



SAPIENZA
UNIVERSITÀ DI ROMA

Spatiotemporal control of bacterial turbulence via light-driven activity

Scuola di Dottorato Vito Volterra
Dottorato di Ricerca in Fisica (XXXVIII cycle)

Carlo Giorgetti

ID number 1803511

Advisor

Prof. Roberto Di Leonardo

Academic Year 2025/2026

Thesis defended on 25/05/2026
in front of a Board of Examiners composed by:

Prof. Gabrielli Andrea (chairman)

Prof. Cammarota Chiara

Prof. Cencini Massimo

Spatiotemporal control of bacterial turbulence via light-driven activity

PhD thesis. Sapienza University of Rome

© 2026 Carlo Giorgetti. All rights reserved

This thesis has been typeset by L^AT_EX and the Sapthesis class.

Author's email: carlo.giorgetti98@gmail.com

Abstract

Active matter systems, from bacterial suspensions to cytoskeletal networks, spontaneously develop collective motion fueled by the continuous injection of energy at the single unit scale. In dense suspensions of motile bacteria, this collective motion takes the form of active turbulence: a chaotic, spatiotemporally disordered flow characterized by transient vortices and jets. While the steady-state properties of active turbulence are increasingly well understood, a fundamental question remains open: how does the collective dynamic respond when activity is instead modulated in space and time? Answering this question is essential both for understanding how the characteristic scales of active turbulence are selected by boundary conditions and for uncovering whether dense active systems can encode and retain information about their history. Spatial modulation of activity probes the first aspect, by testing whether permeable boundaries defined by light, rather than solid walls, can still select coherent flow structures. Temporal modulation probes the second, by revealing what happens to the collective state when activity is removed and later restored, in particular, whether the passive suspension preserves orientational information that biases the recovery. Beyond their fundamental interest, answers to these questions open avenues for the design of spatiotemporal protocols that exploit the internal structure of active fluids to steer collective motion.

This thesis addresses this question experimentally, using dense suspensions of photokinetic *Escherichia coli* as a model system. In these bacteria, motility is powered by a light-driven proton pump, enabling precise and reversible switching between active and passive states through illumination. This unique capability allows the exploration of regimes that are inaccessible in systems with fixed activity levels.

In the first part, I demonstrate that spatially structured illumination can produce virtual confinement of active turbulence. Circular illuminated regions embedded in a dark, non-motile background generate coherent vortex dynamics analogous to those observed under physical geometric confinement, but in a fully reconfigurable manner. Virtual confinement is inherently transient: motile bacteria progressively escape the illuminated region, depleting it. Nevertheless, an azimuthal decomposition of the vorticity field reveals a crossover radius of approximately $60\text{ }\mu\text{m}$ below which the confinement selects a coherent rotation and above which bulk turbulent conditions are recovered.

In the second part, I exploit temporal modulation of activity to uncover phenomena with no previously reported counterpart in active matter. By cycling a dense bacterial suspension between active and passive states, I observe directional memory: the flow field after reactivation retains partial correlation with its pre-quench configuration. By systematically varying bacterial density, I show that memory emerges sharply above a threshold density and is linked to structural arrest in the passive state. The nematic overlap function reveals that at high packing fractions, bacterial orientations freeze on experimental timescales, providing a structural template that biases the flow direction upon reactivation. This connection between active turbulence and the physics of glasses shows that dense active systems can

retain and transmit directional information across activity cycles, opening perspectives for the design of temporal protocols that exploit structural memory to steer collective motion.

Contents

1	I propel myself, therefore I am active	1
1.1	Living as <i>E. coli</i> at low Reynolds number	3
1.2	Properties of motile active matter	4
1.2.1	Anomalous pressure and boundary accumulation	4
1.2.2	Control of active matter	5
1.2.3	Light control of active matter	6
1.3	Collective motion in active matter: features and perspectives	8
1.4	Structure of the thesis	12
2	Geometric confinement of collective motion: from physical to virtual	13
2.1	Geometric confinement of collective active matter	13
2.2	Virtual confinement in optically controlled active turbulence	16
2.2.1	Experimental protocol	16
2.2.2	Density dynamics	18
2.2.3	Nematic ordering in structured confinement	20
2.2.4	Velocity dynamics	22
2.2.5	Vortex structure	23
2.3	Conclusions and perspectives	27
3	Temporal analysis of active turbulence	29
3.1	Onset of turbulence	29
3.1.1	Dry continuum modeling	30
3.1.2	Wet continuum modeling	31
3.1.3	Scale selection: theory versus experiment	33
3.1.4	Temporal development of turbulence	34
3.2	Temporal modulation of activity	35
3.2.1	Experimental protocol	35
3.2.2	Active-to-passive transition	37
3.2.3	Passive-to-active transition	37
3.2.4	Directional memory in the velocity field	38
3.3	Density dependence	39
3.3.1	Characterization of the uniform active state	39

3.3.2	Density dependence of the relaxation dynamics	40
3.3.3	Density dependence of aging and memory	40
3.4	Lagrangian analysis of the velocity field	42
3.4.1	Characterization of the uniform turbulent state	42
3.4.2	Lagrangian memory	43
3.5	Structural origin of memory	45
3.5.1	Structural relaxation	45
3.5.2	Nematic alignment in the passive state	46
3.6	Conclusions and perspectives	48
4	Conclusions and perspectives	49
A	Appendix	53
A.1	Experimental equipment	53
A.1.1	Bacterial strain	53
A.1.2	Growth protocol	54
A.1.3	Microscope	55
A.2	Data analysis	57
A.2.1	Particle Image Velocimetry	57
A.2.2	Local orientation extraction	57
A.2.3	Vortex identification	58
A.3	Supplementary plots for temporal modulation observables	58
	Bibliography	63

Chapter 1

I propel myself, therefore I am active

Precisely defining active matter has become increasingly challenging [1]. It is widely accepted that active matter constitutes one of the most vibrant branches at the boundary between physics, engineering, and biology. The protagonists of active matter are active particles, which can be defined by contrast with their counterpart, passive particles. By taking this perspective, we refer to a class of thermodynamically out-of-equilibrium systems. One of the first definitions of active matter mainly concerned the characteristic of being self-propelled. Active systems (biological or synthetic) were defined as active because they were capable of transforming chemical energy from the environment into direct motion [2-5]. Although this definition is now considered reductive because it does not account for various systems, such as proliferation, growth and division dynamics [6], it will be adopted for the purposes of this thesis. The physical scales at which these systems operate span an enormous range, from the nanoscale of individual proteins to the micron scale of cells, up to the meter scale of animal groups—covering nine orders of magnitude in length (Figure 1.1).

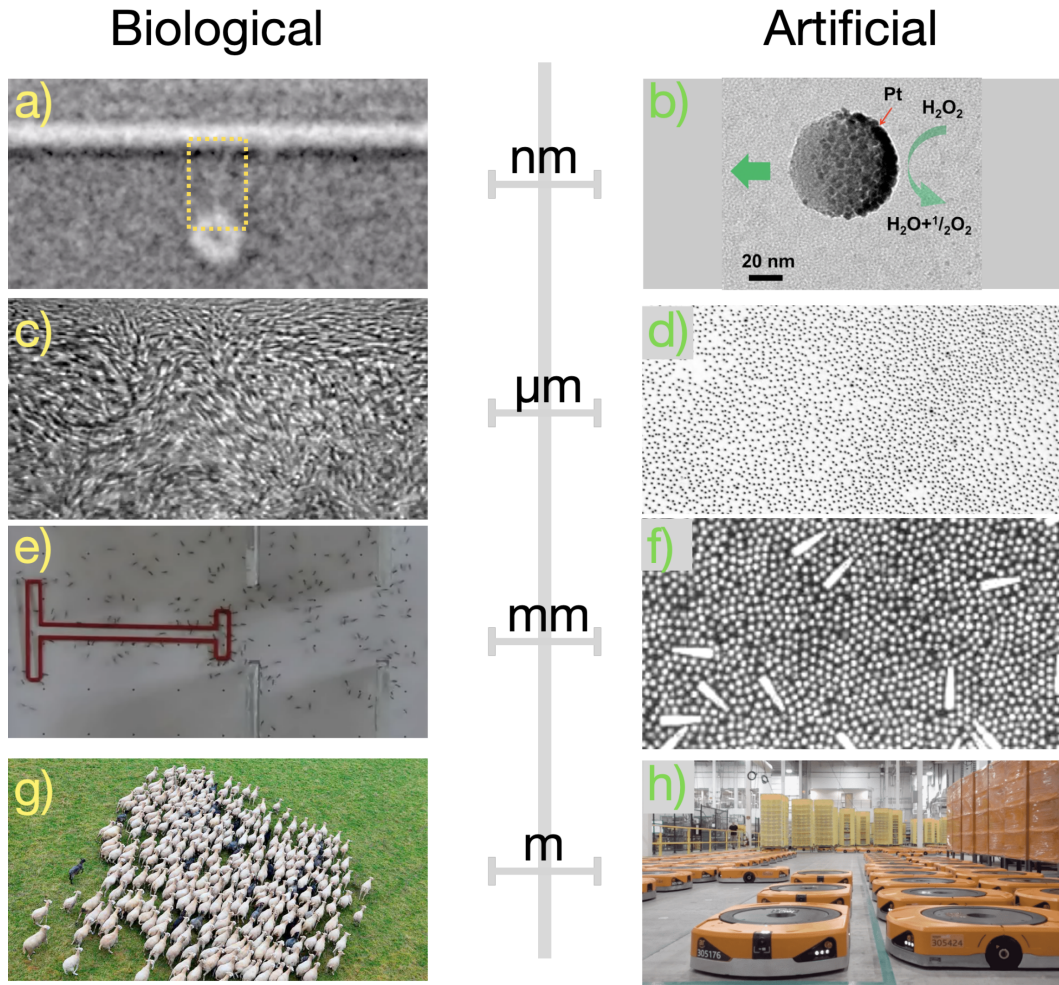


Figure 1.1. Active matter length scales. Active matter spans a wide range of length scales, from the nanometer scale to the meter scale. It encompasses not only biological systems but also artificial ones, including nanobots, Janus particles, Quincke rollers, and robots. Adapted from Refs. [7–11] (ordered from right to left, top to bottom).

1.1 Living as *E. coli* at low Reynolds number

Among all possible examples of active matter, one system deserves a special place. *Escherichia coli* (*E. coli*) is a bacterium with a spherocylindrical shape of approximately 2-4 μm in length and 0.8 μm in width that self-propels by rotating the flagella attached to it in a counter-clockwise direction as viewed from outside the cell [12–15]. This movement is generated by the bacterial flagellar motor, a molecular machinery composed of proteins that converts electrochemical energy into mechanical torque [16–18]. The energy source is the proton motive force (PMF), the electrochemical potential difference maintained across the inner membrane. Protons flowing down this gradient, from the periplasm into the cytoplasm, drive the rotation of the motor. The motion of *E. coli* is not purely ballistic but alternates between straight runs and sudden reorientations called tumbles. A network of chemotaxis proteins, centered on the response regulator CheY, modulates the tumbling rate in response to temporal changes in the local chemical environment: the rate decreases when the bacterium swims toward more favourable concentrations and increases when conditions worsen. This biased random walk enables directed migration up chemical gradients, a process known as chemotaxis. An adaptation mechanism, based on the methylation of membrane-bound receptors, resets the signaling baseline on a timescale of seconds, allowing the bacterium to respond to concentration changes rather than absolute levels [19, 20].

Given the dimensions, timescales, medium properties, and density at which bacteria live, their motion occurs at low Reynolds number [21, 22]. In this regime, inertial effects are negligible and the fluid dynamics are governed by the Stokes equations:

$$\begin{aligned}\eta\nabla^2\mathbf{u} - \nabla p + \mathbf{f} &= 0 \\ \nabla \cdot \mathbf{u} &= 0\end{aligned}\tag{1.1}$$

where $\mathbf{u}$ is the velocity field, p the pressure field (which enforces the incompressibility constraint), η the viscosity of the fluid, and $\mathbf{f}$ the external force density per unit volume acting on the fluid.

By examining the flow field generated by a bacterium, a multipole expansion can be performed. At leading order, since the bacterium is force-free, the flow is described by the gradient of the Stokeslet, the fundamental solution of Eq. (1.1). This is equivalent to describing the swimmer as a force dipole: two equal and opposite forces, one at the head and one at the tail, both pointing outwards from the body. This kind of microswimmer is classified as a *pusher*. The velocity field generated by such a dipole, confirmed experimentally, reads [23] (Figure 1.2):

$$\mathbf{u}(\mathbf{r}) = \frac{p}{8\pi\eta r^2} (3(\hat{\mathbf{e}} \cdot \hat{\mathbf{r}})^2 - 1) \hat{\mathbf{r}}\tag{1.2}$$

where $\mathbf{p} = p\hat{\mathbf{e}}$ is the strength of the dipole moment, proportional to the force magnitude and the distance between the two application points, directed along the axis of the force pair. A pusher expels fluid along its swimming axis and draws it in from the sides. Conversely, if the forces point inward, the swimmer is classified as a *puller*, like for the alga *Chlamydomonas reinhardtii* [24].

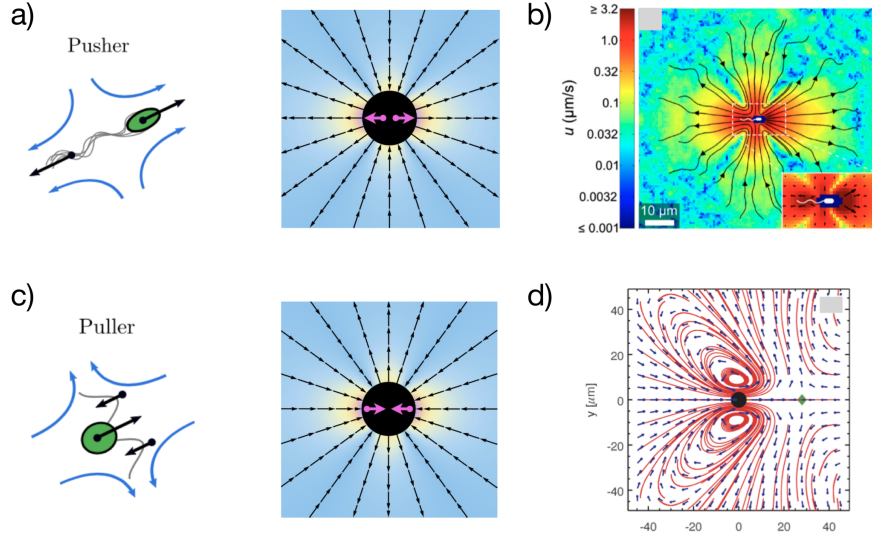


Figure 1.2. Far-field velocity field of microswimmers. a–b) *E. coli*. The measured flow field generated by the microswimmer closely matches the pusher (force dipole) solution, Eq (1.2). c–d) *Chlamydomonas reinhardtii*. The flow field resembles the puller solution of the Stokes equations. Figures adapted from [23, 24].

One of the remarkable features of this low-Reynolds-number world is that the velocity field of the fluid is instantaneously established: it does not propagate but is instead quasi-static. This means that all elements in the fluid at these scales interact with one another constantly through hydrodynamic coupling.

1.2 Properties of motile active matter

1.2.1 Anomalous pressure and boundary accumulation

Being out of equilibrium produces dramatic consequences that cannot be observed in equilibrium systems. A first concept that is fundamentally altered is that of pressure: in active systems the force of self-propulsion contributes to the mechanical pressure, invalidating the existence of an equation of state. In particular, the pressure of an active system depends on the details of the particle–wall interaction, not solely on bulk properties [25]. This stands in stark contrast to equilibrium systems, where the equation of state is a bulk quantity.

Another hallmark effect is the accumulation of active particles at boundaries. Unlike gas molecules at equilibrium, the spatial organization of self-propelled particles can be very sensitive to the boundaries of their container due to their persistent motion [26–28]. An active particle interacting with a wall continues to push against it until rotational diffusion reorients it away, leading to an excess density near surfaces that has no equilibrium analogue.

1.2.2 Control of active matter

Historically, thermodynamics has optimized work extraction from equilibrium systems using machines and control parameters. These systems, however, are strictly bounded by the foundational laws of classical thermodynamics. Inherently out of equilibrium, active systems defy standard thermodynamic constraints, presenting unprecedented opportunities for microscopic control and work extraction.

Geometric control. One instrument that can be used for controlling active particles is geometry. Curvature can play a crucial role in the confinement and rectification of motile microswimmers [29–32]. Several achievements have been obtained in recent years: efficient cargo transport, steady-state spinning of micro-ratchets, and the generation of stationary currents [33–37] (Figure 1.3a-1.3b).

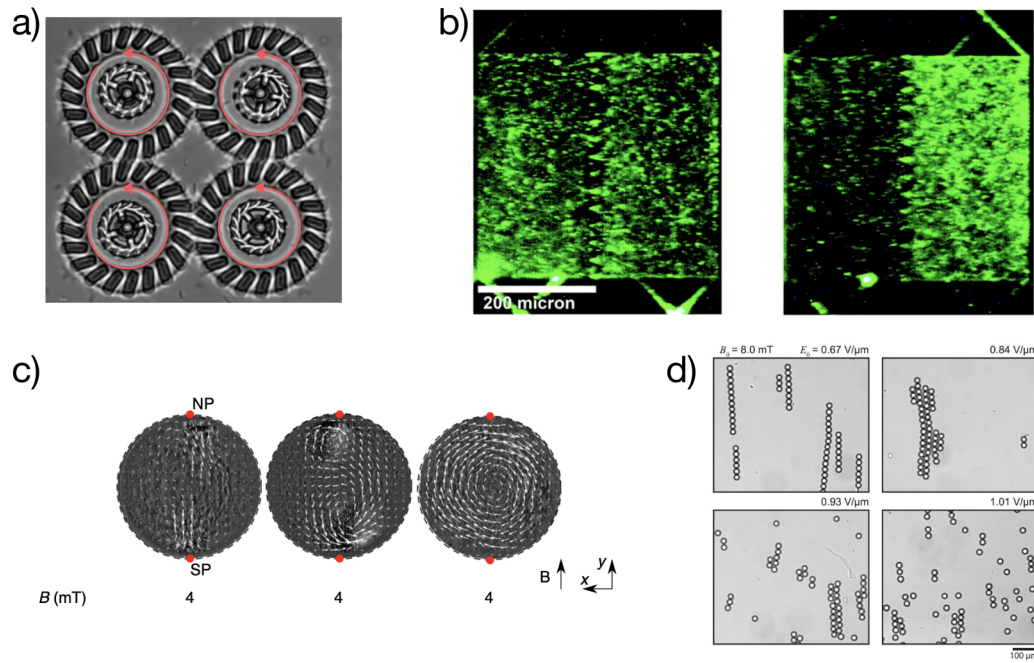


Figure 1.3. Control of active matter. a–b) Geometric control: confinement and rectification using microfabricated structures. The nonequilibrium properties of the system allow the rotation of ratchets and density accumulation by means of curvature. c–d) Electromagnetic control: in droplets of oil, the presence of a magnetic field for magnetotactic bacteria can induce a collective motion. In magnetic Quincke rollers, the presence of a voltage in a constant magnetic field can disrupt the active chain. Adapted from [36–39].

External field control. While geometric confinement provides passive control through spatial constraints, external fields enable *active, dynamic control* by modulating particle behavior in space and time. Fields can be turned on or off, spatially patterned, and temporally modulated, offering a versatility that fixed boundaries cannot match. One example of a controlling field are the electric, magnetic and electromagnetic (light) field. Several microorganisms and engineered colloids are sensitive to magnetic fields [38, 40–43]. Magnetic

control has proven a valuable tool for investigating and directing collective phenomena in active matter [39, 44]. Nevertheless, it lacks spatial selectivity, since the field acts uniformly on all responsive particles within the sample (Figure 1.3c-1.3d).

Light offers perhaps the most versatile external control modality: it can be rapidly switched (on microsecond timescales), precisely patterned in space (with diffraction-limited resolution), modulated in intensity, and delivered non-invasively. These properties make light ideal for both fundamental studies and potential applications.

1.2.3 Light control of active matter

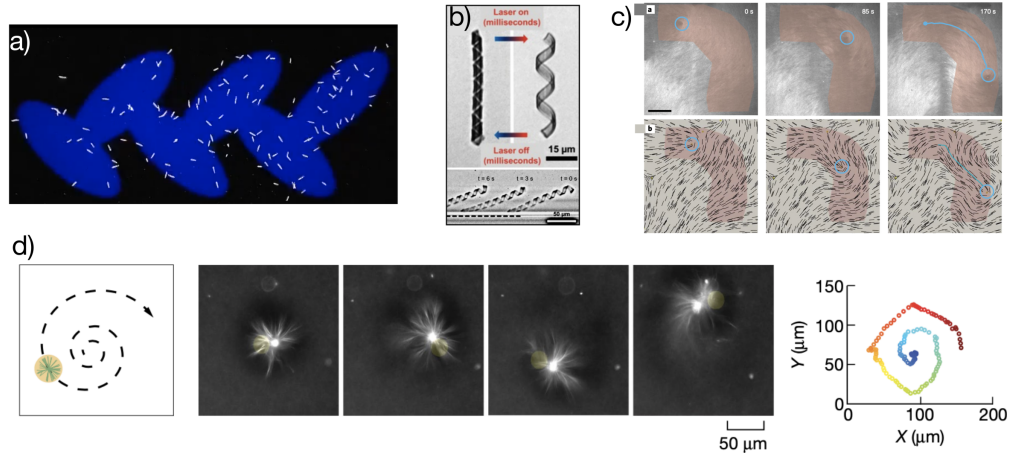


Figure 1.4. Light control of active matter. Examples of light-responsive active systems spanning different material classes and control mechanisms. **a)** Algae can be confined and accumulated using a light confinement. **b)** Light can induce non-reciprocal deformation of rods, inducing a motion. **c–d)** Light can move defects in active nematic either with a static pattern or using a programmable path. Adapted from [45–48].

The key requirement for light-based control of active matter is a mechanism that couples light to the motility of the active particle under investigation [49]. Once such a coupling mechanism is established, light has demonstrated its versatility in controlling diverse active systems, from the confinement of motile microalgae, motion of microbots to the directed transport of topological defects in active nematics [45–48] (Figure 1.4).

Living organisms have evolved diverse strategies to respond to light, including photosynthesis, photosensitive ion channels, and conformational switching. Most relevant to this thesis are light-driven proton pumps such as proteorhodopsin. In the year 2000, a transmembrane protein called proteorhodopsin (PR) was discovered and sequenced [50]. When bound to all-*trans* retinal, PR functions as a proton pump that transports H^+ from the cytoplasm to the extracellular space, against the electrochemical gradient, using energy extracted from incident light. Once the gene sequence was known, it became possible to construct a plasmid encoding PR and transform bacteria using standard molecular biology techniques, creating

photokinetic strains of *E. coli* [51]. In the absence of other sources of energy and oxygen, the bacterium is unable to perform respiration and pump protons out of the cell. Under these conditions, the PMF vanishes and the flagellar motor cannot rotate, rendering the bacterium non-motile. When illuminated with green light, the action of proteorhodopsin restores the PMF and the bacterium resumes swimming. The speed response to light intensity has been measured in several studies and follows a Michaelis–Menten-type relation [52, 53] (Figure 1.5a):

$$v(I) = v_{max} \frac{I}{I + K} \quad (1.3)$$

where K is the intensity at which the speed reaches half of its maximum value, v_{max} is the maximum speed at saturating intensity. The absorption spectrum of PR-expressing bacteria peaks in the green region of the visible spectrum, with a maximum around 520 nm (Figure 1.5a inset). The fact that light can be modulated with high efficiency and temporal precision enables the exploration of a wide range of phenomena. It has been demonstrated that using a spatially structured illumination pattern, bacterial density distributions can be reshaped to match a prescribed target configuration [52, 54, 55], as shown in Figure 1.5b. Bacteria accumulate in regions where they are slower and tend to vacate regions where they are motile. As predicted by theories of non-interacting bacteria [56], the density in the stationary state, in the presence of a non-uniform but time-independent speed profile, is inversely proportional to the speed:

$$\rho(\mathbf{r})v(\mathbf{r}) = \text{const} \quad (1.4)$$

Beyond static patterns, light also enables exploration of the temporal response of motility [57] and the development of dynamic protocols for shaping bacterial distributions. Using a traveling pattern, it is possible to tune the pattern speed to match the characteristic scales of bacterial motion and transport bacteria accordingly [58–60]. A feedback loop can concentrate bacteria in a designated area and direct them like a shepherd guiding a herd [61] (Figure 1.5c).

