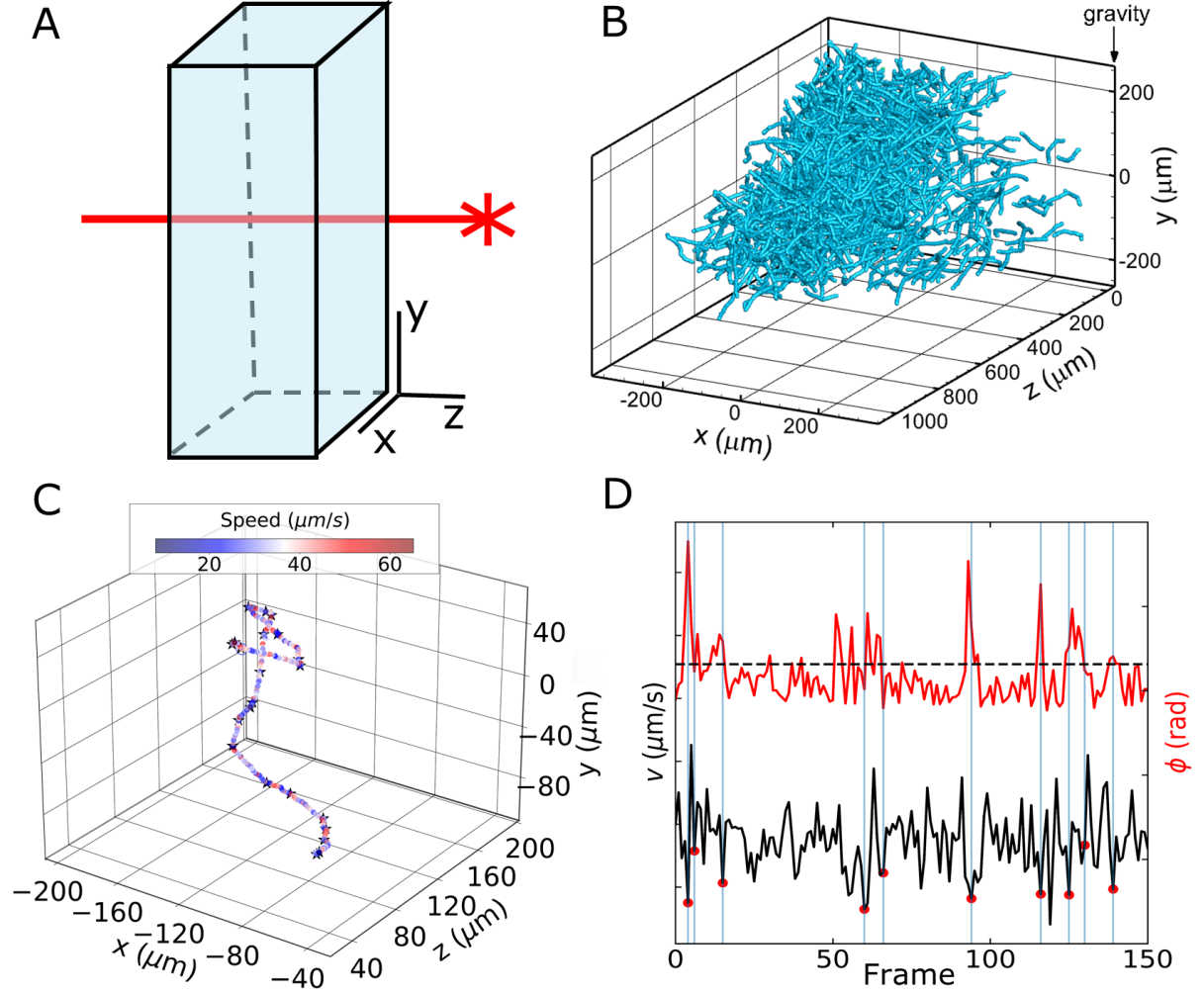


Figure 5.1: (a) Schematic of the sample cuvette with coordinate system. The laser travels along the z -direction, perpendicular to the cuvette’s face, while gravity acts along the y -direction, parallel to the cuvette’s length. (b) Three-dimensional plot of 828 trajectories obtained from a single digital holographic microscopy measurement. The hologram was recorded at 25 FPS for 60 s, resulting in 1500 frames, and was digitally reconstructed. Each trajectory is at least three seconds long. The hologram from which these trajectories were extracted is available at [14]. (c) The longest trajectory among the 828 shown in (b), spanning 352 frames over 14.1 s. The trajectory starts at the end closest to the top of the plot. Black stars denote tumbles detected by the algorithm. (d) Overlay of the instantaneous linear speed and trajectory angle—the angle between successive velocity vectors of the bacterium—on the same plot. Each blue line marks the alignment of a dip in the linear speed with a peak in the trajectory angle, indicating a tumble.