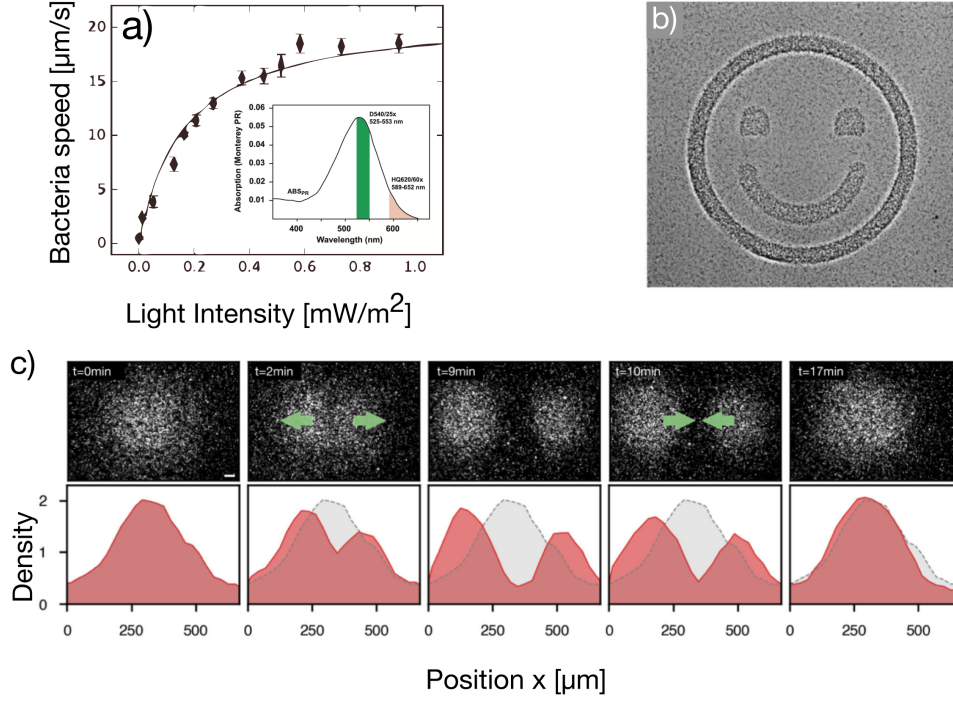


Figure 1.5. Light control of bacteria. a) Proteorhodopsin enables modulation of individual bacterium speed through light absorption, following a relation close to Eq. (1.3). Inset: absorption spectrum. The peak is at 520 nm which corresponds to green in the visible spectrum. b) Using spatial modulation of light, the density distribution can be rearranged so that bacteria accumulate in low-activity zones and create a defined pattern. c) A spatiotemporal modulation allows dynamic reshaping of the density field, accumulating bacteria at the center and moving in a reversible way. Adapted from [51–53, 61].

1.3 Collective motion in active matter: features and perspectives

Up to now, we focused on properties at the level of the single active particle. However, the most fascinating aspect of active matter is the collective behavior that emerges from the interactions of many active particles. This phenomenon is ubiquitous across biological scales and remains a central topic of investigation, precisely because it arises from the intrinsic non-equilibrium nature of these systems.

In some systems, such as flocks of birds, herds of sheep, and schools of fish, a flocking transition is observed in which spontaneous long-range order is established and the group moves collectively along a well-defined direction [62]. This kind of transition is unique to out-of-equilibrium systems and cannot be produced under equilibrium conditions. The first microscopic model describing this phenomenon was formulated by Vicsek [63, 64]. In this model, N self-propelled particles act as continuous spins that tend to align locally with their neighbors. Crucially, unlike equilibrium ferromagnetic systems such as the XY model, these

particles actively move along their orientation vectors. The inherently out-of-equilibrium nature breaks the assumptions of the Mermin-Wagner theorem [65], which forbids continuous symmetry breaking in two-dimensional equilibrium systems, and allows a genuine phase transition in two dimensions [66, 67]. (Figure 1.6a).

In systems interacting solely through repulsive potentials, phase separation cannot occur at equilibrium. However, the persistence of active constituents creates an effective attractive interaction that can drive phase separation between a dilute gas-like state, where particles are isolated, and a dense cluster phase. This phenomenon is known as motility-induced phase separation (MIPS) [68–71] which has been demonstrated experimentally in suspensions of light-activated colloids, Figure 1.6b.

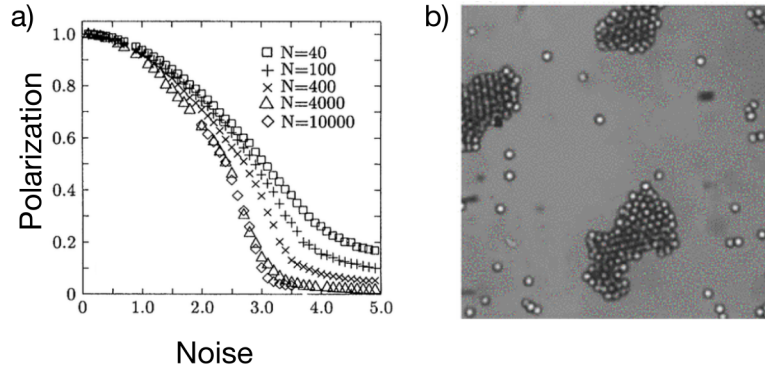


Figure 1.6. Examples of non-equilibrium phase transitions. a) noise-driven transition in the Vicsek model from a disordered state with zero polarization to an ordered state, the flocking state, with finite polarization in a simulation. b) experimental realization of MIPS in a system of light-activated colloids. The solely motility of the system allows the formation of a dense phase of colloids. Adapted from [63, 69].

The collective motion of bacteria displays local alignment (Figure 1.7a), but this order is disrupted at larger scales by chaotic fluid motion arising from hydrodynamic interactions. Bacterial collective flows share with inertial turbulence the visual signature of chaotic, multi-scale vortical motion [72] yet occurs at vanishingly small Reynolds numbers; it is therefore referred to as mesoscale turbulence or *active turbulence* [73, 74]. An analogous phenomenon is also observed in mixtures of microtubules and kinesin motors. In these systems, kinesin motors bind to and walk along microtubules while consuming ATP [75]. The forces exerted by the motors slide neighboring filaments relative to one another, driving both filament motion and fluid flow. The result is a chaotic, turbulent-like state characterized by the proliferation and annihilation of topological defects of charge $\pm 1/2$, with the $+1/2$ defects being motile, as can be observed in Figure 1.8a. To distinguish this nematic-symmetric case from the polar-symmetric bacterial one, this system and its phenomenology are referred to as active nematic turbulence or simply *active nematic* [76].

Although bacterial turbulence and active nematic turbulence share phenomenological similarities with inertial turbulence—both exhibit chaotic flows with vortex structures—they differ in important respects. In inertial turbulence, energy is injected at large scales

and cascades to smaller scales through vortex stretching, until it is dissipated. The 3d power spectrum follows the celebrated $k^{-5/3}$ law predicted by Kolmogorov [72]. In bacterial turbulence, by contrast, energy is injected at the scale of individual swimmers, and finite-size vortices are observed. A hallmark of this is the spatial velocity correlation function, which crosses zero and assumes negative values, indicating a characteristic vortex size (Figure 1.7b). The correlation length of the system however, is defined as the value at which the function reaches $1/e$. This lengthscale is characterized by a maximum of the power spectrum at finite size wavelength. For values bigger than this, an algebraic decay¹ is recorded, with different slope according to the system: for *E. coli* and spermatozoa, the exponent is approximately -3 [77, 78], while for *Bacillus subtilis* it is approximately $-8/3$ [79] as shown in Figure 1.7c. In bacterial suspensions, density acts as a threshold parameter: above a certain onset the vortex correlation length is approximately density-independent, as demonstrated by [80] (Figure 1.7d).

In active nematic systems, universal scaling properties of the power spectrum at large wavenumber emerge with an exponent of -1 , though the exponent can be tuned by experimental conditions [81, 82], Figure 1.8b. Unlike in the bacterial case, active nematic dynamics feature a constant cycle of creation and annihilation of topological defects. For active nematics, both theoretical and experimental investigations have addressed the nature of the transition and its dependence on activity [83, 84] (Figure 1.8c) .

¹Strictly speaking this is not a power law, since the dynamic range in log-log scale is too limited to claim genuine algebraic scaling; we use the term loosely to describe the slope of the low-wavenumber tail

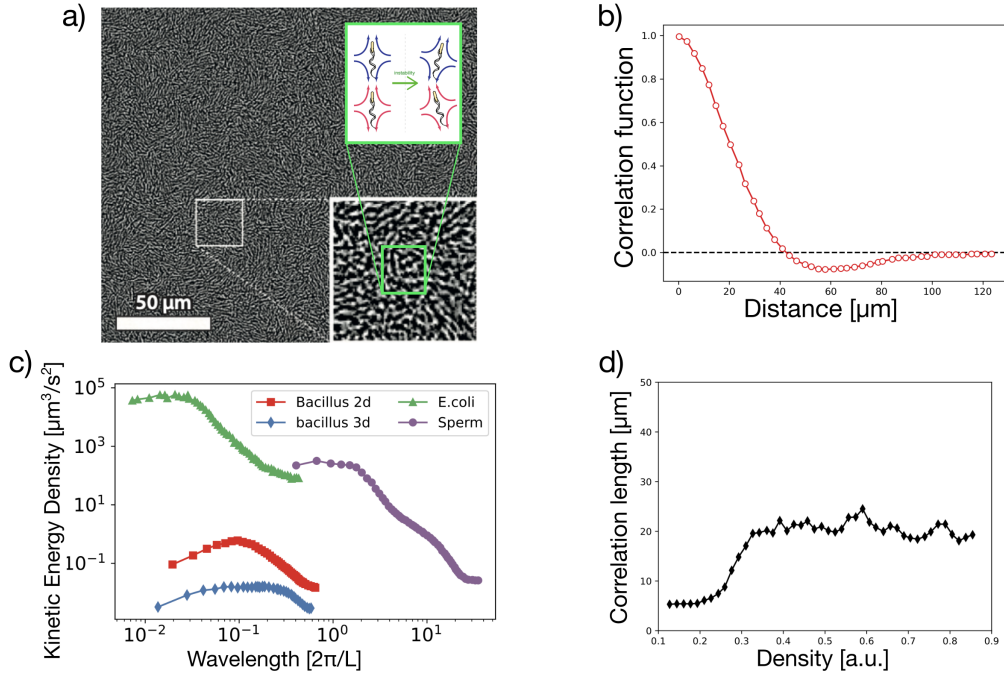


Figure 1.7. Bacterial active turbulence. **a)** Experimental realization of collective motion in a dense suspension of *Bacillus subtilis*. Hydrodynamic interactions destabilize the long range ordering. Adapted from [85, 86]. **b)** Spatial velocity autocorrelation function. It presents negative values, indicating a characteristic vortex size [79]. **c)** Spatial power spectrum of the velocity field for different microorganism species. Despite close phenomenological similarities, no universal scaling exponent is apparent. Data taken from [77–79]. **d)** Correlation length as a function of density. In bacterial suspensions, density plays a threshold role: collective vortices appear only above an onset density, but once formed, the vortex correlation length becomes approximately density-independent. Image from [80].

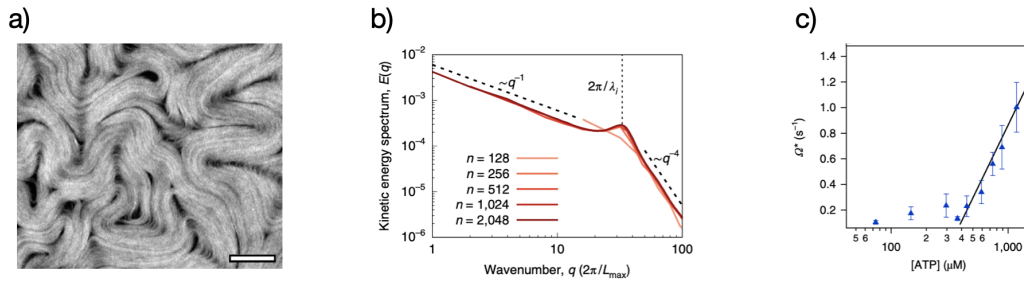


Figure 1.8. Active nematics. **a)** Experimental realization of an active nematic composed of microtubules and kinesin motors (scale bar: 50 μm). Head–tail symmetry and the presence of semi-integer defects (+1/2 and −1/2) are clearly visible. Adapted from [87]. **b)** Spatial power spectrum for a minimal modeling of active nematic. In contrast to bacterial turbulence, a universal scaling at long wavelength with exponent -1 is observed. This exponent is evaluated numerically using large scale simulations. Adapted from [81]. **c)** Growth rate of the instability as a function of ATP concentration, showing a threshold energy input necessary for the onset of active turbulence. Adapted from [88].

1.4 Structure of the thesis

The advances reviewed above demonstrate that light control provides a versatile and precise tool for shaping the behaviour of active particles in the dilute regime, where density distributions can be sculpted and individual trajectories steered. However, the extension of photokinetic control to the dense, collectively moving regime—where hydrodynamic and steric interactions give rise to active turbulence—remains largely unexplored. In this regime, two fundamental questions arise. First, can spatially structured illumination substitute for physical boundaries in selecting coherent flow structures, effectively producing a virtual confinement? Second, what happens to the collective state when activity is removed and subsequently restored—does the turbulent flow recover from a blank slate, or does the passive suspension retain structural information that biases the recovery?

In this thesis, I present experimental results on the control of collective motion in *E. coli* using light, addressing both spatial and temporal modulation of activity.

In **Chapter 2**, I investigate the role of confinement in active turbulence. After reviewing key results on geometric confinement of collective motion, I present experiments on *virtual confinement* achieved through spatial modulation of activity using structured illumination. I demonstrate that circular illuminated regions can transiently confine bacterial vortices, and I characterize the dynamics through density, nematic, and velocity field analysis. An azimuthal decomposition of the vorticity reveals that the confinement efficiency depends on the pattern size, with a crossover radius of approximately $60\text{ }\mu\text{m}$ above which the bulk turbulent state is recovered. I also explore an inverted configuration, where a dark region acts as a permeable obstacle within an active bath, and characterize the stress-induced deformation of the boundary.

In **Chapter 3**, the focus shifts to the temporal response of active turbulence. I first review theoretical models describing the onset of bacterial turbulence and active nematic turbulence, with emphasis on the predictions of linear stability analysis. I then present experiments on temporal modulation of activity, in which the bacterial suspension is driven through cycles of active and passive states by switching the illumination on and off. These experiments reveal that the suspension exhibits directional memory: the flow directions are partially preserved through the dark interval, and the time required to recover the turbulent state grows with the duration of the passive period. By systematically varying bacterial density, I show that this memory effect is linked to structural arrest in the passive state, establishing a connection between active turbulence and glass-like dynamics.

Chapter 2

Geometric confinement of collective motion: from physical to virtual

Abstract

This Chapter addresses the first experimental problem investigated during this PhD research: virtual confinement of collective motion in bacterial suspensions. The problem is motivated by literature on collective motion under geometric physical confinement, where remarkable phenomena emerge from the interplay between activity, hydrodynamics, and boundary geometry. Building on these foundations, we present experiments in which spatial modulation of bacterial activity through structured illumination gives rise to virtual confinement, and we characterize the resulting dynamics through density, nematic order, velocity, and vorticity analyses. The main finding is that the system reproduces the coherent vortex dynamics characteristic of geometric confinement, and an azimuthal decomposition of the vorticity identifies a crossover radius above which bulk turbulent conditions are recovered. However, virtual confinement is inherently transient: density depletion of the active region suppresses collective motion.

2.1 Geometric confinement of collective active matter

At the level of collective motion, geometric confinement profoundly alters the phenomenology, giving rise to effects with no counterpart in the unconfined system. One of the most striking demonstrations of confined active turbulence was reported by Goldstein's lab [89, 90], who studied dense suspensions of *Bacillus subtilis* confined within circular microchambers. As discussed in Chapter 1, unconfined active turbulence is characterized by chaotic flows with a well-defined correlation length. Under circular confinement, the

system is forced to select a single coherent vortex whose size is set by the confining geometry (Figure 2.1a). The mechanism combines hydrodynamic and steric contributions: bacteria arriving at the boundary align with one another and with the wall through direct contact, and the resulting azimuthal current is sustained by hydrodynamic entrainment of the interior fluid. Subsequent work showed that this confined motion decomposes into discrete modes as the confinement diameter is varied [91], and that individual vortices can couple through structured multi-chamber geometries [92].

The extraction of coherent unidirectional flow from a chaotic active bath has also been demonstrated in racetrack geometries, where boundary-driven flows enable persistent bulk transport [93]. This rectification principle has been exploited for the directed transport of asymmetric microstructures [94, 95].

When circular confinements are arranged in periodic lattices connected by narrow channels, even richer collective phenomena emerge. In [96] it was showed that the coupling between confined vortices can produce either *ferromagnetic* ordering (all vortices rotating in the same sense) or *antiferromagnetic* ordering (alternating rotation directions), depending on the channel width and lattice spacing as illustrated in Figure 2.1b. Soft confinement via pillar arrays achieves qualitatively similar effects: periodic obstacle arrays can template vortex lattices with controllable inter-vortex interactions, accessing both ferromagnetic and antiferromagnetic ordering [97, 98] (Figure 2.1c).

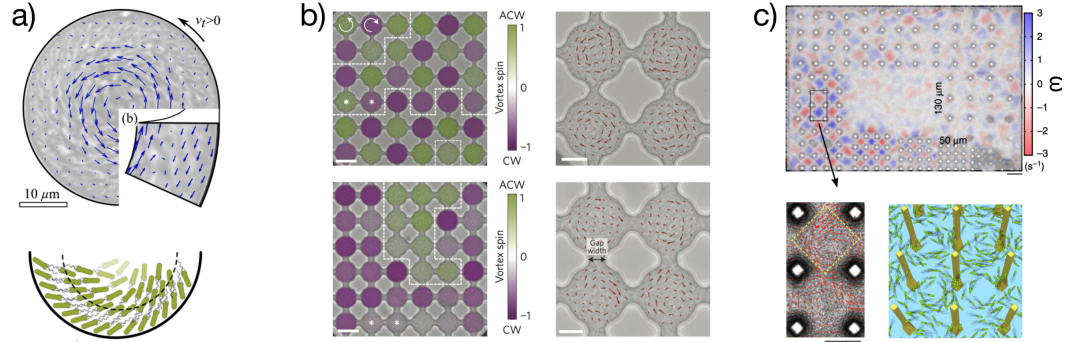


Figure 2.1. Geometric confinement of bacterial active turbulence. a) Dense *Bacillus subtilis* under circular confinement self-organizes into a single coherent vortex. The vortex size is set by the confining geometry rather than by the intrinsic correlation length of the unconfined state. b) Periodic arrays of coupled chambers produce ferromagnetically ordered vortex lattices according to channel width and lattice spacing. c) Soft confinement via pillar arrays templates vortex lattices with tunable ordering, including both ferromagnetic and antiferromagnetic configurations as illustrated by the vorticity map of the bacterial fluid flow field. Adapted from [89, 96, 97].

Active nematic under circular confinement is subject to an additional constraint imposed by the Poincaré–Hopf theorem: the total topological charge enclosed by a boundary must equal the Euler characteristic of the surface, which is $+1$ for a disk and $+2$ for a sphere. As a consequence, a minimum of two unannihilated $+1/2$ defects are always present in a confined active nematic disk, and four in the case of a three-dimensional vesicle [99]. This

topological constraint fundamentally alters the collective dynamics, producing coherent circulating flows as a result of the motility of the $+1/2$ defects [87, 100] (Figure 2.2a). In overlapping toroidal confinements, the active nematic self-organizes into circular flows that spontaneously select a clockwise or counterclockwise chirality [101], Figure 2.2b. Under quasi-one-dimensional confinement, Figure 2.2c, the system undergoes a transition from the bulk active nematic state to a regime dominated by shear instabilities [102]. Recent advances in photopolymerization techniques have enabled the study of active nematics in precisely defined complex geometries [103, 104]. Araki et al. [105] demonstrated that microfabricated triangular pillar arrays can serve as nucleation sites for topological defects, enabling self-assembled flow structures with programmable topology, Figure 2.2d. These experimental findings have been complemented by numerical studies exploring optimal confinement geometries for activity control [106].

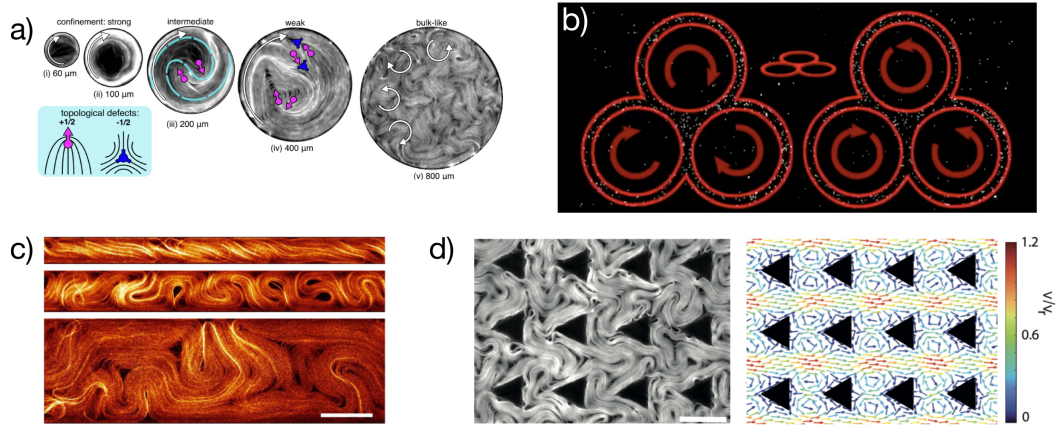


Figure 2.2. Active nematics under geometric confinement. a) Under circular confinement, the Poincaré-Hopf theorem requires the total topological charge to equal $+1$. At small radii, two unannihilated $+1/2$ defects orbit the center; as the radius increases, additional $\pm 1/2$ defect pairs proliferate and the system approaches the bulk turbulent state. b) Flow paths in three partially overlapping toroids. A frustrated or coherent condition can appear. c) Confinement along one direction forces the system from an unstable shear alignment regime to active turbulence regime. Scale bar 100 μm . d) Asymmetric obstacle arrays introduce a spatial rectification mechanism, generating a net flow along the direction of broken symmetry. Scale bar 100 μm . Adapted from [100–102, 104].

2.2 Virtual confinement in optically controlled active turbulence

While geometric confinement is a robust tool for controlling active matter, it is inherently static: boundaries must be fabricated in advance and cannot be reconfigured during an experiment. External fields, and in particular structured illumination, offer a path toward reconfigurable, real-time control. Several realizations of light-based confinement have been demonstrated for microalgae [45] and filamentous bacteria [107].

Here, we address the question of whether virtual confinement can be achieved in the regime of active turbulence, where bacterial motility is modulated by spatially structured light. Taking inspiration from the microfluidic experiments reviewed above, we investigated the effect of circular activity patterns projected onto a dense bacterial suspension. This section is organized as follows: we first describe the experimental setup and methodology, then present the observations and data analysis, focusing first on the density and the orientation, and then on the velocity field and the resulting vortex structures.

2.2.1 Experimental protocol

Detailed information on the bacterial strain, growth protocol and optical setup is provided in Appendix A.1. A smooth-swimming strain, in which the tumbling dynamics has been eliminated by deletion of the *cheY* gene, was used throughout. All experiments were performed at an optical density of 80 OD₆₀₀ (see Appendix A.1.2 for methodology). 3 μ L of sample were loaded into 20-micron thick 4-chamber glass slide (Leja Products B.V., Nieuw-Vennep, The Netherlands¹), sealed with vaseline to prevent oxygen ingress, which would otherwise restore endogenous respiration and compromise the light-controlled motility and imaged by brightfield microscopy. The illumination pattern was projected onto the sample using a digital micromirror device (DMD) coupled with the objective. The DMD was calibrated using a fluorescent layer, from which a homography transformation from DMD to camera coordinates was determined, enabling precise localization of the activity mask. The size of one pixel of the DMD is 0.38 μ m. Acquisitions were performed at 30 fps for a total duration of 3 min. Before each acquisition, the sample is illuminated uniformly from above, reshuffling the suspension and restoring a spatially homogeneous density. The uniform light is then removed, and after a waiting period of 30 s the structured illumination pattern is projected from below via the DMD.

Images were preprocessed according to the following pipeline (Figure 2.3). Each frame was first normalized by the temporal mean of the background intensity, which removes slow illumination fluctuations. A static background image was then subtracted to correct for contamination from dust in the optical path, after which the resulting image was divided by a low-pass filtered background to compensate for spatial non-uniformity of the illumination. Eventually, each frame was normalized to the interval [0, 1].

¹Specifics are reported on the website of the product [here](#)

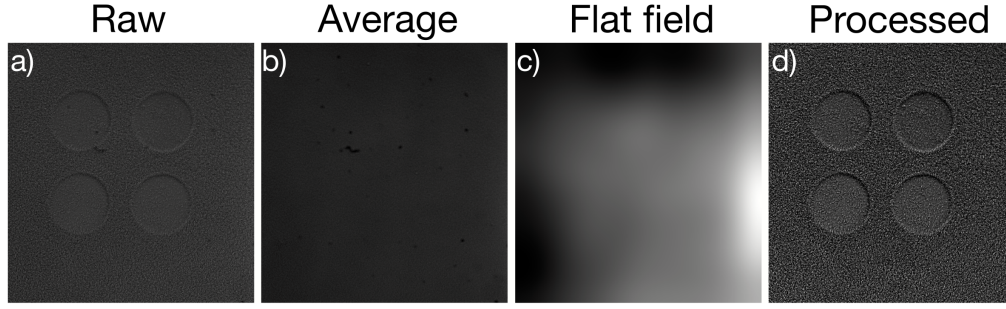


Figure 2.3. Image preprocessing pipeline. a) Raw brightfield image. b) Static background used for subtraction. c) Flat-field correction. d) Normalized to $[0,1]$ processed image. The procedure better highlights features of the system.

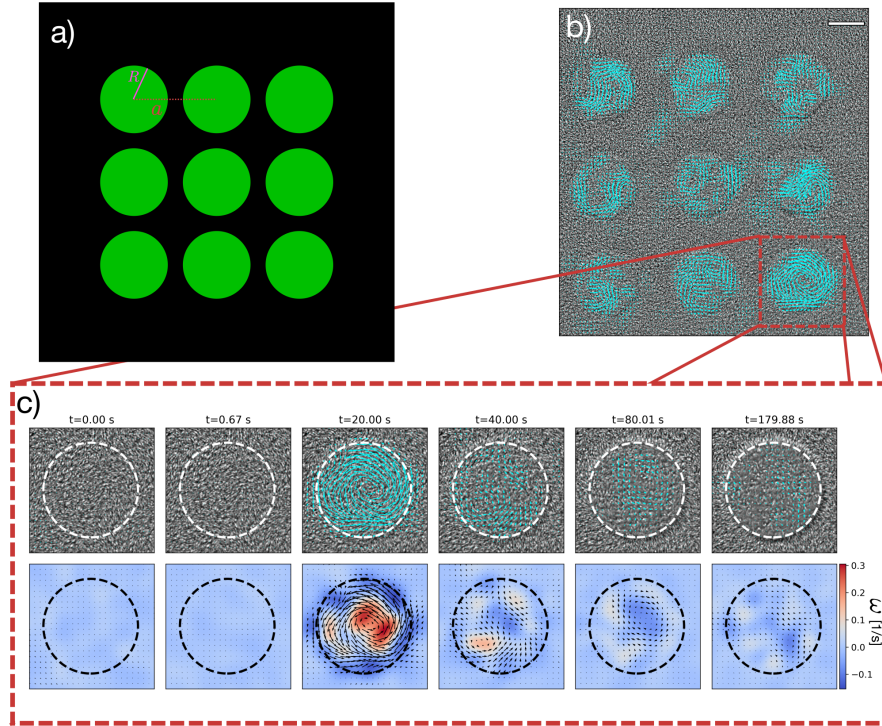


Figure 2.4. Virtual confinement of bacterial active turbulence. a–b) The illumination pattern consists of a square-lattice array of circular spots of radius R , projected with lattice constant $a > 3R$. The spatial modulation of light intensity correctly maps to a spatial modulation of bacterial activity, selectively activating the illuminated regions. Scale bar: $50\mu\text{m}$ c) Time evolution of the system following pattern onset for $R = 45.30\mu\text{m}$. Initially, the suspension is quiescent and bacteria are uniformly distributed. Upon illumination, bacteria respond and collective motion develops within the active spots. After reaching a transient maximum, the illuminated area becomes progressively depleted of motile bacteria and collective motion is suppressed.

The illumination pattern consists of a square-lattice array of circular spots of radius R and lattice constant a , Figure 2.4a, with each spot illuminated at an intensity $I = 0.28 \text{ mW/mm}^2$ and the surrounding region kept in darkness. All experiments presented in this section were performed with $a > 3R$, ensuring that individual spots can be treated as independent as observed from the velocity map in Figure 2.4b: preliminary observations showed that for $a \lesssim 2R$, the dark inter-spot regions are activated by mechanical stress transmitted through the bacterial fluid.

For each experiment, a uniformly dense region was selected and data acquisition was initiated simultaneously with pattern projection. The phenomenology is illustrated in Figure 2.4c. Initially, the suspension is in a quiescent state with bacteria uniformly distributed across the field of view. Upon illumination, bacteria within each spot respond to the light stimulus by recovering motility, leading to the development of collective motion. Over time, motile bacteria preferentially escape the active region into the surrounding dark area, progressively depleting the illuminated spots of active cells. Eventually, only non-motile bacteria remain inside the illuminated regions, and collective motion is extinguished.

2.2.2 Density dynamics

As a first characterization, we focus on the depletion dynamics. Since images are acquired in brightfield, direct access to the density field is not possible; however, the local variance of image intensity provides a proxy for it [108].

$$\rho(r, t) = \left\langle (I(\mathbf{r}, t) - \langle I(\mathbf{r}, t) \rangle)^2 \right\rangle_{|\mathbf{r}|=r} \quad (2.1)$$

Where the average is performed in the illuminated spot radially with respect to the center. Figure 2.5a shows the time evolution of the azimuthally averaged variance profile, normalized by the pre-illumination baseline.

At early times, the variance profile is spatially flat, consistent with a homogeneous suspension. As collective motion develops and bacteria begin to escape the illuminated region, the variance inside the spot decreases while a halo of elevated density builds up just outside the boundary. The rate of depletion is quantified by extracting the spatially averaged variance inside each spot as a function of time for different confinement radii as shown in Figure 2.5b. In all cases, a delay of approximately 5 s precedes any measurable response, corresponding to the time required for bacteria to begin swimming and for the collective motion to develop. The subsequent decay is well described by a single exponential for small radii, where the density reaches a plateau within the observation time window. As can be observed in Figure 2.5c, for larger radii, the dynamics is significantly slower and the plateau is not reached, indicating that larger active regions contain a greater absolute number of slowly-responding or non-motile bacteria.

The stationary radial profile of the variance, Figure 2.5d was fitted with a sigmoidal function:

$$\rho(r) = \rho_0 \frac{1}{1 + e^{-(r-\hat{r})/r_b}} + \rho_1 \quad (2.2)$$

to characterize the spatial extent of the transition region. The stationary profile is evaluated by averaging the profile in the last 10 s. The broadening of the profile, r_b , increases approximately linearly with radius for $R > 90 \mu\text{m}$, with a slope of $0.25(3)$, while it remains approximately constant for smaller radii, Figure 2.5e.

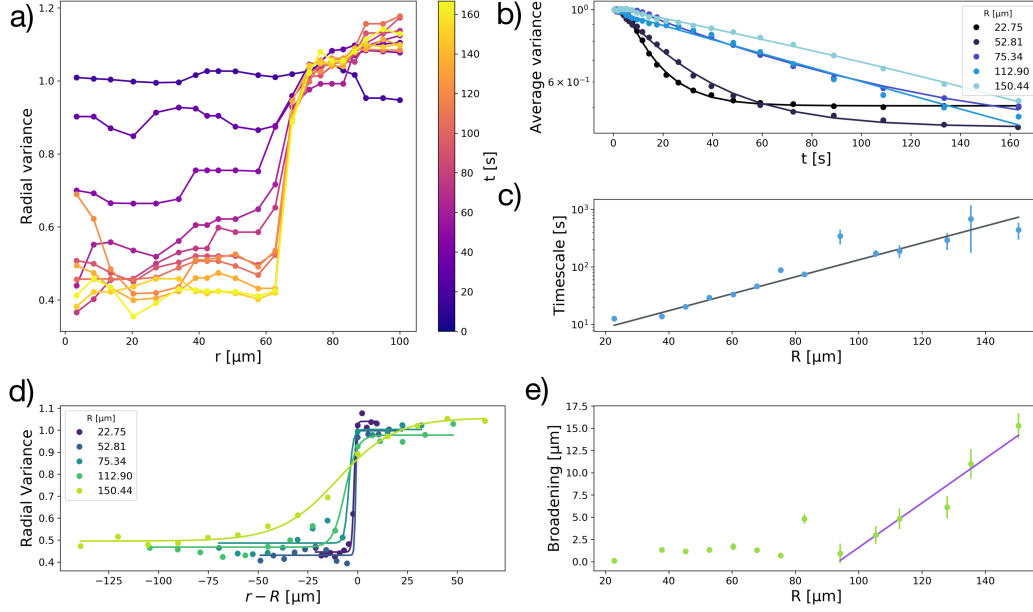


Figure 2.5. Density dynamics of virtual confinement. **a)** Temporal evolution of the azimuthally averaged, Eq. (2.1), normalized variance profile for $R = 45.30 \mu\text{m}$. At the beginning the profile is flat, mirroring the stationary condition. When the suspension starts reacting to light, the system empties inside and density increases outside. Eventually the profile reaches a stationary condition. **b–c)** Temporal dynamics of the spatially averaged variance inside the illuminated spot, for confinement radii ranging from small (fast depletion) to large (slow depletion). All curves show a delay of approximately 5 s before the onset of depletion, followed by an approximately exponential decay. The depletion timescale grows exponentially with R . **d–e)** Stationary radial variance profiles for different confinement radii. The continuous line represents a sigmoidal fit, Eq. (2.2). The broadening width, parameter r_b in Eq (2.2), remains approximately constant for $R \lesssim 90 \mu\text{m}$ and grows linearly for larger radii.

2.2.3 Nematic ordering in structured confinement

Processed images reveal a pronounced tendency for bacteria to align along radial directions relative to the center of each spot, particularly in the vicinity of the boundary. To quantify this structural anisotropy, the orientational field was extracted from the images using the gradient-based nematic tensor algorithm described in Appendix A.2.2. The analysis was performed on squared interrogation windows of size $5.2\,\mu\text{m}$ with 50% overlap, yielding a coarse-grained director field $\hat{\mathbf{n}}(\mathbf{r}, t)$.

The degree of radial alignment was quantified through the azimuthally averaged radial order parameter:

$$S(r, t) = \langle \cos(2\Delta\theta(\mathbf{r}, t)) \rangle_{|\mathbf{r}|=r} \quad (2.3)$$

where $\Delta\theta$ is the angle between the local nematic director and the radial direction from the spot center (see Figure 2.6a). The factor of 2 accounts for the head-tail symmetry of the nematic field. The average is performed azimuthally at fixed distance from the spot center and, where multiple experimental realizations are available, also across realizations.

At early times, $S(r, t)$ is spatially uniform and close to zero, indicating the absence of any preferred orientation (Figure 2.6b). As the system evolves, a pronounced positive peak develops near the boundary of the illuminated region, with values approaching $S \approx 1$, indicating strong radial alignment of bacteria escaping the spot. At intermediate times (Figure 2.6c), reports an example of the dynamics, a region of negative S is also observed slightly inside the boundary, which is a signature of bacteria swimming tangentially—consistent with the formation of a coherent vortex structure. This tangential signal subsequently vanishes as the interior becomes depleted, leaving only the radial alignment peak at the boundary.

Analysis of the stationary profiles for different confinement radii, Figure 2.6d reveals two systematic trends. First, a peak close to $S = 1$ is observed just outside the boundary for all radii investigated, indicating that the escape dynamics at the boundary are robust and size-independent. Second, for larger radii, a region of negative S persists inside the spot even at late times, signaling that a fraction of bacteria continues to swim tangentially and that depletion is not yet complete within the observation window.

It is noteworthy that these observations differ qualitatively from the observations done in [89, 90]. In fact, both experimentally and in simulations, the angle between the boundary and the cell's body is finite and different from 0. This difference is justified by the kind of boundaries: rigid boundaries impose a constraint in the orientation of single bacteria, instead the confinement induced by the presence of other bacteria makes the boundaries permeable (soft) and can allow this high alignment.

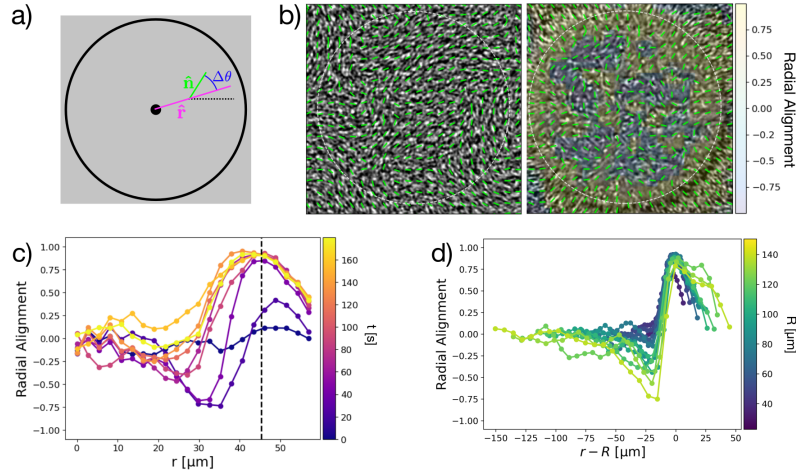


Figure 2.6. Nematic ordering in virtual confinement. **a)** Schematic definition of the angle $\Delta\theta$ between the local nematic director and the radial direction from the spot center, used to construct the radial order parameter S , Eq. (2.3). **b)** Snapshots of the bacterial director field overlaid on preprocessed images at $t = 0$ (disordered, $S \approx 0$, left) and after 3 min of illumination (strongly radially aligned, $S \approx 1$, right), for $R = 45.3 \mu\text{m}$. The director field is represented with lines, to underline the head-tail symmetry of the field. **c)** Time evolution of the azimuthally averaged radial order parameter $S(r, t)$. At early times, S is flat and close to zero. A positive peak develops near the boundary as bacteria escape radially, with a transient region of negative S at interior radii signaling tangential (vortical) swimming. The tangential signal disappears at late times as the interior is depleted. **d)** Stationary radial profiles of S for different confinement radii. A peak near $S = 1$ at the boundary is observed for all sizes. Negative values inside the boundary persist for larger radii, indicating continued tangential bacterial motion and incomplete depletion.

2.2.4 Velocity dynamics

The velocity field was extracted using Particle Image Velocimetry (PIV; see Appendix A.2.1) with $5.2\text{ }\mu\text{m}$ interrogation windows and 50% overlap, matching the spatial resolution of the nematic analysis. The bacteria themselves serve as tracer particles for the velocity field measurement. The velocity field is then filtered with a gaussian filter both in time ($\sigma_t = 0.03\text{ s}$) and in space ($\sigma_s = 10.4\text{ }\mu\text{m}$).

We first characterize the time evolution of the spatially averaged flow speed inside the illuminated region, $m(t) = \langle |\mathbf{u}| \rangle_{\text{inside}}$. As shown in Figure 2.7a, $m(t)$ rises from zero, reaches a maximum, and subsequently decays back to near-zero values, with the decay phase substantially slower than the rise. This non-monotonic behavior reflects the competition between the buildup of collective motion and the progressive depletion of active bacteria from the illuminated region.

The asymmetric temporal profile of $m(t)$ is well captured by the following asymmetric function:

$$m(t) = \begin{cases} m_{\max} \exp \left[-\frac{(\log(t) - \mu)^2}{2\sigma_1^2} \right] + c & t < e^\mu \\ m_{\max} \exp \left[-\frac{(\log(t) - \mu)^2}{2\sigma_2^2} \right] + c & t \geq e^\mu \end{cases} \quad (2.4)$$

The maximum speed increases monotonically with confinement radius, Figure 2.7b, following a saturation law:

$$m_{\max}(R) = (m_\infty - m_0) \frac{R}{R + R_0} + m_0 \quad (2.5)$$

This behavior is consistent with the expectation that in a larger active area, collective motion can develop more fully before depletion suppresses it, while in smaller spots the depletion dynamics outcompetes the growth of turbulent correlations. The number of independent realisations available at each radius ranges from up to nine at the smallest values of R down to a single realisation at the largest radii ($R \gtrsim 100\text{ }\mu\text{m}$). A direct estimate of the experiment-to-experiment variability of $m_{\max}$ is therefore not available in the large- R regime. However, since at large radii the active region exceeds the intrinsic vortex size and the flow inside the spot becomes statistically equivalent to bulk active turbulence (cf. Sec. ?? and Fig. 2.8), a natural proxy for the variability is provided by the temporal fluctuations of the speed measured under uniform illumination at maximum intensity. In that reference experiment the speed has mean $11.32(1)\text{ }\mu\text{m s}^{-1}$ and standard deviation $5.91(1)\text{ }\mu\text{m s}^{-1}$. The non-monotonic ordering of $m_{\max}$ between adjacent confinement radii (e.g. $R = 52$ vs. $R = 75\text{ }\mu\text{m}$, or $R = 112$ vs. $R = 150\text{ }\mu\text{m}$) is comparable to or smaller than this scale, which suggests that it is not a systematic deviation from the saturation law of Eq. (2.5) but rather a manifestation of the intrinsic temporal variability of the bulk turbulent state. Three characteristic timescales are extracted from the fit: the peak time $t_m = e^\mu$, Figure 2.7c, the rising time $\tau_1 = t_m (1 - e^{-\sigma_1})$ (characterizing the onset of collective motion), and the decay time $\tau_2 = t_m (e^{\sigma_2} - 1)$, Figure 2.7d. Notably, the ratio τ_2/τ_1 is approximately constant across all confinement radii (the inset of Figure 2.7d shows a linear dependence between the two timescales).

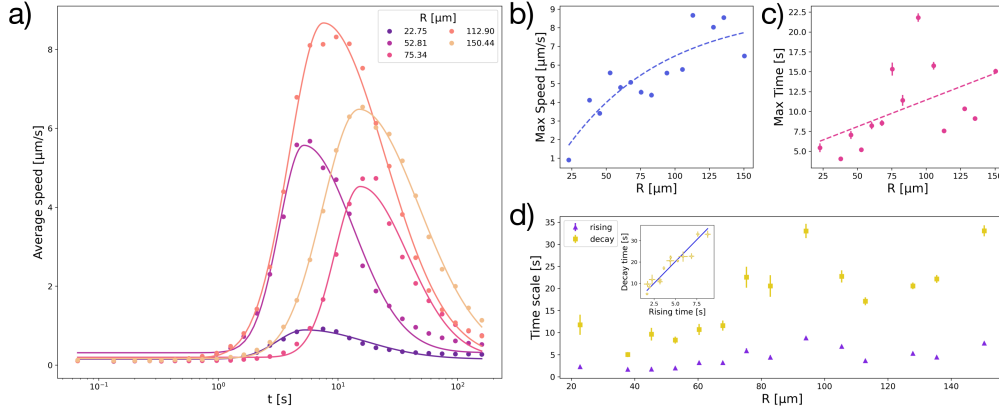


Figure 2.7. Velocity dynamics inside the confined active regions. **a)** Representative time evolution of the spatially averaged speed inside the illuminated region, with the asymmetric log-normal fit of Eq. (2.4) superimposed as a continuous curve. The non-monotonic profile reflects the competition between turbulence buildup and density depletion. **b–d)** Fit parameters as a function of confinement radius R : the maximum speed, the time to peak t_m and the rising, τ_1 and decay, τ_2 timescales all increase with R . Inset of **d**: the ratio τ_1/τ_2 is approximately constant across radii. The maximum speed increases monotonically with R , saturating at large radii.

2.2.5 Vortex structure

The analysis of the spatially averaged speed quantifies the overall energy of the flow but does not resolve its spatial organization. We therefore performed a structural analysis of the vortex field using the Okubo–Weiss criterion (see Appendix A.2.3). Vortices were identified by thresholding the Okubo–Weiss parameter at 30% of its standard deviation in the frame of maximum speed, followed by an erosion–dilation morphological filter to suppress noise and a connected-component algorithm to isolate individual vortices.

Representative vorticity maps and the corresponding Okubo–Weiss thresholding are shown in Figure 2.8a. As expected, the averaged number of vortices present simultaneously increases with the confinement area. For each radius, the temporal evolution of both the number of vortices and their average area mirrors the speed dynamics, following an approximately log-normal profile. The maximum number of vortices scales quadratically with the confinement radius (equivalently, linearly with the confinement area) as observed in Figure 2.8c, consistent with a bulk turbulent state in which vortices tile the available space without a preferred arrangement.

By contrast, the average vortex area is approximately independent of the confinement size, with a mean value of $581(14) \mu\text{m}^2$, Figure 2.8d. This value is compatible with the square of the zero-crossing of the spatial velocity autocorrelation function in the unconfined state ($\xi_0^2 = 594(26) \mu\text{m}^2$). For spot radii smaller than the intrinsic vortex size, the average vortex area is reduced, suggesting that the confining geometry constrains its size.

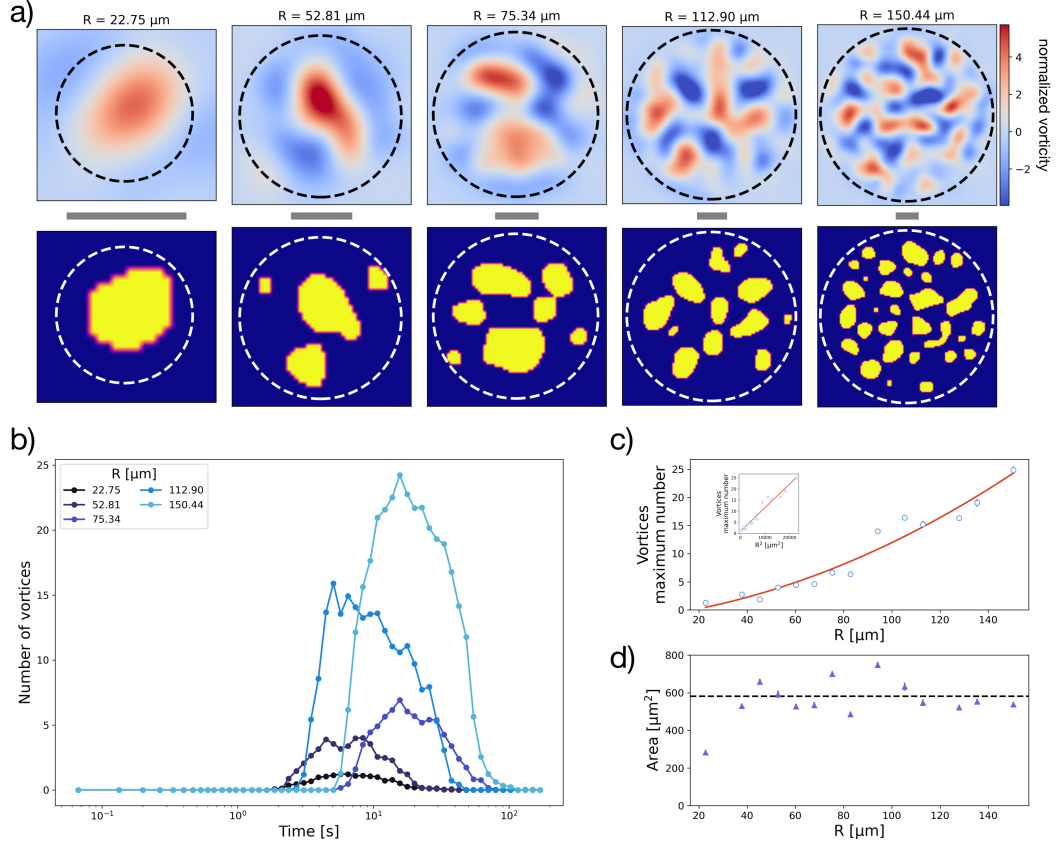


Figure 2.8. Vortex statistics inside the confined active regions. **a)** Representative vorticity maps normalized to vortex detection threshold (top) and the associated thresholded Okubo-Weiss parameter maps (bottom) for different confinement radii. The number of simultaneously present vortices increases with the available area. Scale bar: 40 μm . **b)** Temporal evolution of the averaged vortex count, exhibiting a log-normal profile analogous to the speed dynamics. **c)** Maximum vortex count as a function of R : the scaling is quadratic in R (equivalently linear in the area, inset), as expected for a space-filling bulk turbulent state. **d)** Average vortex area as a function of R : the area is approximately constant across confinement sizes and agrees with the squared zero-crossing length ξ_0^2 of the unconfined velocity autocorrelation function (dashed black line), confirming that the vortex size is an intrinsic property of the turbulent state.