The swimming behavior of planktonic SM3 in 3D was observed using DHM. From a single DHM run we were able to obtain hundreds of trajectories from a suspension of bacteria in MB in a quartz cuvette (Figure 5.1(a) and 5.1(b)). When shown together on the same plot, the trajectories form a tangled mass, making it hard to discern individual trajectories (Figure 5.1(b)). Farther from the focal plane of the magnifying objective, which is positioned at $z = 0 \text{ }\mu\text{m}$ (Figure 2.1 of the Methods), extensive spreading of the interference fringes makes it harder to reconstruct and detect bacteria, resulting in fewer trajectories. The effect of this is seen in the conical tapering of the trajectories in Figure 5.1(b). Still, DHM provided us with a reproducible method for detecting and tracking hundreds of swimming bacteria.

Individual 3D trajectories of SM3 were analyzed using an in-house tracking algorithm, as previously used (see the Methods) [3]. The analysis for the longest trajectory from Figure 5.1(b) is shown in Figures 5.1(c) and 5.1(d). As in 2D [3], SM3 performs run-and-tumble, swimming in straight segments (runs) for periods of time with instances of direction changing events (tumbles) interspersed between runs (Figure 5.1(c)). A plot of linear speed and trajectory angle as a function of frame number (Figure 5.1(d)) shows that events detected as tumbles by the algorithm (black stars in Figure 5.1(c)) correspond to pronounced dips in linear speed and peaks in angular change. The average run of this trajectory is $33.4 \text{ }\mu\text{m/s}$. Bacteria executing a tumble decrease their linear speed by at least 50%. These findings confirm that SM3 performs a robust run-and-tumble motility in 3D.

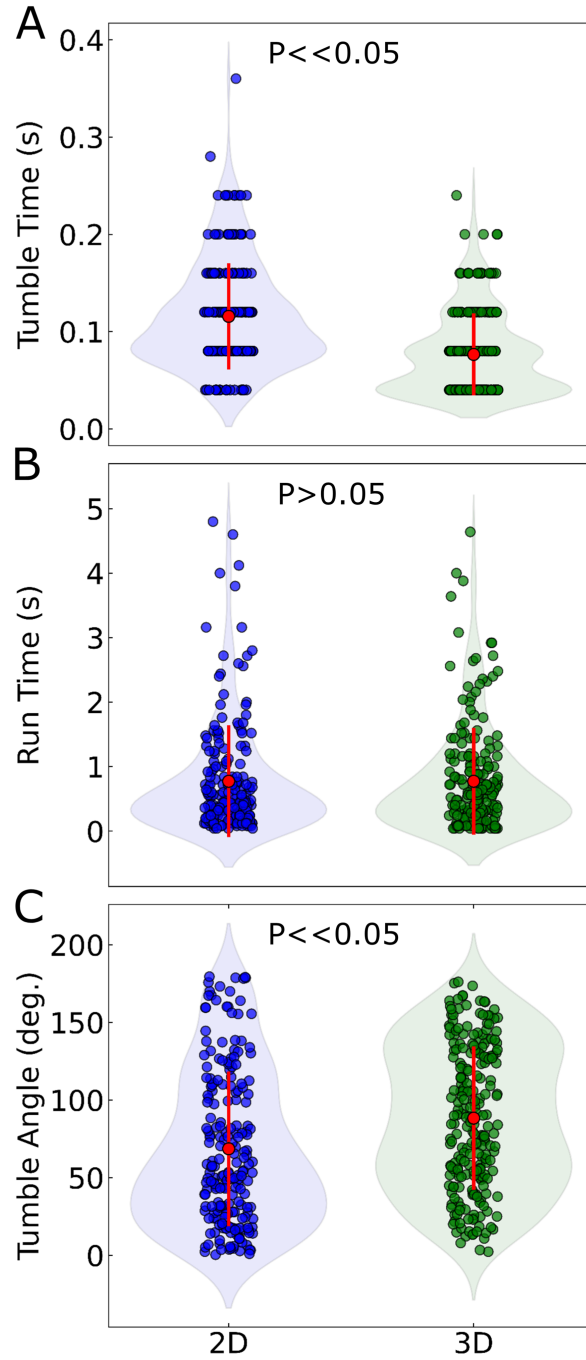


Figure 5.2: Jitter plots for the (a) tumble time, (b) run time, and (c) tumble angle using both two dimensional (2D) and three dimensional (3D) measurement techniques. Corresponding violin plots illustrate the data distributions. The mean is represented by a red data point, and the standard deviation is indicated by a red vertical line in each plot. The p-value, based on a two-sided Welch's t-test, is displayed at the top of each plot. The 2D jitter plots are based on 209, 199, and 209 data points, respectively, and were obtained using phase-contrast microscopy. The 3D jitter plots were based on 232, 207, and 232 data points, respectively, and were obtained using digital holographic microscopy.

5.2.2 Run-and-Tumble Parameters Are Similar in 2D and 3D Experiments

We compared the run-and-tumble parameters obtained by our algorithm for both 2D and 3D tracking methods. Analysis of the trajectories for both methods produced jitter plots of the run-and-tumble parameters (Figures 5.2(a)-5.2(c)). The 2D data comes from our original tracking study for SM3 in motility buffer [3]. The distribution of tumble times (Figure 5.2(a)) is discrete since tumbles were measured in 0.04 s increments as determined by the frame rate. The distribution of the run times (Figure 5.2(b)) and tumble angles (Figure 5.2(c)) are continuous, as they are less affected by the limited frame rate. The measured tumble time in 3D was measured to be slightly ($p < 0.05$) less than in 2D, with an average tumble time of 0.08 ± 0.04 s in 3D compared to an average of 0.12 ± 0.05 s in 2D. Additionally, the average tumble angle was slightly greater ($p < 0.05$) in 3D, the average being $88 \pm 46^\circ$, compared to the previously measured average of $69 \pm 50^\circ$ in 2D. The run time, however, showed no significant difference between the two data sets ($p > 0.05$), and the average value was, coincidentally, identical: 0.77 ± 0.83 s in 3D and 0.77 ± 0.86 s in 2D. Finally, the average linear speed in 3D, 37.1 ± 3.0 $\mu\text{m/s}$, was measured to be slightly higher than the average 30.4 ± 7.5 $\mu\text{m/s}$ measured in 2D. These results mark a first attempt at noting the differences in measured run-and-tumble parameters between the 2D and 3D tracking methods.

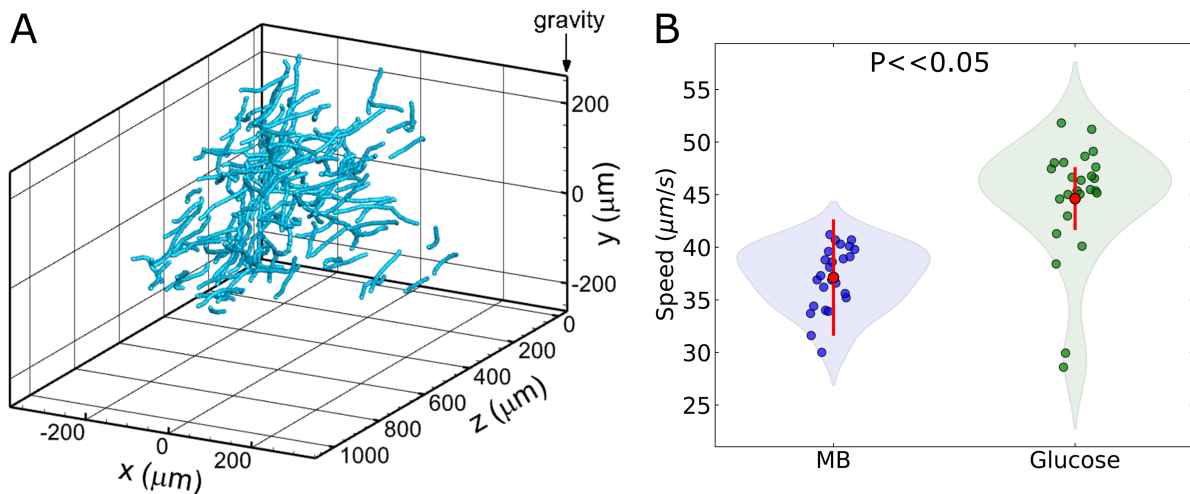


Figure 5.3: (a) Three-dimensional tracks of SM3 exposed to 10 mM glucose. The hologram was recorded at 25 FPS for 60 s, resulting in 1500 frames, and was digitally reconstructed. Each trajectory is at least three seconds long. (b) Jitter plot of the average linear speed for 25 trajectories in both MB and glucose. The mean is represented by a red data point, and the standard deviation is indicated by a red vertical line in each plot. The p-value, based on a two-sided Welch’s t-test, is displayed at the top of the plot.