Azimuthal decomposition of vorticity

To characterize the spatial structure of the flow more systematically, we performed an azimuthal mode decomposition of the vorticity field. The circular symmetry of the confinement makes this a natural basis for the problem, and analogous decompositions have been employed in the study of geometrically confined bacterial and active nematic systems [91, 98].

In polar coordinates (r, θ) centered on each spot, the vorticity field is decomposed as:

$$\omega(r, \theta, t) = \sum_{n=-\infty}^{\infty} \omega_n(r, t) e^{in\theta} \quad (2.6)$$

The complex coefficients are linked by the realness of the vorticity field, $\omega_{-n} = \omega_n^*$. Each coefficient is extracted as:

$$\omega_n(r, t) = \frac{1}{2\pi} \int_0^{2\pi} \omega(r, \theta, t) e^{-in\theta} d\theta \quad (2.7)$$

The mode expansion retains the full radial and temporal dependence of each coefficient, in contrast to the radial-basis approach of [91]. By Parseval's theorem, the spatially average enstrophy $\Omega(t) = 1/A \int_A |\omega|^2 d^2r$ decomposes as:

$$\Omega = \frac{2}{R^2} \int_0^R \left(|\omega_0|^2 + 2 \sum_{n=1}^{\infty} |\omega_n|^2 \right) r dr \equiv \Omega_0 + 2 \sum_{n=1}^{\infty} \Omega_n \quad (2.8)$$

where Ω_n is the mean squared contribution of the n -th mode to the enstrophy.

For the numerical evaluation, the vorticity field at each frame was interpolated onto a polar grid and Eq. (2.7) was applied. Figure 2.9a shows reconstructions of representative configurations using the first fourth modes. The $n = 0$ mode corresponds to net rotation about the spot center, while higher modes describe increasingly complex multi-vortex configurations. To determine the minimum number of modes required for an accurate reconstruction, we evaluated the cumulative reconstruction residual:

$$\mathcal{L}_N = \frac{\left\langle \left(\Omega - \Omega_0 - 2 \sum_{n=1}^N \Omega_n \right)^2 \right\rangle}{\langle \Omega^2 \rangle} \quad (2.9)$$

where the average denotes a time average and an additional average is performed over independent realizations. As shown in the inset of Figure 2.9b, the reconstruction residual for $R = 45.30 \mu\text{m}$ falls below a few percent already for $N = 4$, confirming that four modes are sufficient to capture the dominant vorticity structure for this confinement size. To quantify the dominance of individual modes, the following two quantities are evaluated as a function of the confinement radius:

$$\epsilon = \left\langle \frac{1}{T} \int_0^T \Omega_n dt \right\rangle \quad \eta = \left\langle \frac{\int_0^T \Omega_n dt}{\int_0^T \Omega dt} \right\rangle \quad (2.10)$$

where the averaged is done over multiple realizations (Figures 2.9c–2.9d respectively). For small radii ($R \lesssim 60 \mu\text{m}$), the $n = 0$ mode carries a disproportionately large fraction of the enstrophy, indicating that the confinement selects a coherent rotation. As R increases beyond $60 \mu\text{m}$, the relative contributions of the modes become approximately equal, indicating that the confinement no longer imposes a preferred vortex structure and that bulk turbulent conditions are recovered. Note that panels c and d are not related by a single multiplicative factor: panel c reports the ensemble average of the time-averaged contribution, ϵ , whereas panel d reports the ensemble average of the relative contribution within each realisation, η of Eqs.(??). Because the total enstrophy fluctuates across realisations (inset of Figure 2.9c), $\epsilon \neq \eta$, and the relative ordering of two modes at a given R can therefore differ between the two panels

This conclusion is reinforced by comparison with the same decomposition performed in the unconfined uniform active state (triangles in Figure 2.9d). For this comparison we selected a point at the center of the ROI and performed the decomposition according to Eq. (2.7). The integrated contribution are then evaluated for fixed radii to match the one found in the experiments. The dependence on R in this case represents a finite-window effect. For $R \lesssim 60 \mu\text{m}$ the confined and unconfined mode distributions are clearly distinct, while they converge for larger radii. The crossover at $R \approx 60 \mu\text{m}$ thus marks the transition between the confinement-dominated regime and the bulk turbulent regime.

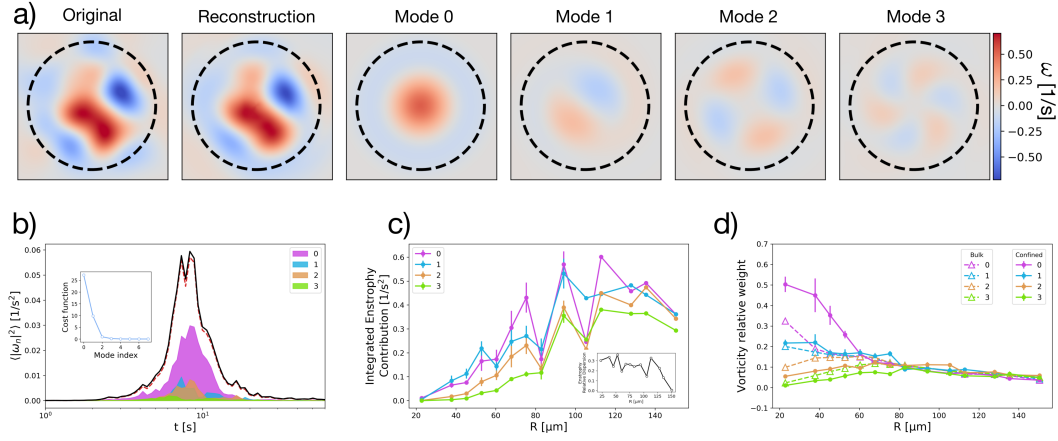


Figure 2.9. Azimuthal decomposition of the vorticity field. **a)** Reconstruction of a representative vorticity frame for $R = 45.30 \mu\text{m}$ using modes $n = 0, 1, 2, 3$ sequentially. Four modes are sufficient to reproduce the main structural features of the flow. **b)** Time evolution of the azimuthal mode contributions $\Omega_n(t)$ to the enstrophy. The sum $\Omega_0 + 2 \sum_{n=1}^3 \Omega_n$ (dashed line) closely agrees with the directly computed enstrophy (solid line). Inset: reconstruction residual $\mathcal{L}_N$ as a function of the number of modes included, confirming convergence for $N = 4$. **c–d)** Time-integrated enstrophy contributions per mode, unnormalized **c)** and normalized by the total enstrophy **d)**, as a function of confinement radius. The relative contribution of the $n = 0$ mode decreases monotonically with R and saturates to the bulk value above $R \approx 60 \mu\text{m}$. Triangles in **d)** show the bulk unconfined values for comparison. The apparent R dependence reflects the finite-window cutoff introduced to make the bulk values directly comparable with the confined case. Inset in **c)**: Dispersion of averaged enstrophy for different R . The different values that the system can have justifies an inversion of trend that can be observed going from the unnormalized to the normalized mode contribution

2.3 Conclusions and perspectives

Circular modulation of bacterial activity through structured illumination produces a virtual confinement that recapitulates, in a transient and reconfigurable manner, the phenomenology observed under physical geometric confinement. The soft boundary formed by the non-motile bacteria surrounding the active region is permeable but functionally distinct from the active interior, enabling the selection of coherent vortex dynamics within the illuminated spots.

The density analysis reveals that depletion of the active region is the fundamental mechanism that limits the duration of virtual confinement. The orientational analysis shows that bacteria escaping the illuminated region adopt a strong radial alignment with respect to the spot center, in contrast to what has been observed in experiments of confinement with hard boundaries [89, 90]. The soft, permeable boundary made of non-motile cells does not impose a constraint on the orientation of bacteria at the boundary, but instead allows steric interactions to align the escaping bacteria along the radial direction.

The azimuthal vorticity decomposition identifies a clear crossover at $R \approx 60 \mu\text{m}$: below this radius, the $n = 0$ mode (net rotation) dominates the enstrophy and the flow is coherently organized by the confinement; above it, the mode contributions equalize and bulk turbulent conditions are recovered.

The physical origin of virtual confinement deserves further experimental scrutiny. Two mechanisms could contribute to the effective boundary: steric interactions, arising from direct physical contact between motile bacteria and the dense layer of non-motile cells in the dark region, and hydrodynamic interactions, mediated by the long-range flow fields that pushers generate in the surrounding fluid. These two mechanisms respond differently to changes in sample geometry. Steric forces are contact interactions that depend primarily on the local packing fraction in the plane and are largely insensitive to the chamber height. Hydrodynamic interactions, by contrast, are strongly modified by the no-slip boundaries at the top and bottom of the sample: in thinner films, the confining walls screen the dipolar flow of each swimmer, reducing both its range and its coupling to distant neighbors. Varying the sample height at fixed area density would therefore selectively suppress the hydrodynamic contribution while leaving steric interactions unchanged, providing a direct way to disentangle the two.

Finally, the transient nature of virtual confinement raises the question of whether active feedback could be used to maintain the active region at constant density, effectively producing a steady-state virtual confinement. Such a scheme would enable long-duration studies of the flow structure within confined active turbulence without the confounding effect of depletion, and would create a direct bridge between the spatial control results presented here and the temporal modulation experiments discussed in Chapter 3.

Chapter 3

Temporal analysis of active turbulence

Abstract

This Chapter demonstrates that dense bacterial active turbulence exhibits directional memory when activity is cyclically switched on and off through light. I first review coarse-grained models of active turbulence, focusing on the Toner-Tu-Swift-Hohenberg and wet kinetic theory approaches, and their predictions for the onset of collective motion and the associated dispersion relations. I then present experiments in which a dense bacterial suspension is driven through cycles of active and passive states. The flow field after reactivation retains partial correlation with its pre-quench configuration, demonstrating directional memory, and the reactivation time grows with the duration of the passive period. By systematically varying bacterial density, I show that memory emerges sharply above a threshold density of approximately 45 OD and is linked to structural arrest in the passive state: at high packing fractions, bacterial orientations freeze on experimental timescales, providing a structural template that guides the flow upon reactivation.

3.1 Onset of turbulence

The temporal properties of active turbulence—how collective motion develops, relaxes, and recovers—are intimately connected to the mechanisms that drive its onset. In this section, I review the main coarse-grained models of active turbulence, focusing specifically on the dispersion relations and instability criteria that set the relevant length and time scales. These predictions will serve as a reference for interpreting the experimental observations of relaxation and reactivation dynamics presented in the subsequent sections.

3.1.1 Dry continuum modeling

One of the first continuum descriptions of flocking was proposed by Toner and Tu [109, 110], who derived hydrodynamic equations from symmetry considerations. The flock is described as a fluid in which isotropy and mass conservation are preserved, but Galilean invariance and total momentum are broken (for this reason this model is referred to as dry model). The equations of motion read:

$$\begin{aligned} \frac{\partial \mathbf{u}}{\partial t} + \lambda_1(\mathbf{u} \cdot \nabla)\mathbf{u} + \lambda_2(\nabla \cdot \mathbf{u})\mathbf{u} + \lambda_3 \nabla |\mathbf{u}|^2 &= -\alpha \mathbf{u} - \beta |\mathbf{u}|^2 \mathbf{u} - \nabla P \\ &+ D_B \nabla \cdot (\nabla \mathbf{u}) + D_T \nabla^2 \mathbf{u} \\ &+ D_2 (\mathbf{u} \cdot \nabla)^2 \mathbf{u} + \mathbf{f} \\ P &= P(\rho) = \sum_n \sigma_n (\rho - \rho_0)^n \\ \frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{u}) &= 0 \end{aligned}$$

Absence of Galilean invariance allows multiple advection terms with independent coefficients. Equations admit two stationary solutions for the velocity field: an isotropic state $u_0 = 0$ and a flocking state $u_0 = \sqrt{-\alpha/\beta}$. The sign of α determines the stability of each one (in fact it can assume negative values). Density is linked to the velocity field via continuity equation and an equation of state for the pressure.

To describe bulk active turbulence, Dunkel and collaborators [79, 111] proposed a mean-field model that incorporates the local alignment and a pattern-formation mechanism inspired by the Swift–Hohenberg equation [112]:

$$\begin{aligned} \partial_t \mathbf{u} + \lambda \mathbf{u} \cdot \nabla \mathbf{u} &= -\nabla p - \alpha \mathbf{u} - \beta |\mathbf{u}|^2 \mathbf{u} + \Gamma_0 \nabla^2 \mathbf{u} - \Gamma_2 \nabla^4 \mathbf{u} \\ \nabla \cdot \mathbf{u} &= 0 \end{aligned} \quad (3.1)$$

with $\nabla^4 = \nabla^2 \nabla^2$, $\beta > 0$, $\Gamma_2 > 0$, and $\Gamma_0 < 0$. The negative sign of the diffusive term, combined with the higher-order correction, produces a band of unstable wave vectors that selects a finite vortex size. The pressure field p is determined by enforcing the incompressibility condition. As in the Toner–Tu framework, Galilean invariance is broken, with $\lambda > 1$ for a suspension of pushers [113]. This model is also known as the Toner–Tu–Swift–Hohenberg (TTSH) model.

Linear stability analysis ($\mathbf{u} = \mathbf{u}_0 + \delta \mathbf{u} e^{\sigma t + i \mathbf{q} \cdot \mathbf{r}}$) of the isotropic state yields the dispersion relation:

$$\sigma = -\alpha - \Gamma_0 q^2 - \Gamma_2 q^4 \quad (3.2)$$

As shown in Figure 3.1a, a band of wave vectors exists for which the growth rate σ is greater than 0, indicating that perturbations at these scales grow exponentially (Figure 3.1a). The most unstable mode defines an intrinsic length scale:

$$\xi_0 = \frac{2\pi}{q_{\max}} = 2\pi \sqrt{\frac{2\Gamma_2}{-\Gamma_0}}$$

This length scale will be directly relevant to the interpretation of our reactivation experiments: the TTSH model predicts that collective motion should first develop at this characteristic scale, which sets a lower bound on the time required for the turbulent state to be established.

Stability analysis of the flocking state reveals an additional instability for transverse perturbations, with dispersion relation:

$$\sigma = -\Gamma_0 q^2 - \Gamma_2 q^4 + 2\alpha \cos(\theta) + i\lambda v_0 q \cos(\theta) \quad (3.3)$$

where θ is the angle between the wave vector and the flocking direction. The fact that the two fixed points become simultaneously unstable signals the existence of spatially inhomogeneous dynamic attractors.

Using a spectral shell decomposition of the energy spectrum, Bratanov et al. [114] demonstrated that this model can reproduce the non-universality of the power spectrum decay observed in experiments (Figure 3.1b). More recently, the model has been used to investigate the intermittency of vortex dynamics [115] and active turbulence in structured environments, in a manner analogous to the experiments discussed in the previous Chapter [116]. An extension incorporating density modulation, proposed by [117], coupled the velocity field to a density equation with a motility-induced feedback, enabling the study of the interplay between MIPS and turbulence.

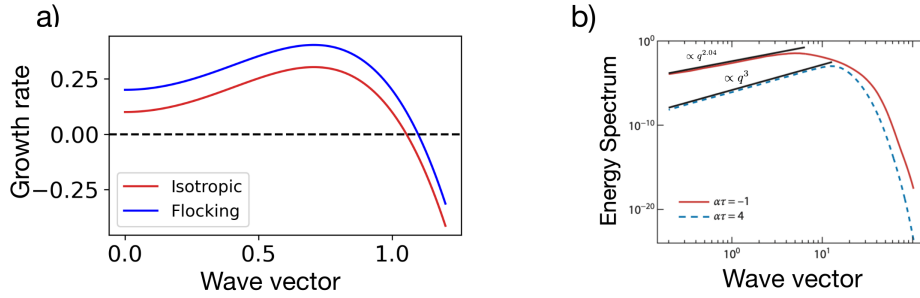


Figure 3.1. Relaxation modes of the TTSH model. **a)** Linear stability analysis of the isotropic and aligned state, Eqs. (3.2)-(3.3). A band of wave vectors exists for which the real part of the growth rate is positive, indicating instability. The most unstable mode selects the intrinsic vortex scale ξ_0 . **b)** A shell decomposition analysis of equations Eq. (3.1) predicts a non-universal scaling of the power spectrum at long wavelengths. Adapted from [114].

3.1.2 Wet continuum modeling

An alternative approach retains momentum conservation by explicitly coupling the swimmers to the surrounding fluid [118–121]. A standard formulation fully solves the Stokes hydrodynamic equations [122, 123]. At the meanfield level, the suspension can be described using the single-particle probability function $\Psi(\mathbf{r}, \hat{\mathbf{p}}, t)$. Many-body interactions enter through a self-consistent coupling: Ψ determines the active stress that drives the fluid velocity and the resulting flow in turn advects and reorients each swimmer. Considering a run-and-tumble swimmer with position $\mathbf{r}$ and orientation $\hat{\mathbf{p}}$, the evolution of the probability function follows the equation:

$$\frac{\partial \Psi}{\partial t} + \nabla_{\mathbf{r}} \cdot (\dot{\mathbf{r}} \Psi) + \nabla_{\hat{\mathbf{p}}} \cdot (\dot{\hat{\mathbf{p}}} \Psi) = -\lambda \Psi + \int \frac{\lambda}{\Omega_d} \Psi d\hat{\mathbf{p}}$$

where λ is the tumbling rate. The orientational moments of Ψ define the macroscopic fields: the concentration ρ , the polarization $\mathbf{m}$, and the nematic tensor $\mathbf{Q}$.

Following the formulation of Weady et al. [124], the single-particle dynamics are prescribed as:

$$\begin{aligned}\dot{\mathbf{r}} &= v\hat{\mathbf{p}} + \mathbf{u} - D_t \nabla_{\mathbf{r}} \log(\Psi) \\ \dot{\mathbf{p}} &= (\mathbf{1} - \hat{\mathbf{p}}\hat{\mathbf{p}}) \cdot (\nabla_{\mathbf{r}} \mathbf{u} + 2\zeta_0 c \mathbf{Q}) \cdot \hat{\mathbf{p}} - D_r \nabla_{\hat{\mathbf{p}}} \log(\Psi)\end{aligned}$$

capturing self-propulsion at speed v , alignment with the local flow and nematic order [125, 126], and translational and rotational diffusion (D_t , D_r). The fluid obeys the Stokes equations (Eq. (1.1)) with a stress tensor comprising active, rigidity, and steric contributions:

$$\begin{aligned}\Sigma &= \Sigma_a + \Sigma_r + \Sigma_s \\ \Sigma_a &= \sigma_a \rho \mathbf{Q}, \quad \Sigma_r = \sigma_r \rho \mathbf{S} : \mathbf{E}, \quad \Sigma_s = -\sigma_s \rho^2 (\mathbf{Q} \cdot \mathbf{Q} - \mathbf{S} : \mathbf{Q})\end{aligned}\tag{3.4}$$

The coefficient σ_a sets the strength of the active stress, with positive values for pushers and negative for pullers. The coefficient σ_r governs the rigidity stress, which arises because rod-like swimmers reorient under flow gradients and thereby couple back to the fluid; this term depends on the rate-of-strain tensor $\mathbf{E} = \frac{\nabla \mathbf{u} + (\nabla \mathbf{u})^T}{2}$. The coefficient σ_s encodes the steric stress generated by direct contact between cells at finite packing fraction. The fourth-order tensor $\mathbf{S} = \int \hat{\mathbf{p}}\hat{\mathbf{p}}\hat{\mathbf{p}}\hat{\mathbf{p}} \Psi d\hat{\mathbf{p}}$ is the fourth orientational moment of the single-particle distribution, and contracts with the second-rank tensors $\mathbf{E}$ and $\mathbf{Q}$ as $(\mathbf{S} : \mathbf{E})_{ij} = S_{ijkl} E_{kl}$.

Solving the full kinetic theory is computationally demanding. A practical approach consists in integrating out the orientational degrees of freedom and closing the resulting moment hierarchy. In the isotropic regime, closure at second order is adequate; in the nematic regime where the polarization is 0, the Bingham closure $\Psi_B = Z^{-1} \exp(\mathbf{B} : \hat{\mathbf{p}}\hat{\mathbf{p}})$, also referred to as the *B-model*, provides a better approximation.

Linear stability analysis of the isotropic state for a pusher suspension, neglecting rotational diffusion, yields:

$$\sigma = -\lambda + \frac{\sigma_a \rho_0}{5} - \left(\frac{15v_0}{7\sigma_a \rho_0} + D_t \right) q^2 + \mathcal{O}(q^4)$$

Two features distinguish this dispersion relation from that of the TTSH model. First, the most unstable mode occurs at $q = 0$, predicting that instabilities develop at the largest available scale rather than at a selected intrinsic scale. Second, the instability requires a minimum density for the onset of collective motion:

$$\rho_c = \frac{5\lambda}{\sigma_a}$$

The aligned state is also unstable, presenting at least one length scale at which the real part of the growth rate is positive (Figure 3.2). This long-wavelength instability, known as the Simha–Ramaswamy instability [113], is a hallmark of active systems with no passive analogue.

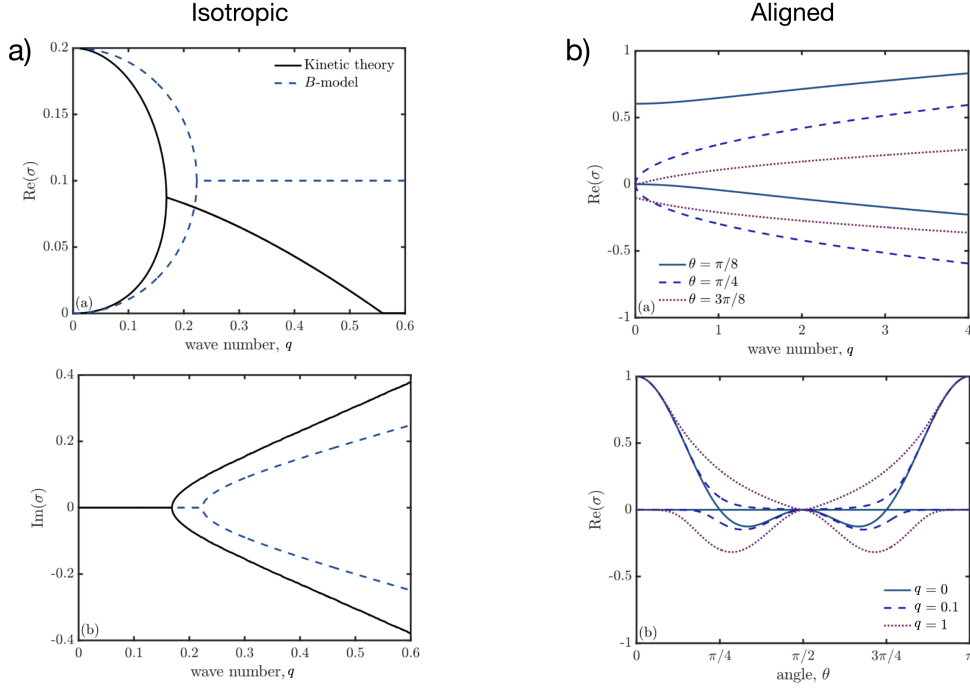


Figure 3.2. Linear stability analysis of the wet active nematic model. a–b) Dispersion relation for the isotropic state and the aligned state. In the first case the most unstable mode occurs at $q = 0$, in contrast to the TTSH prediction. In the case of aligned state, there is always at least one value with positive real part of the growth rate. Adapted from [124].

3.1.3 Scale selection: theory versus experiment

The two modeling frameworks make qualitatively different predictions for the characteristic scale of active turbulence: the TTSH model selects an intrinsic scale, whereas the wet kinetic theory predicts instability at the largest available scale, with the vortex size set by the system boundaries. Several experiments have addressed this discrepancy by varying the confinement height of the sample [127, 128] (Figure 3.3a–3.3b). For the case of *Escherichia coli* a linear dependence with the confinement height is found, presenting bigger macroscale structures for bigger confinements. The critical concentration for the onset of turbulence depends on the sample height H , scaling as [129]:

$$\frac{\rho}{\rho_c} = 1 + \frac{3}{10} \left(2\pi \frac{v}{H\lambda} \right) + \frac{1}{5} \left(2\pi \frac{v}{H\lambda} \right)^2$$

These results support the wet modeling picture, in which the smallest confining dimension sets the relevant scale for the onset of collective motion. A similar observation has been done also in the case of active nematic, [130]. Even though the two phenomena are different, an increase of the correlation length with the confinement height is observed (Figure 3.3c–3.3d).

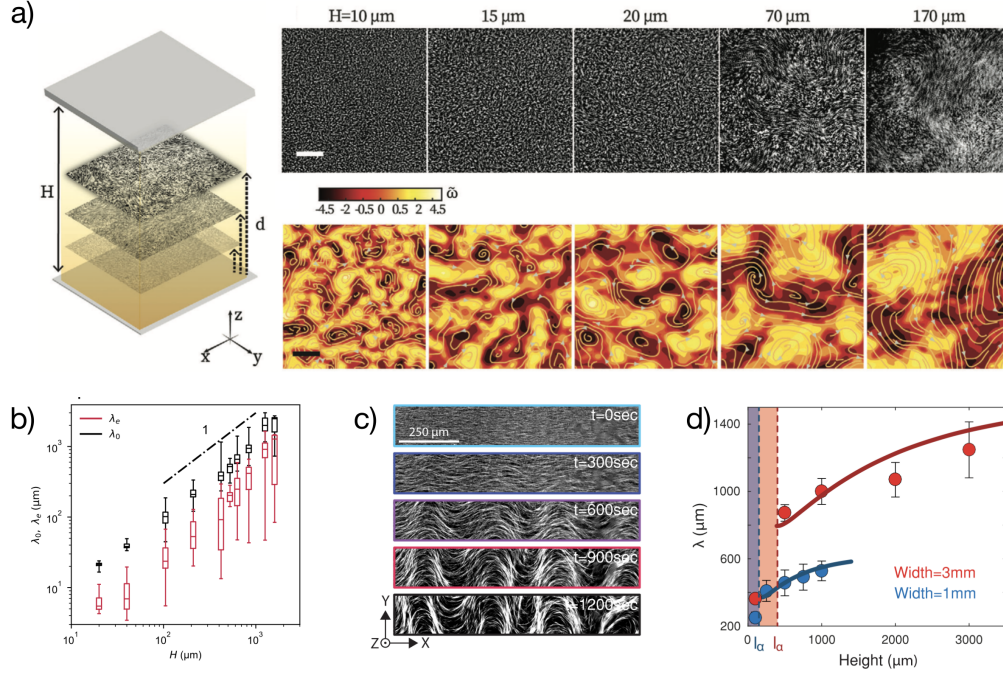


Figure 3.3. Experimental investigation of bacterial turbulence onset. **a)** Experimental observation of height influence on correlation length for a suspension for *E. coli* bacteria. The higher the sample, the bigger the vortex size, as evidenced by the vorticity map and the isostream lines (adapted from [127]). **b)** The bacterial correlation length and vortex size extracted by the spatial velocity autocorrelation function for *E. coli* suspension scales linearly with the sample height, in accordance with predictions of wet continuum modelings (adapted from [128]). **c–d)** In active nematics, the characteristic length scale is also set by the smallest confining dimension, showing an increase with it (adapted from [130]).

3.1.4 Temporal development of turbulence

Beyond the steady-state properties, the route by which the system develops collective motion is also of interest. In bacterial systems, the literature is limited: Peng et al. [77] used photokinetic bacteria to monitor the approach to the stationary turbulent state, mapping out the phase diagram as a function of density and activity. They found that the transition from an isotropic to a turbulent state proceeds through a two-stage process: the bacterial fluid first develops local nematic order, which then couples to coherent local flows.

However, key questions remain open: How does the flow field evolve when a suspension transitions between active and passive states? What information is preserved during these transitions? Does the system retain memory of its previous configuration? The dispersion relations reviewed above predict characteristic growth rates for the onset of turbulence, but they do not address whether the system's history—encoded in bacterial positions and orientations—affects the recovery dynamics. To address these questions, we exploit the temporal control afforded by photokinetic bacterial strains.

3.2 Temporal modulation of activity

3.2.1 Experimental protocol

As observed in the virtual confinement experiments of Chapter 2, using spatially modulated illumination from below via the DMD leads to non-uniform activation of the sample: only the field of view is activated, creating density modulation that can compromise the reliability of measurements of the uniform state. For this reason, the experiments presented in this Chapter employ uniform illumination from above, which covers a wider area (see Appendix A.1.3). Although this sacrifices the spatial precision of the DMD, temporal modulation is achieved by regulating the LED intensity with an Arduino microcontroller.

The bacterial strain used also differs from that in Chapter 2. During preliminary experiments, the formation of non-motile bacterial clusters was observed. Inspired by the works on adhesion [131–135] we attributed the process to fimbriae-mediated adhesion. A strain with deleted fimbriae (AUYBG strain, see Appendix A.1.1) was indeed used throughout. This modification substantially reduced cluster formation while preserving photokinetic behavior and swimming characteristics. The growth protocol is described in Appendix A.1.2.

3 μ L of bacterial suspension were loaded into a 20-micron 4-chamber slide (Leja Products B.V., Nieuw-Vennep, The Netherlands) and sealed with vaseline, as described in Chapter 2. Image preprocessing followed the protocol described in Chapter 2. The temporal modulation protocol is illustrated in Figure 3.4:

- 1) **Uniform state:** The sample is in the turbulent state under uniform illumination.
- 2) **Dark period:** Light is removed for a variable waiting time t_w .
- 3) **Reactivation:** Light is restored.

The time at which illumination is removed defines $t = 0$. The frame rate of acquisition is 25 fps. To avoid memory saturation, data were acquired during the first 40 s after light removal and the last 40 s before reactivation, enabling measurements with waiting times up to 5 min. Velocity fields were extracted using PIV with 10.4 μ m interrogation windows and 50% overlap. The velocity is then filtered with a gaussian filter of width 0.04 s in time and 5.2 μ m in space. In this initial section, I will present the analyses conducted on a sample with a density of 83 OD₆₀₀ and then proceed to vary the latter. The intensity of the LED is equal to 0.78 mW/mm².

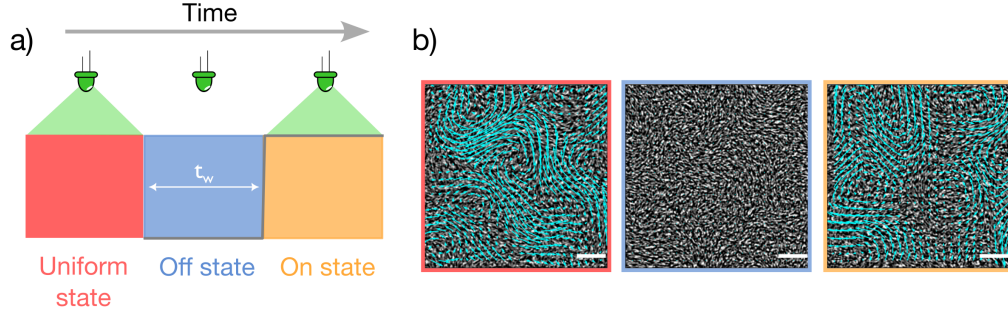


Figure 3.4. Protocol for temporal modulation of activity. a–b) The illumination is cycled through three phases: uniform activation, a dark period of variable waiting time t_w (the passive state), and reactivation (the on state). In the first phase, uniform turbulence is recorded. During the passive phase, the transition to a quiescent state is captured. Finally, the reawakening of collective motion is monitored upon reactivation. Snapshots take respectively at 1 s after the sampling started, 240 s after illumination is removed and 60 s after reactivation. Scale bar: 20 μm .

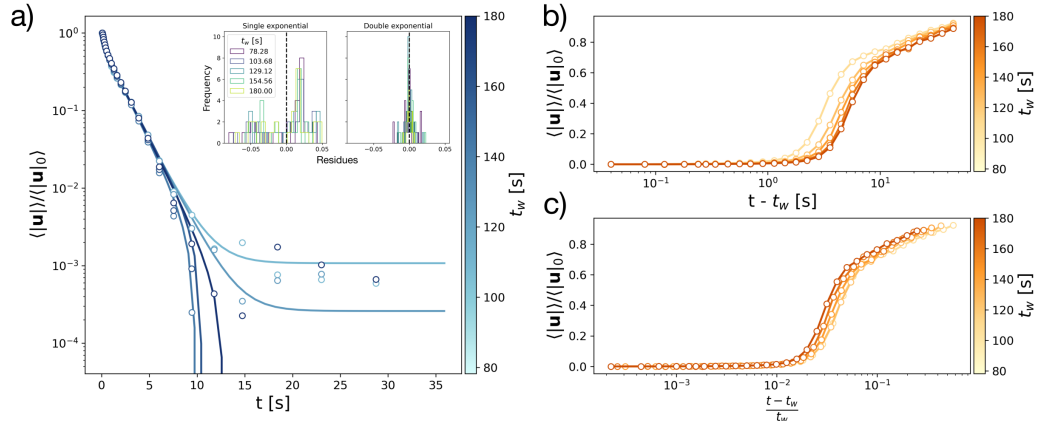


Figure 3.5. Speed dynamics under temporal modulation. a) Speed decay following light removal exhibits bi-exponential kinetics, Eq. (3.5) as confirmed by the distribution of the residues in the inset. The decay time are $\tau_1 = 0.40(3)\text{s}$ and $\tau_2 = 2.6(1)\text{s}$ where the parenthesis number is given by the average error over multiple realizations. Inset: Histogram of residues with single and double exponential fit for every curve. The distribution of residues approaches a gaussian when a double exponential fit is used. Instead the broadening of the residues for the single exponential one captures the trend of overestimating and underestimating of the fit function. b) Speed recovery as a function of waiting time t_w : longer dark periods require longer reactivation times. c) The same recovery curves plotted against the rescaled time t/t_w at the highest density investigated (83 OD). The improved overlap suggests time–waiting-time superposition; this hypothesis is tested quantitatively in Section 3.3.3 and is found not to hold across the explored density range.

3.2.2 Active-to-passive transition

Upon light removal, the bacterial suspension undergoes rapid relaxation from active turbulence to a passive state. The spatially averaged speed $m(t) = \langle |\mathbf{u}| \rangle$ (Figure 3.5a) is well described by a bi-exponential decay:

$$m(t) = \frac{m_0 - m_\infty}{1 + r} \left(e^{-t/\tau_1} + r e^{-t/\tau_2} \right) + m_\infty \quad (3.5)$$

where m_0 is the initial active speed, m_∞ the residual passive motion, and r the relative amplitude of the two components. In order to confirm the quality of the fit, we observed the residues of the fitting procedure and compare them with the one of a single exponential fit (Inset Figure 3.5a). As it is evidenced, in the first case residues are normally distributed around 0, whereas for the single fit, the histogram seems to be bimodal, mirroring the underestimate and overestimate at different times. The reduced χ^2 evaluated as the sum of the squared residues divided by the number of degrees of freedom differs by more than an order of magnitude, being on average 1.6×10^{-5} and 5.0×10^{-8} respectively.

Two timescales are extracted: a fast component $\tau_1 = 0.40(3)$ s and a slow component $\tau_2 = 2.6(1)$ s. The fast timescale τ_1 is compatible with the flagellar motor dynamics [136]. The slow timescale τ_2 likely reflects the decay of the collective hydrodynamic flow once the driving force diminishes: as individual bacteria slow down, the coherent velocity field, sustained by the collective interactions among many swimmers, decays on a timescale longer than that of the individual one.

3.2.3 Passive-to-active transition

When light is restored after a waiting time t_w , the suspension returns to the active turbulent state. The reactivation dynamics depend on t_w (Figure 3.5b): longer dark periods require longer recovery times. A natural hypothesis, motivated by glassy systems, is that the recovery curves rescale as a function of t/t_w onto a single master curve, indicative of time–waiting-time superposition. A visual inspection of Figure 3.5c, in which the recovery curves at 83 OD are plotted against t/t_w , suggests that this rescaling improves the overlap of the curves. Whether this holds quantitatively, and whether it survives variation of bacterial density, is investigated in Sec. 3.3.3.

The accessible t_w range is bounded from both sides. For short waiting times ($t_w \lesssim 10$ s), the average speed has not yet decayed to zero, so the system retains residual activity that accelerates recovery. For long waiting times (of order minutes), bacteria begin to disassemble torque-generating units from flagellar motors [137], introducing an additional slow timescale associated with motor reassembly.

3.2.4 Directional memory in the velocity field

We now ask whether directional information is also preserved through the passive period. To probe this, we define the two-time autocorrelation function of the normalized velocity field:

$$C(t_0, \tau) = \langle \hat{\mathbf{u}}(\mathbf{r}, t_0) \cdot \hat{\mathbf{u}}(\mathbf{r}, \tau + t_0) \rangle \quad (3.6)$$

where $\hat{\mathbf{u}} = \mathbf{u}/|\mathbf{u}|$ is the unit velocity direction, $t_0 > 0$ is the reference time after light removal, and $\tau > t_w$ is the lag time extending into the reactivation period. Since the system is non-stationary during relaxation, we retain explicit dependence on both t_0 and τ .

The correlation map $C(t_0, \tau)$ reveals a pronounced maximum occurring shortly after reactivation (Figure 3.6a). This maximum is present for reference times t_0 corresponding to the early passive state, when the velocity field still carries directional information, but vanishes for later t_0 values when the residual velocity field is dominated by noise.

The maximum of the correlation map, evaluated over both t_0 and τ , is shown as a function of t_w in Figure 3.6b. For short waiting times, this value is high, reflecting strong persistence of flow direction. The function then reaches a plateau that remains constant over a wide range of t_w . This plateau value, which we term the **memory** of the system, indicates that directional information is preserved even after prolonged periods in the passive state. This quantity will also be referred to as the Eulerian memory, reflecting the fixed-point methodology used for its evaluation.

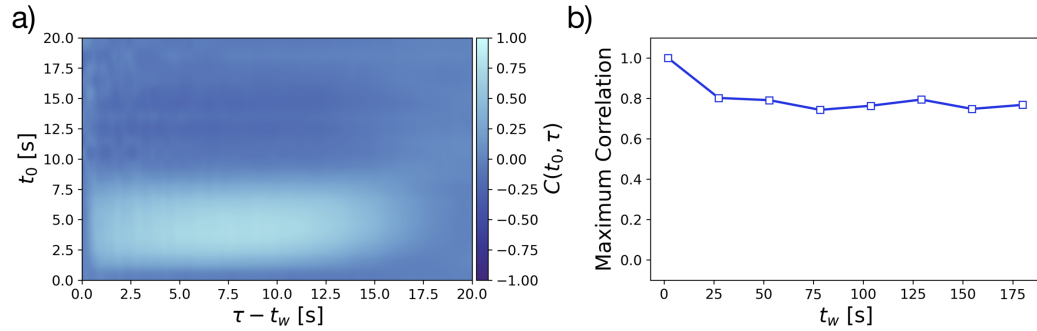


Figure 3.6. Directional memory in Eulerian velocity fields. **a)** Two-time correlation map $C(t_0, \tau)$ showing a pronounced maximum after reactivation for $t_w = 104$ s **b)** Maximum correlation as a function of waiting time. A plateau (memory) persists for $t_w > 10$ s

3.3 Density dependence

To understand the physical origin of memory, we systematically varied the bacterial density from 10 OD to 94 OD (from now on OD refers to OD₆₀₀). After the preparation of the dense mother suspension according to the protocol described in Appendix A.1.2, dilutions to the target density were performed.

3.3.1 Characterization of the uniform active state

We first characterized the uniform active state as a function of density. The normalized spatial velocity autocorrelation function

$$C(r) = \frac{\langle \mathbf{u}(\mathbf{x} + \mathbf{r}, t) \cdot \mathbf{u}(\mathbf{x}, t) \rangle}{\langle |\mathbf{u}(\mathbf{x}, t)|^2 \rangle} \quad (3.7)$$

was computed for each density, Figure 3.7a. Since the system is in a stationary condition, an average over time is also computed. Two characteristic lengths were extracted: the $1/e$ decay length ξ_d , Figure 3.7b, and the first zero-crossing ξ_0 (when present), Figure 3.7c. Direct inspection of the velocity fields and the correlation functions indicates that correlated collective motion is absent below 20 OD, which sets the threshold for active turbulence.

The spatially averaged speed $\langle |\mathbf{u}| \rangle$ increases monotonically with density (Figure 3.8a), exhibiting a sharp increase near 80 OD, consistent with previous observations [77]. The speed fluctuations σ_u scale linearly with the mean speed (Figure 3.8b), indicating that relative fluctuations remain approximately constant: $\sigma_u / \langle |\mathbf{u}| \rangle = 0.55(1)$ across all densities.

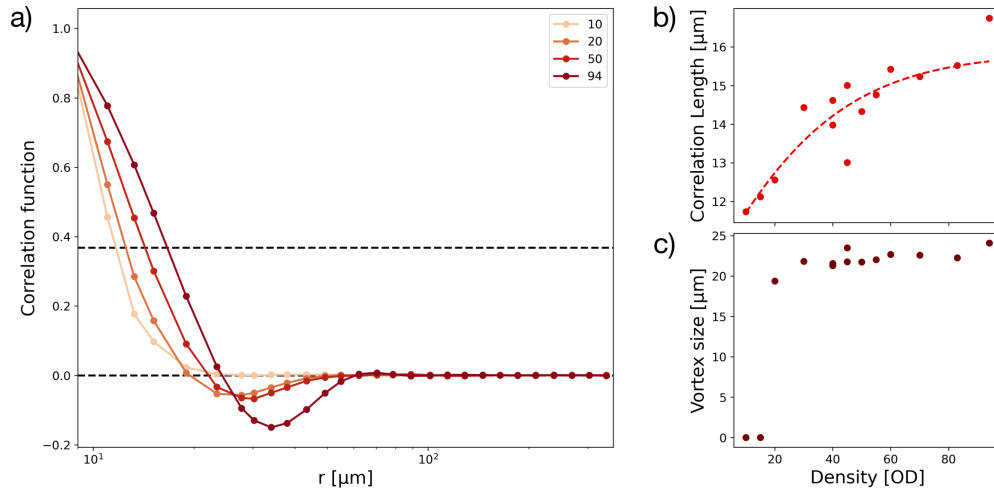


Figure 3.7. Density dependence of uniform active turbulence. a) Normalized spatial correlation function for different densities. As density increases, data assume negative values and the curves relax with a longer timescale. b–c) Density dependence of the correlation lengths ξ_d and ξ_0 . A zero-crossing is not observed below 20 OD; above this threshold, active turbulence is fully developed.

3.3.2 Density dependence of the relaxation dynamics

The two decay timescales extracted from the bi-exponential fit (Eq. (3.5)) of the speed decay are shown in Figure 3.8c. Neither τ_1 nor τ_2 exhibits a systematic dependence on density. The value of τ_2 at 60 OD can be considered an outlier. The relative weight of the slow component, $|r|/(1 + |r|)$, increases with density and saturates above 20 OD as observed in Figure 3.8d. This suggests that the slow relaxation channel becomes prominent only in the turbulent regime.

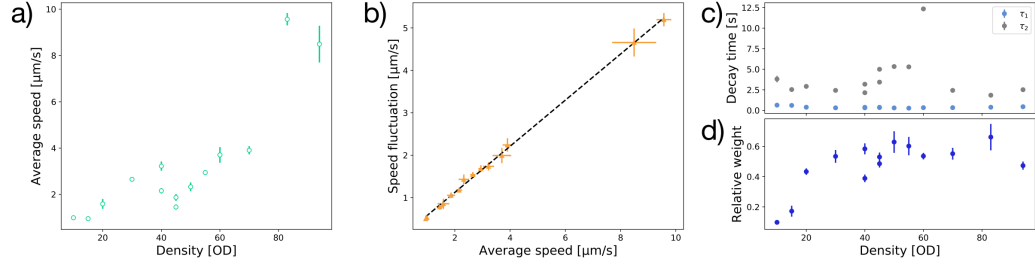


Figure 3.8. Density dependence of speed dynamics. **a)** Average speed in the uniform active state as a function of density, with a marked increase near 80 OD. **b)** Linear relationship between speed fluctuations and mean speed. **c)** Decay timescales τ_1 and τ_2 as a function of density. The two timescales are approximately constant across densities. **d)** Relative weight of the slow relaxation component, $|r|/(1 + |r|)$ from fit results using Eq. (3.5). The increase and saturation above 20 OD indicates that in the turbulent regime the slow relaxation becomes relevant. Error bars represent the experiment-to-experiment standard deviation.

3.3.3 Density dependence of aging and memory

To assess quantitatively whether the recovery curves collapse onto a master curve when time is rescaled by t_w , we computed the integrated squared dispersion of the recovery curves from their mean profile, both with and without rescaling. Let $m_{t_w}(\tau)/m_0$ denote the normalized speed recovery for a given waiting time t_w , where the lag variable is either $\tau = t - t_w$ (no rescaling) or $\tau = (t - t_w)/t_w$ (rescaling). For each definition we compute the average $M(\tau) = \langle m_{t_w}(\tau)/m_0 \rangle_{t_w}$ over the available waiting times and the dispersion of the individual curves around it:

$$R = \frac{1}{\tau_{\max}} \int_0^{\tau_{\max}} \left\langle \left(\frac{m_{t_w}(\tau)}{m_0} - M(\tau) \right)^2 \right\rangle_{t_w} d\tau. \quad (3.8)$$

A smaller R indicates a tighter collapse. To exclude the short- t_w regime where residual activity contaminates the recovery (cf. Section 3.2.3), only waiting times $t_w > 60$ s are included.

Results are shown in Figure 3.9a. For densities below approximately 72 OD, the unrescaled dispersion is smaller than the rescaled one, meaning that the t/t_w rescaling makes the collapse *worse* rather than better in this range. The two become comparable only at higher density. This is also visible in the raw recovery curves shown for all densities in Appendix A.3. Taken together, these observations indicate that a clean time–waiting-time superposition of the type observed in glassy systems is not present in the explored density range. The qualitative slow-down of the recovery with t_w , visible in Figure 3.5b, therefore cannot be interpreted as evidence of aging-like scaling, and is reported here as a fact whose mechanistic interpretation goes beyond the scope of this Chapter.

The Eulerian memory increases smoothly with density (Figure 3.9b). The evaluation is performed in a regime where no significant dependence on t_w is evident. A sigmoidal fit places the midpoint of the transition at approximately 60(12) OD, with a width of 22(18) OD; the quoted errors are obtained from the fit. Notably, this threshold lies well above the onset density for active turbulence (20 OD), indicating that memory is not simply a consequence of collective motion but requires an additional ingredient related to the packing of cells in the passive state. The structural origin of this requirement is investigated in Sec. 3.5.

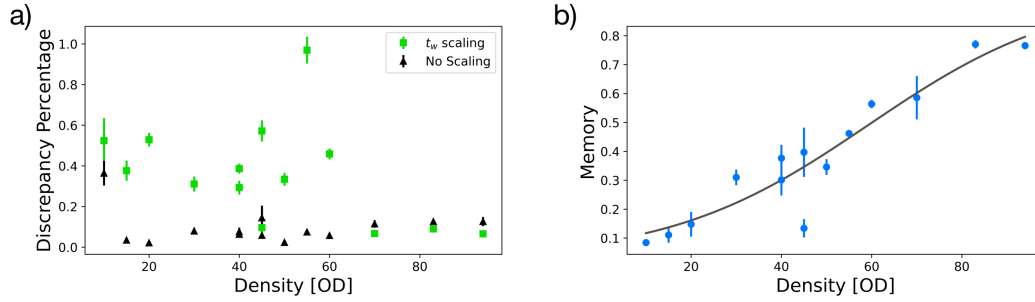


Figure 3.9. Quantitative test of the aging hypothesis and density dependence of memory. **a)** Integrated squared dispersion R of the recovery curves around their average, computed using Eq. (3.8), plotted as a function of bacterial density. Black triangles: R for the unrescaled curves ($\tau = t - t_w$). Green squares: R for the rescaled curves ($\tau = (t - t_w)/t_w$). The rescaled R is larger than the unrescaled one for $\rho \lesssim 72$ OD, indicating that the t/t_w rescaling does *not* improve the collapse in that regime; the two become comparable only at higher density. The expected signature of aging-like time–waiting-time superposition is therefore not observed across the explored density range. **b)** Eulerian memory as a function of density. A smooth transition to a memory-bearing state occurs well above the active-turbulence onset of 20 OD. A sigmoidal fit (solid line) places the midpoint of the transition at 60(12) OD with a width of 22(18) OD; quoted errors are obtained from the fit. Error bars on the data points represent the average uncertainty across realizations.