5.2.3 SM3 Chemotaxes in 3D

We exposed SM3 to a high concentration of glucose to observe its chemotactic behavior. The trajectories of SM3 exposed to 10 mM glucose (Figure 5.3(a)) are noticeably smoother than those in MB (Figure 5.1(b)). The lower number of trajectories in Figure 5.3(a) as compared to Figure 5.1(b) is due to variation in the quality of the recorded holograms, and not because of motility differences. When exposed to high concentrations of glucose, SM3 rarely tumbles, resulting in long and straight runs. Since so few tumble events were measured, a robust statistical analysis of the run-and-tumble parameters was not possible. Instead, we compared the average trajectory speed for 25 trajectories with and without added glucose (Figure 5.3(b)). The average speed of SM3 is 44.6 ± 5.5 μm/s when exposed to 10 mM glucose and 37.1 ± 3.0 μm/s in MB. The statistically significant ($p < 0.5$) increase in average speed, coupled with visual smoothening of trajectories, is evidence of a chemotactic response of SM3 to glucose.

5.3 Discussion

We hypothesize that the differences in parameters are caused by biological variation, and not by the dimension of the method of data acquisition (2D versus 3D). It is known that the tumble time, tumble angle, and average speed can be measured accurately using a 2D slicing approach [2]. Interestingly, the average run times that we measured are nearly identical in 2D and 3D despite others reporting that the run time can be lower than the true value by a factor of two using 2D slicing [2]. If a run lasts for, on average, one second, then one would need a 2D trajectory lasting several seconds to measure an accurate average run time. The trajectories we measured using 2D slicing (Figure 3.2(a)) were tens of seconds long and had several runs. Therefore, it seems wrong to assume that 2D slicing would inaccurately measure the average run speed by such a large margin, and our data comparison verifies this (Figure 5.2(b)). 2D slicing seems to be an acceptable means of measuring run-and-tumble parameters.

It seems more plausible that the differences in the averages could be caused by sample variation. Since the 3D data used to produce the jitter plots in Figure 5.2 were obtained from a single biological replicate, the average could reflect the conditions of that particular experiment more than the true behavior of SM3. The same applies to the 2D data, which was also obtained from a single biological replicate. Further tracking data, both in 2D and 3D, with more biological replicates would discern whether the observed differences are caused by sample variation or from the dimensionality of the measurement technique.

The differences between run-and-tumble parameters also cannot be explained by differences in how the tracking algorithm detected tumbles for the 2D and 3D data. Tumble events were determined based on linear speed and direction change criteria. In our previous work [3], candidate tumble events were first determined as those in which the bacterium slowed down by approximately 40% ($\Delta v/v_{min} > 0.7$). For our 3D analysis, we increased the threshold so that bacteria were required to halve their linear speed ($\Delta v/v_{min} > 1$) for an event to be a candidate tumble. For both studies, the threshold was empirically

chosen as the value that detected events that closely resembled tumbles by eye. We found that changing the threshold value from 0.7 to 0.5 when analyzing the 3D data returned more accurate detections. However, changing the threshold could have reduced the overall number of tumbles detected. This would have the effect of artificially increasing the average run time compared to the results obtained in 2D. However, since the average run times in 2D and 3D were similar (Figure 5.2(b)), the change in threshold is unlikely to have affected the frequency of tumble detections.

To automate the 3D tracking process, we changed how the algorithm applied the direction change criteria. Bacteria run with their long axis parallel to the swimming direction. Therefore, changes in the orientation of the cell body can be directly related to the instantaneous angular change of the trajectory. Previously, in 2D, we required that the instantaneous angular velocity, $\omega = d\theta/dt$, exceed 20% above baseline for an event to be considered a tumble. This was done manually by visualizing the coincidence of dips and peaks in the speed trace of the trajectory (Figure 3.2(d)), which was laborious and inefficient. Also, the baseline of the angular velocity data varies significantly between different trajectories, making it impossible to use a constant threshold when automating the analysis. For our current 3D analysis, we adjusted the algorithm to filter candidate tumbles that passed the speed criterion, based on the angle calculated between successive linear velocity vectors, with each set of velocity vectors having a corresponding angle [15]. If the candidate tumble occurred when the angle exceeded 35° for two sets of velocity vectors, then the event was considered to be a tumble. In instances when the angle exceeded 35° for only one set of velocity vectors, the angle between the vector sum of the two vectors directly before and after was calculated and compared to 35° to reduce false detections from noise in the trajectory (see Methods). The current algorithm has the benefit of being broadly applicable to many different trajectories and, as a result, is more forgiving than our previous criterion, resulting in more detected tumbles. Again, however, it seems that changing the direction change criterion had a limited effect on the