3.4 Lagrangian analysis of the velocity field

The Eulerian analysis probes the velocity field at fixed spatial positions. We now complement this with a Lagrangian perspective, which tracks virtual fluid elements along their trajectories and can reveal information about single-particle-level dynamics that is not accessible from the Eulerian framework alone [138–141]. Specifically, we ask: does memory manifest at the level of individual trajectories, and does the Lagrangian analysis provide a more precise estimate of the memory transition density?

A set of N virtual tracers $\{\mathbf{r}_i\}$ is advected according to:

$$\mathbf{r}_i(t) = \mathbf{r}_i^{(0)} + \int_0^t \mathbf{u}(\mathbf{r}_i(\tau), \tau) d\tau \quad (3.9)$$

The velocity field is interpolated spatially and temporally using cubic interpolation, and the equations are integrated with a fourth-order Runge–Kutta method at the frame rate. The spatial averages are replaced by averages along the trajectories:

$$\bar{f} = \frac{1}{N} \sum_i f(\mathbf{r}_i)$$

3.4.1 Characterization of the uniform turbulent state

Representative trajectories for different densities are shown in Figure 3.10a. The trajectories exhibit no preferential direction, and the spatial extent of the traced paths increases with density, consistent with the increasing average speed. Given the trajectories, the mean squared displacement (MSD) is evaluated according to the formula:

$$\text{MSD}(\tau) = \overline{\langle |\mathbf{r}_i(\tau + t_0) - \mathbf{r}_i(t_0)|^2 \rangle_{t_0}} \quad (3.10)$$

Where two kind of averages are performed: the inner average is over the reference time t_0 and the outer average is over the set of tracers. A linear fit in the log-log scale reveals two regimes (Figure 3.10b–3.10d): for short time ($\tau < 1$ s) data are proportional to τ^2 . The long time regime ($\tau > 1$ s) presents dependence proportional to τ . The crossover time $\tau_{\text{cross}} \approx 1$ s is observed across all densities above the turbulence threshold. Changes in density affect the value at zero lag extracted from the linear fit procedure of the MSD, Figure 3.10d, reflecting the variation in speed.

To characterize the persistence of trajectories, we computed the tangent autocorrelation function parametrized by arc length s :

$$C_t(s) = \overline{\langle \hat{\mathbf{t}}_i(s + s_0) \cdot \hat{\mathbf{t}}_i(s_0) \rangle_{s_0}} \quad \hat{\mathbf{t}}_i = \frac{d\mathbf{r}_i}{ds} \quad (3.11)$$

where $\hat{\mathbf{t}}_i$ is the unit tangent vector along the i -th trajectory. The maximum length that can be sampled increases with density, reflecting the longer path lengths traversed at higher speeds, Figure 3.10e. At low densities, a zero-crossing is observed that is absent at high density values. The persistence length of the trajectories is extracted by extrapolating the value at which the tangent correlation function decays to $1/e$. As can be observed in Figure 3.10f, it increases with density, although a clear quantitative trend is not evident. This measurement in fact contains both the structural and spatial properties of the velocity flow field.

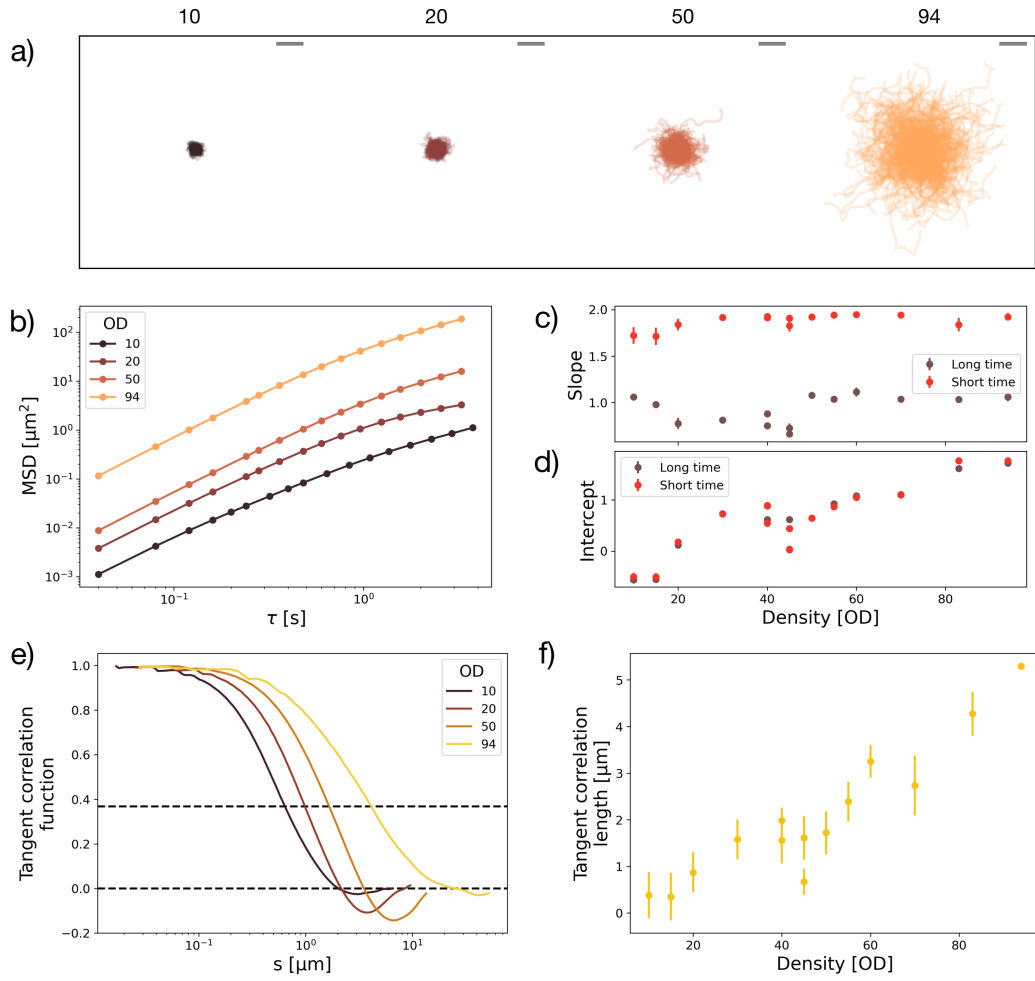


Figure 3.10. Lagrangian analysis of uniform active turbulence. **a)** Superimposition of trajectories for different densities indicated by the title in OD units (scale bar: $10\ \mu\text{m}$). Trajectories appear isotropic, with a spatial extension reflecting the speed. **b)** Mean squared displacement showing two regimes for short and long times. **c–d)** Result of linear fitting for short and long time regimes. The slope of the log-log fit is close to 2 for short times, 1 for longer. The value at zero lag follows the same trend of the speed. **e)** Tangent autocorrelation function. Dashed lines indicate $1/e$ and zero crossing. The definition domain reflects the longer path lengths. **f)** Persistence length evaluated from trajectories as a function of density. The average have been performed over different realizations of the experiment. There is not a clear trend with density, although the persistence length tends to increase with it.

3.4.2 Lagrangian memory

For each virtual tracer, two trajectory segments are computed: one during the transition to the passive state, after light removal, and one after reactivation (with the initial position of the second segment set to the final position of the first). This matches the reference window used for the Eulerian correlator of Sec. 3.2.4, ensuring that the two memory estimates are constructed from the same time intervals. The tangent correlation between the two segments

defines the Lagrangian memory correlator:

$$C_{\text{lag}}(s_1, s_2) = \overline{\hat{\mathbf{t}}_{\text{before}}(s_1) \cdot \hat{\mathbf{t}}_{\text{after}}(s_2)}$$

where s_1 and s_2 are arc-length coordinates along the pre-quench and post-reactivation segments, respectively. Each trajectory segment is smoothed in the arc-length parametrization to suppress noise (see Figure 3.11a for an operative definition of the correlator and Figure 3.11b for example trajectories). The Lagrangian memory is extracted by maximizing C_{lag} over s_1 and s_2 .

The maximum of the Lagrangian correlator, as a function of density, reveals a transition at 45(2) OD with a width of 2.1(9) OD (Figure 3.11c). The Lagrangian estimate may be more accurate because it directly probes the trajectory-level correlations without the spatial averaging inherent in the Eulerian framework, which can smear the transition. The two observables do not exhibit a linear relationship. For the remainder of this Chapter, I adopt the Lagrangian estimate as the reference value for the memory transition density.

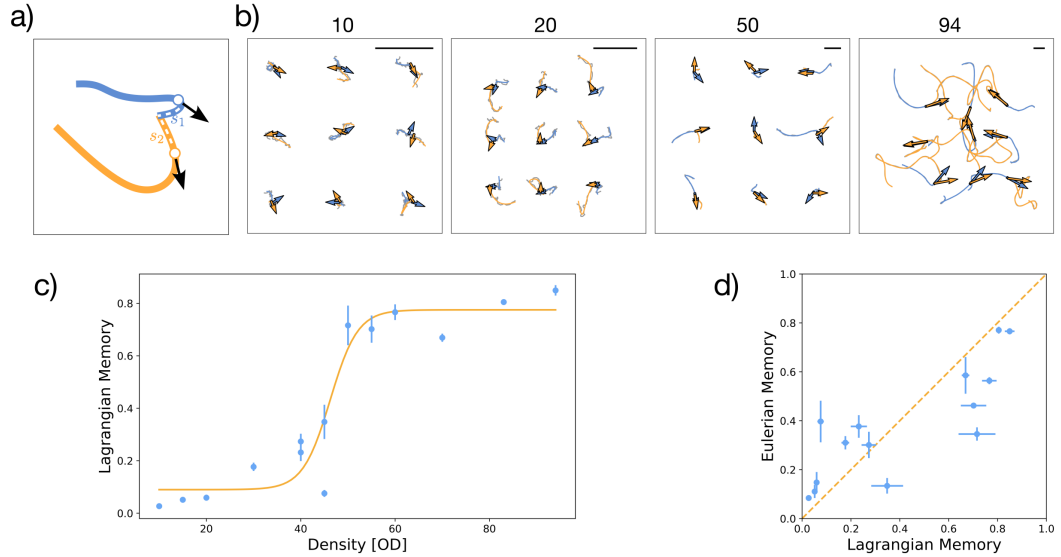


Figure 3.11. Lagrangian memory. **a)** Scheme for the evaluation of the lagrangian memory. For each virtual tracer, the tangent vectors before light removal and after reactivation are correlated **b)** Examples of trajectories for representative densities. The trajectory (gray line) is divided in two strands and smoothed (blue and orange). Scale bar: 5 μm . **c–d)** Lagrangian memory as a function of density, with superimposed sigmoid fit, and Eulerian memory. A transition is observed at 45(2) OD with a width of 2.1(9) OD. The error associated is the one from fitting procedure. Compared to the Eulerian estimate, the transition is sharper.

3.5 Structural origin of memory

The density dependence of memory suggests a connection to the structural properties of the passive state. At high densities, as observed by [131, 132], orientational and translational degree of freedom are suppressed, even in presence of activity. If bacterial orientations are frozen in the passive state, they would provide a structural template that biases the flow direction upon reactivation, explaining both the existence and the density dependence of memory. To test this hypothesis, we performed two complementary analyses characterizing bacterial organization in the absence of activity.

3.5.1 Structural relaxation

Inspired by approaches used in colloidal glass physics, we characterized density fluctuations in the passive state using the intermediate scattering function (ISF), evaluated through a differential dynamic microscopy framework [15, 142, 143]:

$$f_q(\tau) = \frac{\langle I_{\mathbf{q}}(t_0 + \tau) I_{\mathbf{q}}^*(t_0) \rangle_{|\mathbf{q}|=q, t_0}}{\langle |I_{\mathbf{q}}(t_0)|^2 \rangle_{|\mathbf{q}|=q, t_0}} \quad (3.12)$$

Here $I_{\mathbf{q}}$ is the Fourier transform of the image intensity. The function is evaluated over a 30 s window in the deep passive state, before reactivation, ensuring that the average speed has decayed to zero. For this analysis, we consider experiments where $t_w > 60$ s.

The static structure factor $S(q) = \langle |I_{\mathbf{q}}|^2 \rangle$ (inset of Figure 3.12) exhibits a peak at a wave vector $\bar{q} \approx 1 \mu\text{m}^{-1}$, corresponding to the characteristic inter-bacterial spacing. We evaluated $f_q(\tau)$ at $q = 2.34 \mu\text{m}^{-1}$, a value at which the structure factor has decreased from its peak and where preliminary observations showed more distinct relaxation dynamics.

The ISF reveals a clear density dependence of structural relaxation (Figure 3.12a): as density increases, the decay becomes progressively slower, and at the highest densities the function does not fully decorrelate within the available time window. This arrest of density fluctuations is characteristic of slow dynamics. However, the limited time window (30 s) restricts the reliability of exponential fits for the slowest relaxation timescales, which can exceed the measurement duration. To obtain a robust, window-independent measure of the relaxation dynamics, we computed the normalized integrated relaxation time:

$$t_i = \frac{1}{T} \int_0^T f_q(\tau) d\tau$$

This quantity captures the fraction of the observation window during which density fluctuations remain correlated, without requiring an extrapolation beyond the data. As shown in Figure 3.12c, t_i increases with density and saturates near 40 OD, indicating that above this density the structural relaxation time exceeds the experimental observation window—consistent with arrested dynamics.

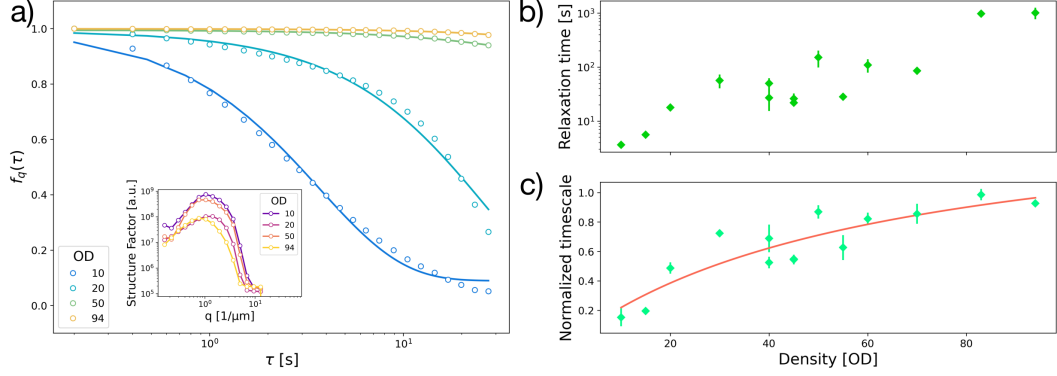


Figure 3.12. Structural relaxation in the passive state. **a)** Intermediate scattering function for different densities, evaluated at $q = 2.34 \mu\text{m}^{-1}$. At high densities, the function does not fully decorrelate within the observation window, indicating arrested dynamics. The exponential fit is superimposed. Inset: static structure factor $S(q)$. **b)** Exponential fit timescales as a function of density. Note that for the highest densities, the extracted timescales exceed the measurement window and should be interpreted with caution. **c)** Normalized integrated relaxation time t_i . For densities above 40 OD, arrested dynamics on this time window is observed.

3.5.2 Nematic alignment in the passive state

The ISF probes the relaxation of translational density fluctuations, but memory in the velocity field is expected to originate from the persistence of *orientational* order. To directly assess whether bacterial orientations are frozen in the passive state, we analyzed the local alignment using the structure tensor algorithm described in Appendix A.2.2. A coarse-grained director field $\hat{\mathbf{n}}(\mathbf{r}, t)$ was extracted from the brightfield images with the same window size and overlap as the PIV analysis. Importantly, unlike the velocity field, the nematic director can be reliably extracted even in the deep passive state, since it depends only on image gradients rather than on frame-to-frame displacements (Figure 3.13a).

We define the nematic overlap function:

$$F_n(\tau) = \langle \cos(2\theta(\mathbf{r}, \tau)) \rangle \quad \text{with} \quad \theta(\mathbf{r}, \tau) = \arccos[\hat{\mathbf{n}}(\mathbf{r}, t^*) \cdot \hat{\mathbf{n}}(\mathbf{r}, \tau + t^*)] \quad (3.13)$$

where t^* is a reference time chosen as the time that maximizes the Eulerian velocity correlation (Eq. (3.6)) with respect to t_0 , which corresponds to the earliest frame at which the speed is still well defined. The factor of 2 accounts for the head-tail symmetry of the nematic field.

As shown in Figure 3.13b, $F_n(\tau)$ exhibits a rapid initial decay to a plateau value in the passive state, followed by further decorrelation upon reactivation as active stresses randomize bacterial orientations. The plateau value, which we term the **freezing parameter**, quantifies the degree to which orientational order is preserved in the passive state. It is important to notice that this parameter is not well defined for low densities, for which the evaluation is done by averaging the function over 10 s before light is turned on again. This parameter does not depend appreciably on t_w , as can be observed in Figure A.6 in Appendix, confirming that it is an intrinsic property of the passive substrate.

The freezing parameter increases monotonically with density (Figure 3.13c), presenting a slower increase for densities above 40 OD. The relationship between the freezing parameter and the Lagrangian memory is shown in Figure 3.13d. A transition in memory is observed at a freezing parameter value of 0.37(4) with a width of 0.04(3), where the associated error is extracted from a fit with a sigmoidal function. When the nematic overlap is high enough, orientational information from the active state is preserved, which is sufficient to bias the reactivation dynamics and produce a measurable memory. For low values, orientational relaxation in the passive state is fast enough to erase the directional information before reactivation can probe it.

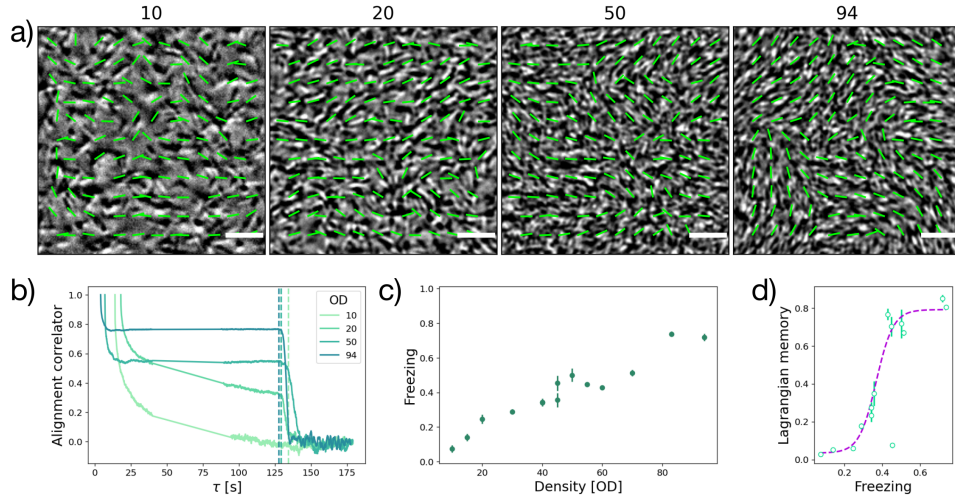


Figure 3.13. Nematic relaxation and freezing parameter. **a)** Nematic director field superimposed on preprocessed images for different densities at $t = 4$ s from the initial sampling (scale bar: 10 μ m). **b)** Nematic overlap function $F_n(\tau)$: a rapid decay to a plateau is followed by further decorrelation upon reactivation (dashed line). The plateau value (freezing parameter) increases with density. **c)** Density dependence of the freezing parameter. A monotonic behaviour can be observed with density, reaching a solid value for density bigger than 40 OD. Error bars represent the average uncertainty across repetitions. **d)** Freezing parameter versus Lagrangian memory with superimposed sigmoidal fit. A sharp transition in memory occurs at a freezing parameter of 0.37(4) with a width of 0.04(3), indicating that memory requires a threshold level of structural arrest.

3.6 Conclusions and perspectives

The experiments presented in this Chapter demonstrate that bacterial active turbulence exhibits directional memory under temporal modulation of activity. The velocity field after reactivation retains significant directional correlation with its pre-quench configuration. The strength of this memory depends on bacterial density, with a transition occurring at 45 OD. Importantly, this threshold lies well above the onset density for active turbulence (20 OD), demonstrating that memory is not a consequence of collective motion itself but requires an additional ingredient. This ingredient is structural arrest in the passive state. Two independent lines of evidence support this conclusion. First, the intermediate scattering function exhibits arrested relaxation at high densities, with relaxation times exceeding the observation window above 40 OD. Second, the nematic director field retains substantial orientational order in the passive state, as quantified by the freezing parameter, which grows slowly above 40 OD. Several questions remain open for future investigation.

The role of tumbling in modulating memory is an important open direction. Previous studies [144, 145] have shown that the fraction of tumbling bacteria alters the strength of collective motion. Characterizing how memory depends on the tumbling rate would clarify whether the relevant structural arrest occurs at the level of individual bacterial orientations or at the level of the collective nematic order.

Direct microscopic validation through tracking of fluorescent bacteria would enable measurement of single-particle orientations in the passive state, providing a direct test of the structural-arrest hypothesis at the individual-bacterium level.

Finally, the ability to retain and read out directional memory suggests the possibility of designing optimal temporal protocols—analogous to swift protocols in stochastic thermodynamics—that exploit the memory effect to guide the recovery of collective motion along prescribed directions. Such protocols could combine the spatial control demonstrated in Chapter 2 with the temporal modulation presented here, enabling full spatiotemporal control of active turbulence.

Chapter 4

Conclusions and perspectives

This thesis set out to investigate what happens to the collective dynamics of a dense bacterial suspension when the activity that sustains it is no longer held constant but is instead modulated (spatially in one set of experiments, temporally in the other). The two studies were conceived independently, yet the results converge on a single physical insight: in dense active systems, the structural degrees of freedom of the bacterial population in the absence of activity play a decisive role in shaping the collective response.

Summary of results

The virtual confinement experiments of Chapter 2 established that spatially patterned illumination can substitute for solid boundaries in selecting coherent flow structures. Circular active regions embedded in a non-motile background develop vortex dynamics whose spatial organization mirrors that observed in microfluidic chambers, despite the absence of any physical wall. Two important differences from physical confinement should be emphasised. First, virtual confinement is inherently transient, since depletion of the illuminated region progressively suppresses collective motion. Second, the soft, permeable boundary supports a pronounced radial alignment of cells at the interface, in contrast to the tangential alignment imposed by rigid walls. The azimuthal decomposition of the vorticity field provided a quantitative criterion for confinement: below a crossover radius of approximately $60\text{ }\mu\text{m}$, the flow is dominated by coherent rotation, while above it the mode spectrum becomes flat and indistinguishable from that of unconfined turbulence.

The temporal modulation experiments of Chapter 3 revealed that dense bacterial turbulence possesses a form of history dependence. When activity is removed and later restored, the recovery is not memoryless: the reactivated flow field is partially aligned with the flow that preceded the quench, and the reactivation timescale grows with the duration of the passive interval. The directional memory is absent at low densities and emerges sharply above approximately $45(2)\text{ OD}$. The nematic overlap analysis identified the structural origin of this memory: at sufficiently high packing fractions, bacterial orientations become arrested

on experimental timescales during the passive state, preserving a structural template that biases the reactivated flow.

Connecting the two experiments

Although the spatial and temporal experiments probe different aspects of the system, they are connected by the central role of density. In the virtual confinement geometry, it is the dense packing at the boundary that governs the escape dynamics and the orientational order of the interface, effects that a dilute system would not exhibit. In the temporal modulation protocol, it is again the dense packing that arrests structural relaxation in the passive state and thereby encodes the directional information that constitutes memory. In both cases, density acts as a mediator between the active driving force and the collective response.

This connection also highlights a tension. The spatial experiments demonstrate that dense packing at a virtual boundary expels bacteria efficiently, depleting the active region. The temporal experiments show that the same dense packing, once activity is removed, freezes orientations and preserves information. The passive suspension is thus simultaneously a sink for active particles (in the spatial context) and a memory substrate (in the temporal context). Reconciling these two roles and potentially exploiting both in a single protocol is an open challenge.

Scope and limitations

Several aspects of this work warrant explicit discussion of their limitations. The virtual confinement experiments were performed at a single bacterial density (80 OD₆₀₀) and a single illumination intensity. How the crossover radius depends on these parameters remains to be established. The transient nature of the confinement also prevented the study of long-time statistical properties of the confined flow, such as vortex reversal rates and their dependence on geometry.

In the temporal modulation experiments, the Lagrangian and Eulerian estimates of the memory yield different transition densities (45(2) and 60(12) OD, respectively) and a different sharpness of the transition. This suggests that the transition density may depend on the observable used to probe it. A more systematic investigation, with finer density sampling near the transition and the inclusion of physical tracer particles, would be needed to establish the nature of the transition.

More fundamentally, the structural-arrest mechanism proposed here rests on indirect evidence: the bacterial orientations are inferred from image gradients rather than measured at the single-cell level. While the consistency of three independent measurements makes the conclusion robust, a direct microscopic validation remains desirable.

Future directions

The most natural extension of this work is to combine the spatial and temporal control modalities into fully spatiotemporal protocols. The discovery of directional memory suggests a concrete strategy: by exploiting orientational arrest in the passive state, one could design illumination sequences that first imprint a desired nematic pattern and then reactivate the system to recover collective motion along prescribed directions. Such protocols would constitute an active-matter analogue of the swift protocols developed in stochastic thermodynamics for optimal driving of colloidal systems, with the crucial difference that the control parameter acts on activity rather than on an external potential.

Several specific open questions could guide this program:

Microscopic validation of structural arrest. Tracking fluorescently labelled bacteria in the passive state would test directly whether orientational freezing occurs at the single-particle level or is an emergent property of the coarse-grained nematic field.

Role of tumbling. Restoring tumbling (e.g. by using a CheY^+ strain) at fixed density would disentangle single-particle orientational relaxation from collective nematic relaxation. If memory is controlled by single-bacterium orientations, tumbling should substantially weaken or destroy it. If, instead, memory is controlled by the collective nematic order, tumbling should have a much smaller effect, since the many-body nematic alignment survives individual reorientations.

Directional control of the fluid. A real-time feedback scheme that shapes the illumination intensity according to the local direction of the velocity field could rectify the collective motion, sustaining coherent transport without the depletion that limits the static modulation explored in Chapter 2.

Appendix A

Appendix

A.1 Experimental equipment

A.1.1 Bacterial strain

The light-driven smooth-swimming *Escherichia coli* strain used in this work is derived from the wild-type strain AB1157. For the creation of the smooth-swimmer strain two genomic deletions were introduced using λ -Red recombination with the recombination plasmid pKD46 [146].

The first deletion removes the *unc* operon, which encodes the F_1F_0 -ATP synthase complex. The *unc* operon was replaced with the chloramphenicol resistance cassette (CmR), amplified from template pKD3 using the following primers:

- Forward: GCGATCGCTTTGGCCAGTTCCGCAGACGCCTGAGCGTAATCTACG
TCGCCGTGTAGGCTGGAGCTGCTTC
- Reverse: CGGCATACCTCGAAGGGAGCAGGAGTGAAAAACGTGATGTCTGTG
TCGCTATGGGAATTAGCCATGGTCC

The second deletion removes the *cheY* gene, which encodes the chemotaxis response regulator responsible for flagellar motor switching. Its deletion produces a smooth-swimming phenotype in which bacteria run without tumbling. The *cheY* gene was replaced with the kanamycin resistance cassette (KnR), amplified from template pKD4 using the following primers:

- Forward: GCAAAAATTAGTGCCGGACAGGCGATACGTATTTAAATCAGGAG
TGTGAAGTGTAGGCTGGAGCTGCTTC
- Reverse: CTGAATGCTCGTCAGCAGGTTTGATTGATGGTTGCATCATAGTCG
CATCCATGGGAATTAGCCATGGTCC

Both deletions were confirmed by PCR. The resulting strain is AB1157 $\Delta unc::cmR$ $\Delta cheY::knR$, hereafter referred to as AUY.

For the experiments described in Chapter 3, an additional deletion of the *fim* operon was performed to eliminate type I fimbriae. To introduce this third deletion, the CmR and KnR antibiotic resistance cassettes employed for AUY were first removed using the FLP recombinase encoded by plasmid pCP20 [147]. The *fim* operon was then deleted through λ -Red recombination using the following primers to amplify the KnR cassette from plasmid pKD4:

- Forward: GCTAACCCAGCACAGCTAGTGCGCGTCTGTAATTATAAGGGAAAA
ACGATGTGTAGGCTGGAGCTGCTTC
- Reverse: TTAGCTTCAGGTAATATTGCGTACCAGCATTAGCAATGTCCTGTG
ATTCATGGGAATTAGCCATGGTCC

The resulting strain, which lacks type I fimbriae, is referred to as AUYB.

Photokinetic behaviour was conferred by transforming these bacteria with the plasmid SAR86p γ , which encodes proteorhodopsin under the regulation of the arabinose-inducible promoter AraC [148]. Functional reconstitution of the light-driven proton pump requires the addition of both L-arabinose (to induce gene expression) and all-*trans* retinal (the chromophore cofactor), as described in the growth protocol below. The transformed strains are identified as AUYG and AUYBG.

A.1.2 Growth protocol

Bacteria were cultured overnight (approximately 16 hours) in Terrific Broth (TB), a nutrient-rich growth medium containing 1.2% tryptone, 2.4% yeast extract, and 0.5% glycerol. The overnight culture was then diluted 1:100 in fresh TB. All-*trans*-retinal (20 μ M) and L-arabinose (1 mM) were added 4 hours post-dilution to ensure proper expression and folding of proteorhodopsin in the cell membrane.

After 6 hours of total growth, bacteria were harvested by centrifugation for 8 minutes at 700 rcf. The pellet was resuspended in 700 μ L of motility buffer. Two different buffer compositions were used depending on the experiment:

Chapter 2 (virtual confinement): double-distilled H₂O with Tween 20 (0.02% v/v).

Chapter 3 (temporal modulation): double-distilled H₂O, phosphate buffer (0.01 M, pH 7.0), EDTA (0.1 mM), and Tween 20 (0.02% v/v).

Tween 20 serves as a surfactant to minimise bacterial adhesion to the glass surfaces in both cases.

Following a 20 min equilibration period, the suspension was transferred to a microcentrifuge tube and centrifuged again for 8 minutes at 700 rcf. The final pellet was resuspended in the appropriate motility buffer at the desired concentration, measured as optical density at 600 nm (OD₆₀₀) measured with the photometer (NanoPhotometer® NP80).

A.1.3 Microscope

The experimental setup is illustrated in Figure A.1. It consists of a custom-built inverted microscope with two independent illumination paths: one for brightfield imaging and one for photokinetic activation of bacteria.

Imaging illumination. Brightfield imaging illumination is provided from above by a red LED M625L4 (Thorlabs M625L4, $\lambda = 625$ nm). This wavelength was chosen to lie outside the absorption spectrum of proteorhodopsin, ensuring that the imaging light does not activate bacteria.

Activation illumination. The light used to activate proteorhodopsin is delivered through two optical paths:

From above: A green LED (Thorlabs M520L4, $\lambda = 520$ nm) is coupled to the imaging path using a beamsplitter. This path provides spatially uniform illumination over an area much larger than the field of view and is used for the temporal modulation experiments described in Chapter 3.

From below: A green LED (Thorlabs M520L2, $\lambda = 532$ nm) is coupled to a Digital Micromirror Device (DMD, DLP5000, Texas Instruments). The DMD enables the projection of spatially structured illumination patterns with a refresh rate of 30 fps. After bandpass filtering at 520 nm, the activation beam passes through a tube lens and is projected onto the sample plane via a dichroic mirror that couples it to the objective. This path is used for the virtual confinement experiments described in Chapter 2.

A long-pass filter placed in front of the camera blocks the green activation light, allowing brightfield imaging without contamination from the activation wavelength. The objective used for both experiments is a Nikon 20 $\times$, NA = 0.50.

The camera is a high-sensitivity CMOS sensor (Hamamatsu Orca-Flash 4.0) with an image resolution of 2048 pixel $\times$ 2048 pixel.

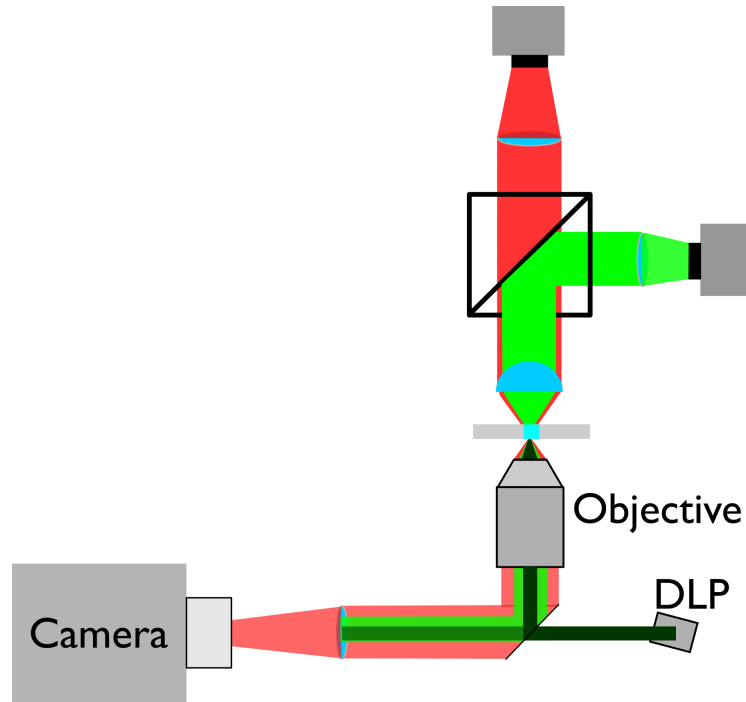


Figure A.1. Experimental setup. Custom-built inverted microscope with two independent green-light activation paths for proteorhodopsin: uniform illumination from above (used for temporal modulation experiments) and spatially structured illumination from below via a DMD projected through the objective via a dichroic mirror (used for virtual confinement experiments). Red illumination from above provides brightfield imaging. The two activation wavelengths (520 nm and 532 nm) are rendered in different shades of green for clarity.

A.2 Data analysis

A.2.1 Particle Image Velocimetry

Particle Image Velocimetry (PIV) is a well-established technique for measuring velocity fields in fluid systems [149]. The method exploits the displacement of intensity features between consecutive image frames to extract the underlying flow field.

The algorithm proceeds as follows (Figure A.2). Each image is divided into a grid of square interrogation windows, with a specified overlap between adjacent windows. For each window, the spatial cross-correlation between the corresponding regions in two consecutive frames is computed via the Fast Fourier Transform. The location of the correlation peak identifies the most probable displacement of the intensity pattern within that window. The displacement is then converted to a velocity by dividing by the time interval between frames.

In our implementation, the interrogation window size and overlap are specified separately for each experiment (see the corresponding sections in Chapters 2 and 3). The bacteria themselves serve as tracer particles, as their density is sufficiently high to provide adequate seeding of the flow field. Sub-pixel accuracy is achieved by fitting a Gaussian function to the correlation peak.

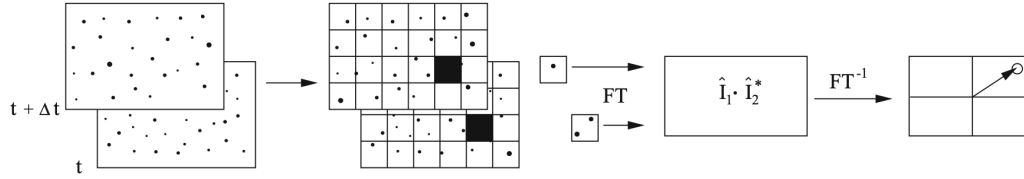


Figure A.2. Particle Image Velocimetry workflow. Two consecutive frames are divided into overlapping interrogation windows. For each window, the cross-correlation function is computed via the Fourier transform. The peak of the correlation identifies the most probable displacement, which is converted to a velocity by dividing by the inter-frame time interval.

A.2.2 Local orientation extraction

The local nematic orientation of bacteria is extracted from brightfield images using a gradient-based structure tensor method. Given an image $I(\mathbf{r})$, the normalized image gradient is computed as:

$$\hat{\mathbf{t}} = \frac{\nabla I}{|\nabla I|}$$

This vector is oriented along the boundary of intensity features (i.e., perpendicular to elongated objects). A 90° rotation yields the vector $\hat{\mathbf{r}} = (t_y, -t_x)$, which is aligned along the long axis of the bacterium. From this vector, the local nematic tensor is constructed as:

$$\mathbf{J} = \hat{\mathbf{r}} \otimes \hat{\mathbf{r}} = \begin{pmatrix} t_y^2 & -t_y t_x \\ -t_y t_x & t_x^2 \end{pmatrix}$$

A coarse-grained nematic tensor $\mathbf{Q}$ is obtained by averaging $\mathbf{J}$ over a square subwindow of specified size:

$$\mathbf{Q} = \langle \mathbf{J} \rangle_{\text{window}}$$

The eigenvalues of $\mathbf{Q}$ are $\lambda_1 \geq \lambda_2 \geq 0$, and the eigenvector associated with the smaller eigenvalue λ_2 defines the direction orthogonal to the gradients, therefore the local nematic director $\hat{\mathbf{n}}$.

A.2.3 Vortex identification

Vortex-dominated regions in the velocity field are identified using the Okubo–Weiss criterion, which evaluates the second invariant of the velocity gradient tensor $\nabla \mathbf{u}$:

$$Q = \frac{1}{2} (|\mathbf{\Omega}|^2 - |\mathbf{\Sigma}|^2)$$

where $\mathbf{\Omega} = \frac{1}{2}(\nabla \mathbf{u} - \nabla \mathbf{u}^T)$ is the antisymmetric (vorticity) part and $\mathbf{\Sigma} = \frac{1}{2}(\nabla \mathbf{u} + \nabla \mathbf{u}^T)$ is the symmetric (strain-rate) part of the velocity gradient tensor. The matrix norm is defined as $|\mathbf{A}|^2 = A_{ij}A_{ij}$ (summation over repeated indices implied). Note that, with respect to the original Okubo–Weiss formulation, the sign convention is inverted so that vortex-dominated regions correspond to $Q > 0$ (rotation exceeds strain), while regions where $Q < 0$ are strain-dominated.

A.3 Supplementary plots for temporal modulation observables

Figures A.3–A.6 present the full density-dependent datasets for the temporal modulation experiments described in Chapter 3. These supplementary plots complement the representative single-density analyses shown in the main text by displaying the raw data for all densities investigated.

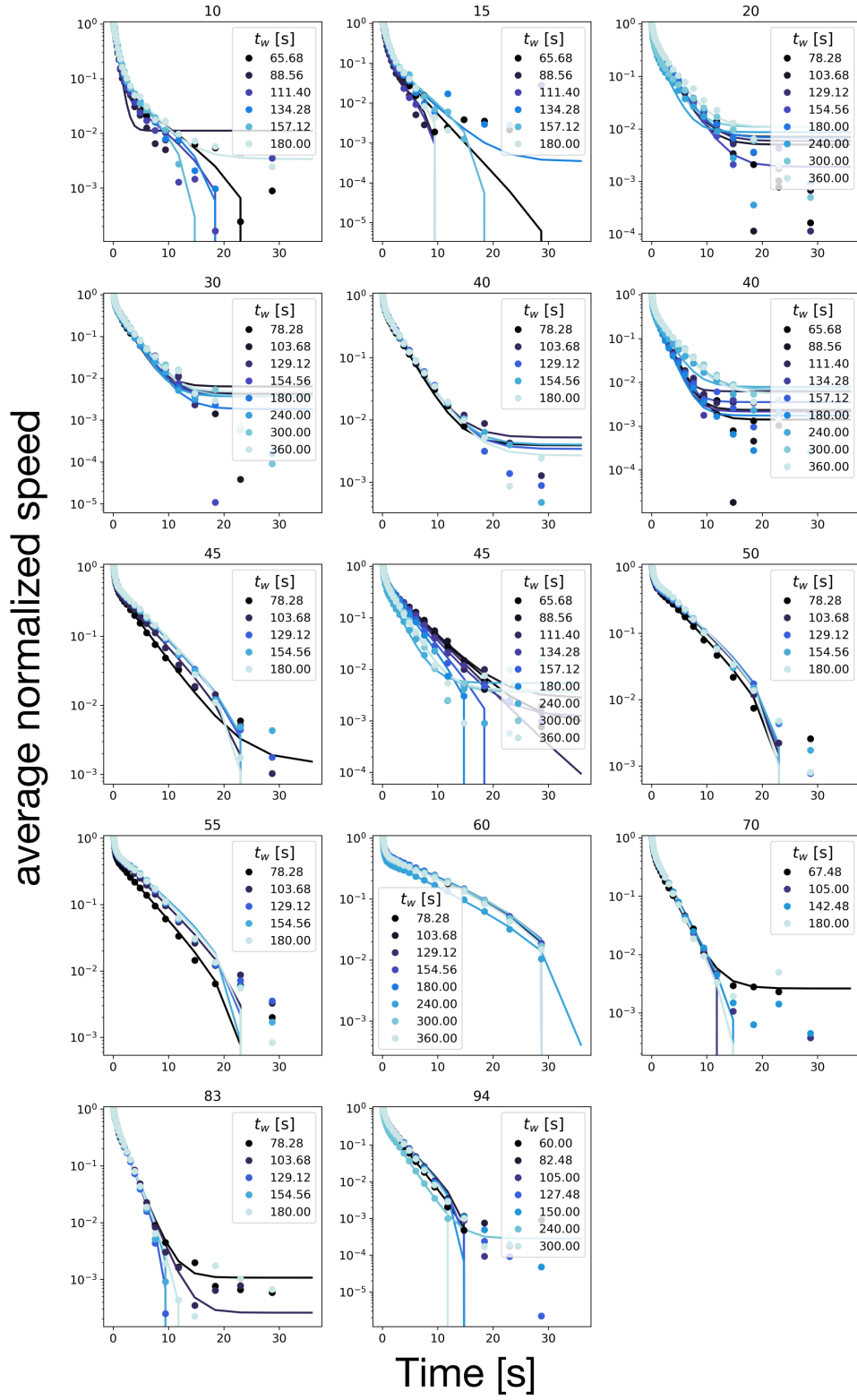


Figure A.3. Speed decay following light removal for all densities. The title represents the density expressed in OD₆₀₀ and the legend the waiting time t_w .

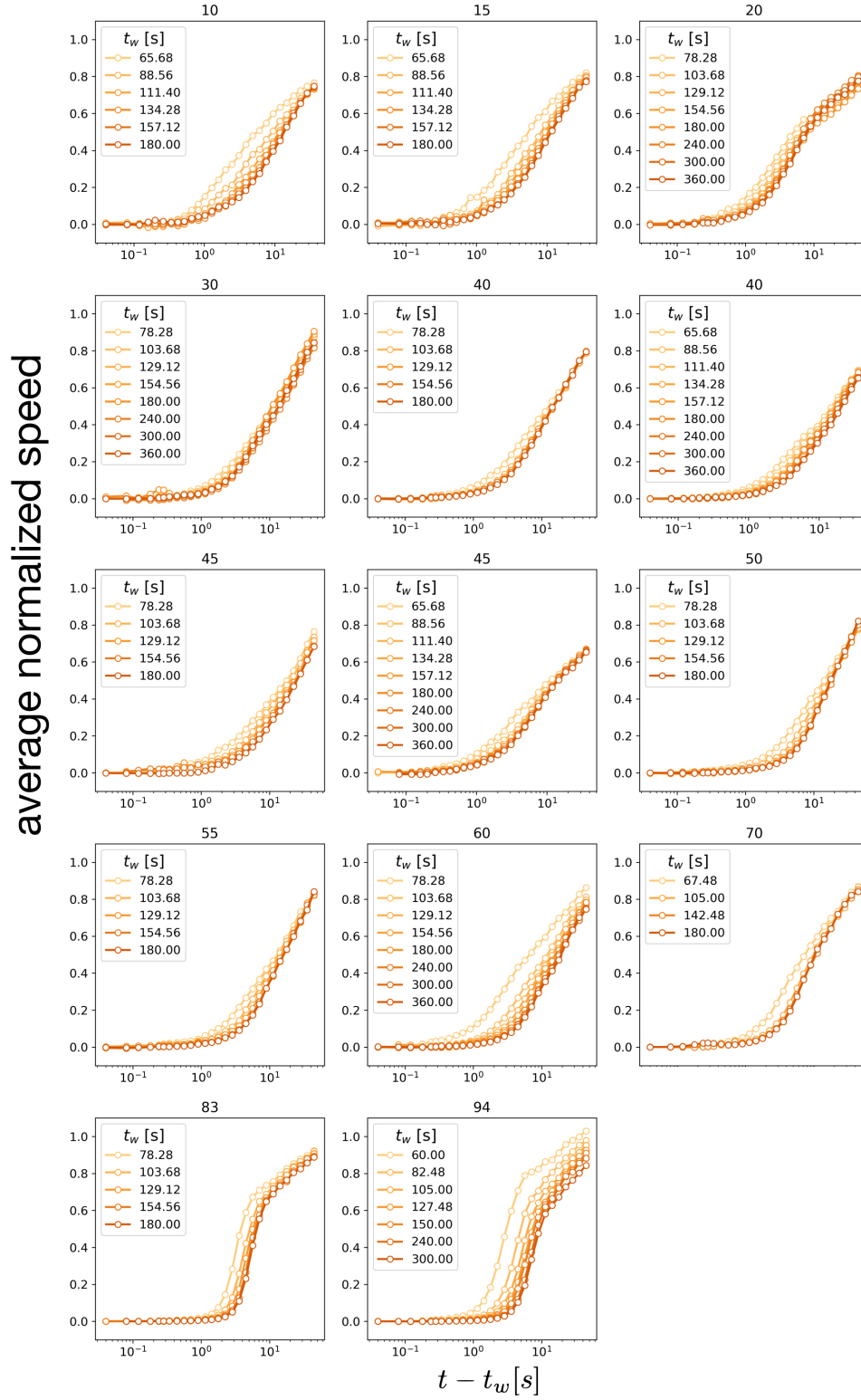


Figure A.4. Speed recovery following reactivation for all densities. The title represents the density expressed in OD₆₀₀ and the legend the waiting time t_w .