frequency of detected tumbles, as the average run time was measured to be unaffected (Figure 5.2(b)).

Apart from changes in the detection algorithm, the algorithm to calculate the tumble times, run times, tumble angles, and average linear speed remained unchanged. Therefore, we conclude that the observed differences in parameters were not introduced by changes to the algorithm.

The 3D chemotactic response of SM3 to a high concentration of glucose in the steady state produces smooth trajectories with an increased average run speed. This response is qualitatively in agreement with our previous results for serine and aspartate in 2D (Figure 3.4). If we were to perform a dose dependency study, making it possible to measure run and tumble times at lower concentrations of glucose, we would expect to measure a suppression of tumbling, increased average run times, and decreased average tumble angles—as previously measured in 2D. Furthermore, it may be interesting to track SM3 navigating a chemical gradient in 3D, in addition to measuring the steady state response as done here.

In conclusion, we have showed that SM3 performs run-and-tumble while swimming in aqueous media in 3D, which was previously known to be the case in 2D. The run-and-tumble parameters in 3D were found to be slightly different than those measured previously in 2D. However, further studies are required to determine if the differences are due to the limited number of biological replicates studied. When exposed to the chemoattractant glucose at a high concentration, SM3 runs along straighter trajectories and at a higher average speed, which indicates a chemotactic response to glucose. These results mark a first attempt at characterizing and comparing the swimming motility of SM3 using both 2D and 3D imaging techniques. The results of these experiments shall aid in our understanding of the motility of SM3 in complex 3D environments.

References

- [1] A. P. Petroff, V. Kelin, and N. Radchenko-Hannafin. Magnetotactic bacteria optimally navigate natural pore networks. *bioRxiv*, pages 2024–11, 2024.
- [2] K. M. Taute, S. Gude, S. J. Tans, and T. S. Shimizu. High-throughput 3D tracking of bacteria on a standard phase contrast microscope. *Nat. Comm.*, 6:8776, 2015.
- [3] S. Johnson, B. Freedman, and J. X. Tang. Run-and-tumble kinematics of *Enterobacter* sp. SM3. *Phys. Rev. E*, 109:064402, 2024.
- [4] L. Turner, W. S. Ryu, and H. C. Berg. Real-time imaging of fluorescent flagellar filaments. *J. Bacteriol.*, 182:2793–2801, 2000.
- [5] N. C. Darnton, L. Turner, S. Rojevsky, and H. C. Berg. On torque and tumbling in swimming *Escherichia coli*. *J. Bacteriol.*, 189:1756–1764, 2007.
- [6] H.C. Berg and D.A. Brown. Chemotaxis in *Escherichia-Coli* Analyzed by 3-Dimensional Tracking. *Nature*, 239:500–504, 1972.
- [7] P. D. Frymier, R. M. Ford, H. C. Berg, and P. T. Cummings. Three-dimensional tracking of motile bacteria near a solid planar surface. *PNAS*, 92:6195–6199, 1995.
- [8] F. C. Cheong, C. C. Wong, Y. Gao, M. H. Nai, Y. Cui, S. Park, L. J. Kenney, and C. T. Lim. Rapid, high-throughput tracking of bacterial motility in 3D via phase-contrast holographic video microscopy. *Biophys. J.*, 108:1248–1256, 2015.
- [9] A. Wang, R. F. Garmann, and V. N. Manoharan. Tracking *E. coli* runs and tumbles with scattering solutions and digital holographic microscopy. *Opt. Express*, 24: 23719–23725, 2016.
- [10] S. A. Matthews, C. Coelho, E. E. Rodriguez Salas, E. E. Brock, V. J. Hodge, J. A. Walker, and L. G. Wilson. Real-time 3D tracking of swimming microbes using digital holographic microscopy and deep learning. *Plos One*, 19:e0301182, 2024.

- [11] M. Bedrossian, M. El-Kholy, D. Neamati, and J. Nadeau. A machine learning algorithm for identifying and tracking bacteria in three dimensions using digital holographic microscopy. *AIMS Biophys.*, 2018.
- [12] J. Gao, J. Carnahan, G. Alexandre, and J. Trolinger. Digital holographic microscopy for volumetric, three-dimensional characterization of bacterial motility. *Interferometry XXI*, 12223:100–110, 2022.
- [13] A. De, W. Chen, H. Li, J. R. Wright, R. Lamendella, D. J. Lukin, W. A. Szymczak, K. Sun, L. Kelly, S. Ghosh, et al. Bacterial swimmers enriched during intestinal stress ameliorate damage. *Gastroenterology*, 161:211–224, 2021.
- [14] S. Johnson, E. Chery, J. Gao, J. George, and J. X. Tang. A representative hologram. *Brown Digital Repository*, 2025. doi: <https://doi.org/10.26300/pxaj-z079>.
- [15] L. Turner, L. Ping, M. Neubauer, and H.C. Berg. Visualizing flagella while tracking bacteria. *Biophys. J.*, 111:630–639, 2016.

CHAPTER 6

CONCLUDING REMARKS

“A journey of a thousand miles begins with a single step.”

—Lao Tzu

This thesis represents a portion of what I worked on in the three years that I spent in the Tang Lab.

When I joined, little was known about SM3’s swimming behavior apart from its basic physiology, growth rate, and swarming proficiency. My first publication, which makes up the contents of Chapter 3, addressed a gap in our understanding of how this novel species moves. Notably, we confirmed that SM3 swims using a run-and-tumble strategy, closely resembling the motility patterns of well-known bacteria like *E. coli*. A particularly fruitful outcome of this work was its contribution to our physical understanding of tumbling events. While the general mechanism of bacterial tumbling was already established in the literature; to our knowledge, no one had previously described tumbling using a kinematic formulation based on the mechanics of body rotation. By comparing swimming and swarming cells, we found that modeling the cell body as a prolate ellipsoid, with forces and torques exerted on it by unbundled flagella, provided an adequate depiction of the physics involved. Larger bacteria, such as swimmers, experience more drag and turn less for a

given applied torque. One limitation that we encountered frequently was being unable to directly image the flagella of SM3. Future measurements using fluorescence tagging and microscopy to view the flagella during a tumble, with high spatial and temporal resolution, may greatly improve our current tumble model.

In collaboration with an undergraduate student from Tougaloo College in Mississippi and a fluid dynamics expert, Dr. Enkeleida Lushi of the New Jersey Institute of Technology, I led an experiment aimed at determining how cell length influences the kinematics of circular trajectories. Together, we discovered that SM3 swimmers, which can elongate, swim in larger circles as their length increases. Furthermore, we provided quantitative data that could be compared with hydrodynamic simulations. Thus, this study was valuable in advancing our physical understanding of near-surface hydrodynamics. At the time of writing this thesis, we are in the process of drafting a manuscript for publication based on this work. Some future investigations might include modifying the simulation so that the full length of the cell is taken into account, and not just the shape. This could be achieved by incorporating more than just two spheres for the cell body. Also, we would like to eventually use our simulation to model more complicated near-surface swimming behaviors. These include tumbling behavior, as well as the wobbling behavior that is often observed for bacteria swimming near the surface with their flagellar bundle at an off-axis angle to the cell body.

More recently, we have turned our attention to 3D tracking of SM3, and the preliminary results presented in Chapter 5 reflect the progress that we have made so far. We were able to generate high-quality tracks of SM3 and gain an initial understanding of its motility in 3D. In the process, I also trained another undergraduate student to use the system, ensuring that the lab can continue to benefit from this technology. At the current moment, more data needs to be collected to ensure the accuracy and reproducibility of these preliminary findings. However, it is exciting to see where this new research endeavor will lead and how it will open new avenues for exploring bacterial motility. I can imagine

multiple experiments that could be conducted with this system: motility in different flow fields, across gradients, or even in response to various external stimuli. The world is the limit!

Certainly, it is difficult to make a significant contribution to any field in a short period of time. However, I am deeply grateful that—through the supportive leadership of my advisor and the helping hands of my peers—I was able to make a tangible contribution to our understanding of bacterial motility. During my final years here at Brown, I have truly grown to love my research and I hope that for you, dear reader, this thesis serves as an inspiration in developing a deeper appreciation for your own work. Science, at the end of the day, is a team effort—one well worth dedicating a lifetime to.