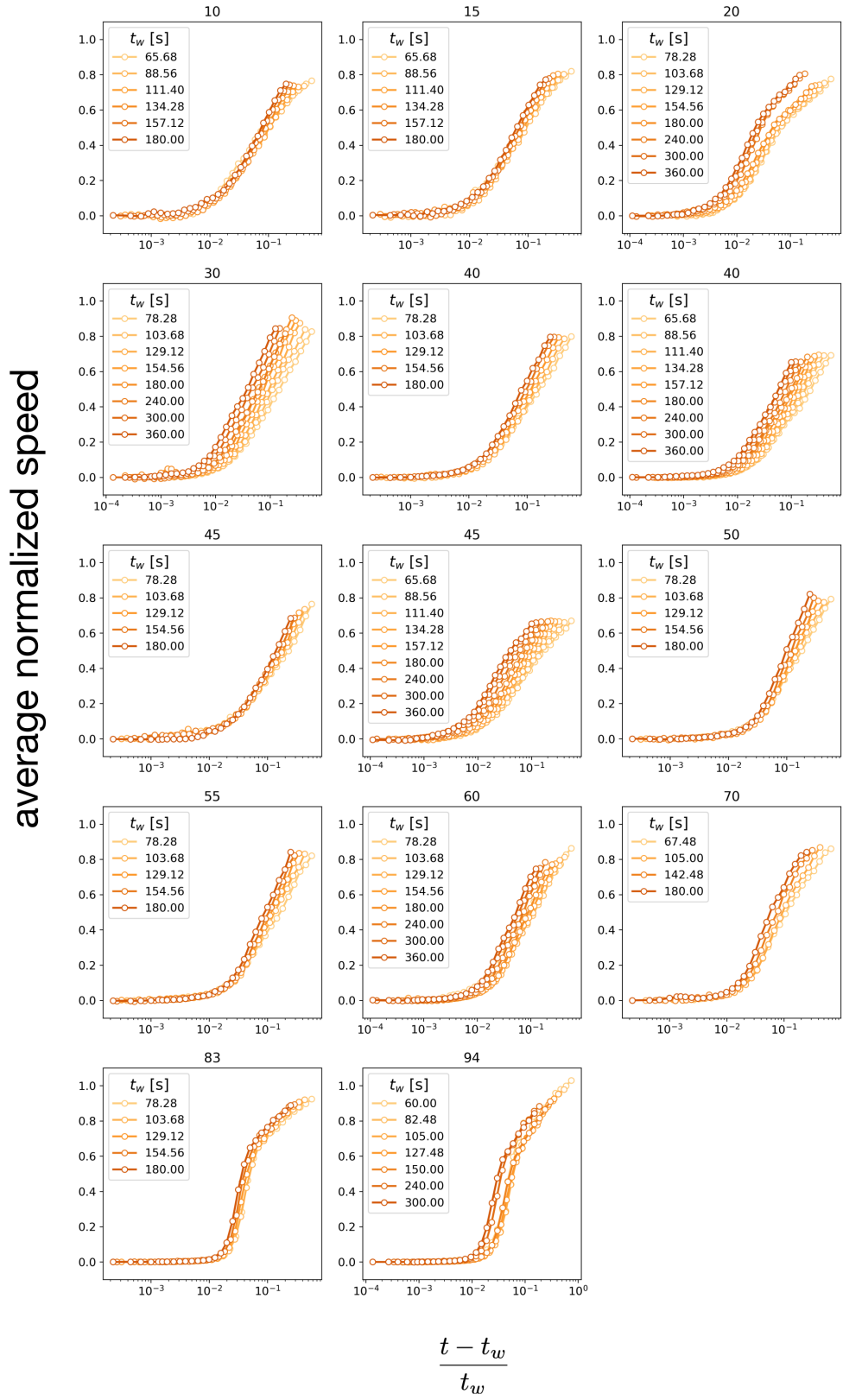


Figure A.5. Speed recovery following reactivation for all densities with normalized timescale. The title represents the density expressed in OD₆₀₀ and the legend the waiting time t_w . The visual inspection across densities confirms that the curves do not collapse onto a single master curve, in agreement with the quantitative analysis of Sec. 3.3.3.

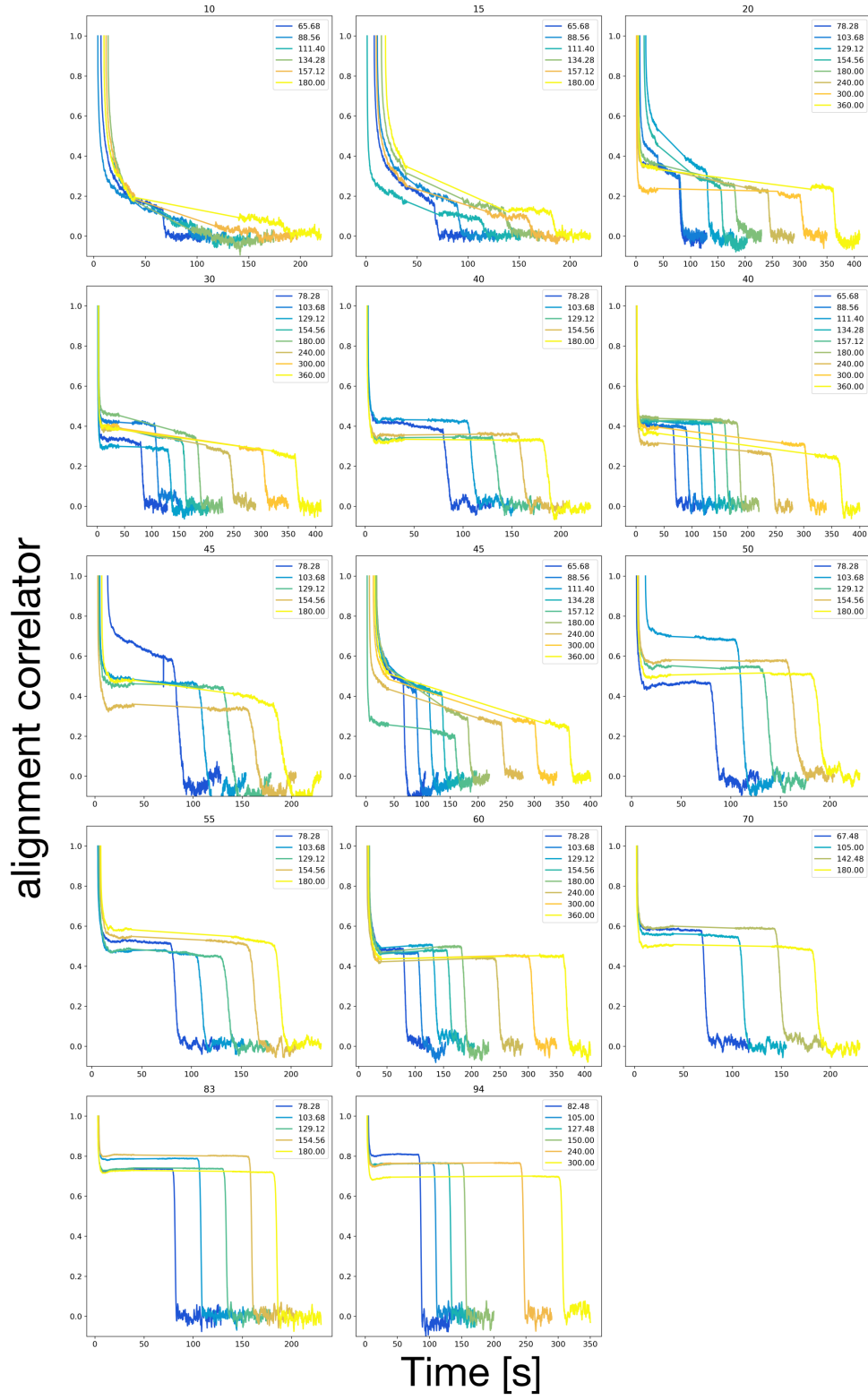


Figure A.6. Nematic overlap function for all densities. The title represents the density expressed in OD₆₀₀ and the legend the waiting time t_w .

Bibliography

1. Vrugt, M. te, Liebchen, B. & Cates, M. E. *What Exactly Is 'Active Matter'?* arXiv: [2507.21621 \[cond-mat\]](#).
2. Bechinger, C. *et al.* Active Particles in Complex and Crowded Environments. *Reviews of Modern Physics* **88**, 045006 (Nov. 23, 2016).
3. Fodor, É. *et al.* How Far from Equilibrium Is Active Matter? *Physical Review Letters* **117**, 038103 (July 13, 2016).
4. Marchetti, M. C. *et al.* Hydrodynamics of Soft Active Matter. *Reviews of Modern Physics* **85**, 1143–1189 (July 19, 2013).
5. Bishop, K. J., Biswal, S. L. & Bharti, B. Active Colloids as Models, Materials, and Machines. *Annual Review of Chemical and Biomolecular Engineering* **14**, 1–30 (June 7, 2023).
6. Hallatschek, O. *et al.* Proliferating Active Matter. *Nature Reviews Physics* **5**, 407–419 (May 31, 2023).
7. Imai, H. *et al.* Direct Observation Shows Superposition and Large Scale Flexibility within Cytoplasmic Dynein Motors Moving along Microtubules. *Nature Communications* **6**, 8179 (Sept. 14, 2015).
8. Ma, X., Hahn, K. & Sanchez, S. Catalytic Mesoporous Janus Nanomotors for Active Cargo Delivery. *Journal of the American Chemical Society* **137**, 4976–4979 (Apr. 22, 2015).
9. Dombrowski, C., Cisneros, L., Chatkaew, S., Goldstein, R. E. & Kessler, J. O. Self-Concentration and Large-Scale Coherence in Bacterial Dynamics. *Physical Review Letters* **93**, 098103 (Aug. 24, 2004).
10. Dreyer, T. *et al.* Comparing Cooperative Geometric Puzzle Solving in Ants versus Humans. *Proceedings of the National Academy of Sciences* **122**, e2414274121 (Jan. 7, 2025).
11. Kline, T. R., Paxton, W. F., Mallouk, T. E. & Sen, A. Catalytic Nanomotors: Remote-Controlled Autonomous Movement of Striped Metallic Nanorods. *Angewandte Chemie International Edition* **44**, 744–746 (Jan. 21, 2005).
12. Turner, L., Ryu, W. S. & Berg, H. C. Real-Time Imaging of Fluorescent Flagellar Filaments. *Journal of Bacteriology* **182**, 2793–2801 (May 15, 2000).

13. Berg, H. C. *E. Coli in Motion* en. 133 pp. ISBN: 978-0-387-00888-2 (Springer, New York, 2004).
14. DiLuzio, W. R. *et al.* Escherichia Coli Swim on the Right-Hand Side. *Nature* **435**, 1271–1274 (June 2005).
15. Cerbino, R. & Trappe, V. Differential Dynamic Microscopy: Probing Wave Vector Dependent Dynamics with a Microscope. *Physical Review Letters* **100**, 188102 (May 5, 2008).
16. Berg, H. C. The Rotary Motor of Bacterial Flagella. *Annual Review of Biochemistry* **72**, 19–54 (June 2003).
17. Darnton, N. C., Turner, L., Rojevsky, S. & Berg, H. C. On Torque and Tumbling in Swimming *Escherichia Coli*. *Journal of Bacteriology* **189**, 1756–1764 (Mar. 2007).
18. Wadhwa, N. & Berg, H. C. Bacterial Motility: Machinery and Mechanisms. *Nature Reviews Microbiology* **20**, 161–173 (Mar. 2022).
19. Wadhams, G. H. & Armitage, J. P. Making Sense of It All: Bacterial Chemotaxis. *Nature Reviews Molecular Cell Biology* **5**, 1024–1037 (Dec. 2004).
20. Min, T. L., Mears, P. J., Golding, I. & Chemla, Y. R. Chemotactic adaptation kinetics of individual *Escherichia coli* cells. *Proceedings of the National Academy of Sciences* **109**, 9869–9874. eprint: <https://www.pnas.org/doi/pdf/10.1073/pnas.1120218109> (2012).
21. Purcell, E. M. Life at Low Reynolds Number. en, 10 (2014).
22. Happel, J. & Brenner, H. *Low Reynolds Number Hydrodynamics* (red. Moreau, R. J.) ISBN: 978-90-247-2877-0 978-94-009-8352-6 (Springer Netherlands, Dordrecht, 1981).
23. Drescher, K., Dunkel, J., Cisneros, L. H., Ganguly, S. & Goldstein, R. E. Fluid Dynamics and Noise in Bacterial Cell–Cell and Cell–Surface Scattering. *Proceedings of the National Academy of Sciences* **108**, 10940–10945 (July 5, 2011).
24. Guasto, J. S., Johnson, K. A. & Gollub, J. P. Oscillatory Flows Induced by Microorganisms Swimming in Two Dimensions. en. *Physical Review Letters* **105**, 168102 (Oct. 11, 2010).
25. Solon, A. P. *et al.* Pressure Is Not a State Function for Generic Active Fluids. en. *Nature Physics* **11**, 673–678 (Aug. 2015).
26. Lauga, E., DiLuzio, W. R., Whitesides, G. M. & Stone, H. A. Swimming in Circles: Motion of Bacteria near Solid Boundaries. *Biophysical Journal* **90**, 400–412 (Jan. 2006).
27. Elgeti, J. & Gompper, G. Wall Accumulation of Self-Propelled Spheres. *EPL (Europhysics Letters)* **101**, 48003 (Feb. 1, 2013).
28. Bianchi, S., Saglimbeni, F. & Di Leonardo, R. Holographic Imaging Reveals the Mechanism of Wall Entrapment in Swimming Bacteria. *Physical Review X* **7**, 011010 (Jan. 27, 2017).

29. Sipos, O., Nagy, K., Di Leonardo, R. & Galajda, P. Hydrodynamic Trapping of Swimming Bacteria by Convex Walls. *Physical Review Letters* **114**, 258104 (June 25, 2015).
30. Takaha, Y. & Nishiguchi, D. Quasi-Two-Dimensional Bacterial Swimming around Pillars: Enhanced Trapping Efficiency and Curvature Dependence. en. *Physical Review E* **107**, 014602 (Jan. 9, 2023).
31. Pérez-Estay, B., Cordero, M. L., Sepúlveda, N. & Soto, R. Accumulation and Depletion of *E. Coli* in Surfaces Mediated by Curvature. *Physical Review E* **109**, 054601 (May 1, 2024).
32. Pellicciotta, N., Bagal, O. S., Cannarsa, M. C., Bianchi, S. & Di Leonardo, R. Wall Torque Controls Propulsion of Curved Microstructures in Bacterial Baths. *Physical Review Letters* **135**, 138302 (Sept. 25, 2025).
33. Di Leonardo, R. *et al.* Bacterial Ratchet Motors. *Proceedings of the National Academy of Sciences* **107**, 9541–9545 (May 25, 2010).
34. Sokolov, A., Apodaca, M. M., Grzybowski, B. A. & Aranson, I. S. Swimming Bacteria Power Microscopic Gears. *Proceedings of the National Academy of Sciences* **107**, 969–974 (Jan. 19, 2010).
35. Reichhardt, C. O. & Reichhardt, C. Ratchet Effects in Active Matter Systems. *Annual Review of Condensed Matter Physics* **8**, 51–75 (Mar. 31, 2017).
36. Vizsnyiczai, G. *et al.* Light Controlled 3D Micromotors Powered by Bacteria. *Nature Communications* **8**, 15974 (June 28, 2017).
37. Galajda, P., Keymer, J., Chaikin, P. & Austin, R. A Wall of Funnel Concentrates Swimming Bacteria. *Journal of Bacteriology* **189**, 8704–8707 (Dec. 2007).
38. Vincenti, B. *et al.* Magnetotactic Bacteria in a Droplet Self-Assemble into a Rotary Motor. *Nature Communications* **10**, 5082 (Nov. 8, 2019).
39. Reyes Garza, R., Kyriakopoulos, N., Cenev, Z. M., Rigoni, C. & Timonen, J. V. I. Magnetic Quincke Rollers with Tunable Single-Particle Dynamics and Collective States. *Science Advances* **9**, eadh2522 (June 30, 2023).
40. Schüler, D. & Frankel, R. B. Bacterial Magnetosomes: Microbiology, Biomineralization and Biotechnological Applications. *Applied Microbiology and Biotechnology* **52**, 464–473 (Oct. 21, 1999).
41. Guillamat, P., Ignés-Mullol, J. & Sagués, F. Control of Active Liquid Crystals with a Magnetic Field. *Proceedings of the National Academy of Sciences* **113**, 5498–5502 (May 17, 2016).
42. Akolpoglu, M. B. *et al.* Magnetically Steerable Bacterial Microrobots Moving in 3D Biological Matrices for Stimuli-Responsive Cargo Delivery. *Science Advances* **8**, eabo6163 (July 15, 2022).

43. Jin, D. & Zhang, L. Collective Behaviors of Magnetic Active Matter: Recent Progress toward Reconfigurable, Adaptive, and Multifunctional Swarming Micro/Nanorobots. *Accounts of Chemical Research* **55**, 98–109 (Jan. 4, 2022).
44. Beppu, K. & Timonen, J. V. I. Magnetically Controlled Bacterial Turbulence. *Communications Physics* **7**, 216 (July 4, 2024).
45. Di Leonardo, R. *et al.* Active Billiards: Engineering Boundaries for the Spatial Control of Confined Active Particles. *Proceedings of the National Academy of Sciences* **122**, e2426715122 (Sept. 23, 2025).
46. Mourran, A., Zhang, H., Vinokur, R. & Möller, M. Soft Microrobots Employing Nonequilibrium Actuation via Plasmonic Heating. en. *Advanced Materials* **29**, 1604825 (Jan. 2017).
47. Ross, T. D. *et al.* Controlling Organization and Forces in Active Matter through Optically Defined Boundaries. en. *Nature* **572**, 224–229 (Aug. 2019).
48. Zhang, R. *et al.* Spatiotemporal Control of Liquid Crystal Structure and Dynamics through Activity Patterning. en. *Nature Materials* **20**, 875–882 (June 2021).
49. Rey, M., Volpe, G. & Volpe, G. Light, Matter, Action: Shining Light on Active Matter. *ACS Photonics* **10**, 1188–1201 (May 17, 2023).
50. Bèjà, O. *et al.* Bacterial Rhodopsin: Evidence for a New Type of Phototrophy in the Sea. *Science* **289**, 1902–1906 (Sept. 15, 2000).
51. Walter, J. M., Greenfield, D., Bustamante, C. & Liphardt, J. Light-Powering *Escherichia Coli* with Proteorhodopsin. *Proceedings of the National Academy of Sciences* **104**, 2408–2412 (Feb. 13, 2007).
52. Arlt, J., Martinez, V. A., Dawson, A., Pilizota, T. & Poon, W. C. K. Painting with Light-Powered Bacteria. en. *Nature Communications* **9**, 768 (Dec. 2018).
53. Pellicciotta, N. *et al.* Colloidal Transport by Light Induced Gradients of Active Pressure. *Nature Communications* **14**, 4191 (July 13, 2023).
54. Frangipane, G. *et al.* Dynamic Density Shaping of Photokinetic E. Coli. en. *eLife* **7**, e36608 (Aug. 14, 2018).
55. Arlt, J., Martinez, V. A., Dawson, A., Pilizota, T. & Poon, W. C. K. Dynamics-Dependent Density Distribution in Active Suspensions. en. *Nature Communications* **10**, 2321 (Dec. 2019).
56. Schnitzer, M. J. Theory of Continuum Random Walks and Application to Chemotaxis. *Physical Review E* **48**, 2553–2568 (Oct. 1, 1993).
57. Bianchi, S., Saglimbeni, F., Frangipane, G., Cannarsa, M. C. & Di Leonardo, R. Light-Driven Flagella Elucidate the Role of Hook and Cell Body Kinematics in Bundle Formation. *PRX Life* **1**, 013016 (Sept. 19, 2023).
58. Koumakis, N., Maggi, C. & Di Leonardo, R. Directed Transport of Active Particles over Asymmetric Energy Barriers. *Soft Matter* **10**, 5695–5701 (2014).

59. Maggi, C., Angelani, L., Frangipane, G. & Di Leonardo, R. Currents and Flux-Inversion in Photokinetic Active Particles. *Soft Matter* **14**, 4958–4962 (2018).
60. Koumakis, N. *et al.* Dynamic Optical Rectification and Delivery of Active Particles. *Soft Matter* **15**, 7026–7032 (2019).
61. Massana-Cid, H., Maggi, C., Frangipane, G. & Di Leonardo, R. Rectification and Confinement of Photokinetic Bacteria in an Optical Feedback Loop. *Nature Communications* **13**, 2740 (Dec. 2022).
62. Bialek, W. *et al.* Statistical Mechanics for Natural Flocks of Birds. *Proceedings of the National Academy of Sciences* **109**, 4786–4791 (Mar. 27, 2012).
63. Vicsek, T., Czirók, A., Ben-Jacob, E., Cohen, I. & Shochet, O. Novel Type of Phase Transition in a System of Self-Driven Particles. *Physical Review Letters* **75**, 1226–1229 (Aug. 7, 1995).
64. Ginelli, F. The Physics of the Vicsek Model. *The European Physical Journal Special Topics* **225**, 2099–2117 (Nov. 2016).
65. Mermin, N. D. & Wagner, H. Absence of Ferromagnetism or Antiferromagnetism in One- or Two-Dimensional Isotropic Heisenberg Models. *Physical Review Letters* **17**, 1133–1136 (Nov. 28, 1966).
66. Jentsch, P. & Lee, C. F. New Universality Class Describes Vicsek’s Flocking Phase in Physical Dimensions. *Physical Review Letters* **133**, 128301 (Sept. 17, 2024).
67. Martin, D. *et al.* Fluctuation-induced first order transition to collective motion. *Journal of Statistical Mechanics: Theory and Experiment* **2024**, 084003 (Aug. 2024).
68. Cates, M. E. & Tailleur, J. Motility-Induced Phase Separation. *Annual Review of Condensed Matter Physics* **6**, 219–244 (Mar. 1, 2015).
69. Buttinoni, I. *et al.* Dynamical Clustering and Phase Separation in Suspensions of Self-Propelled Colloidal Particles. *Physical Review Letters* **110**, 238301. arXiv: [1305.4185 \[cond-mat\]](#) (June 5, 2013).
70. Palacci, J., Sacanna, S., Steinberg, A. P., Pine, D. J. & Chaikin, P. M. Living Crystals of Light-Activated Colloidal Surfers. *Science* **339**, 936–940 (Feb. 22, 2013).
71. Singh, D. P., Choudhury, U., Fischer, P. & Mark, A. G. Non-Equilibrium Assembly of Light-Activated Colloidal Mixtures. *Advanced Materials* **29**, 1701328 (Aug. 2017).
72. Frisch, U. *Turbulence: The Legacy of A. N. Kolmogorov* (Cambridge University Press, 1995).
73. Aranson, I. Bacterial Active Matter. *Reports on Progress in Physics* (May 23, 2022).
74. Alert, R., Casademunt, J. & Joanny, J.-F. Active Turbulence, 28 (2021).
75. Schnitzer, M. J. & Block, S. M. Kinesin Hydrolyses One ATP per 8-Nm Step. *Nature* **388**, 386–390 (July 24, 1997).
76. Doostmohammadi, A., Ignés-Mullol, J., Yeomans, J. M. & Sagués, F. Active Nematics. *Nature Communications* **9**, 3246 (Aug. 21, 2018).

77. Peng, Y., Liu, Z. & Cheng, X. Imaging the Emergence of Bacterial Turbulence: Phase Diagram and Transition Kinetics. en. *Science Advances* **7**, eabd1240 (Apr. 23, 2021).
78. Creppy, A., Praud, O., Druart, X., Kohnke, P. L. & Plouraboué, F. Turbulence of Swarming Sperm. *Physical Review E* **92**, 032722 (Sept. 23, 2015).
79. Dunkel, J. *et al.* Fluid Dynamics of Bacterial Turbulence. *Physical Review Letters* **110**, 228102 (May 28, 2013).
80. Sokolov, A., Aranson, I. S., Kessler, J. O. & Goldstein, R. E. Concentration Dependence of the Collective Dynamics of Swimming Bacteria. en. *Physical Review Letters* **98**, 158102 (Apr. 11, 2007).
81. Alert, R., Joanny, J.-F. & Casademunt, J. Universal Scaling of Active Nematic Turbulence. en. *Nature Physics* **16**, 682–688 (June 2020).
82. Martínez-Prat, B. *et al.* Scaling Regimes of Active Turbulence with External Dissipation. *Physical Review X* **11**, 031065 (Sept. 23, 2021).
83. Lemma, L. M., DeCamp, S. J., You, Z., Giomi, L. & Dogic, Z. Statistical Properties of Autonomous Flows in 2D Active Nematics. *Soft Matter* **15**, 3264–3272 (2019).
84. Hillebrand, M. & Alert, R. Discontinuous Transition to Active Nematic Turbulence. *Nature Communications* **16**, 11169 (Dec. 15, 2025).
85. Wensink, H. H. *et al.* Meso-Scale Turbulence in Living Fluids. *Proceedings of the National Academy of Sciences* **109**, 14308–14313 (Sept. 4, 2012).
86. Nishiguchi, D. *Order and Fluctuations in Collective Dynamics of Swimming Bacteria: Experimental Exploration of Active Matter Physics* ISBN: 978-981-329-997-9 978-981-329-998-6 (Springer Singapore, Singapore, 2020).
87. Guillamat, P., Ignés-Mullol, J. & Sagués, F. Taming Active Turbulence with Patterned Soft Interfaces. *Nature Communications* **8**, 564 (Dec. 2017).
88. Martínez-Prat, B., Ignés-Mullol, J., Casademunt, J. & Sagués, F. Selection Mechanism at the Onset of Active Turbulence. *Nature Physics* **15**, 362–366 (Apr. 2019).
89. Wioland, H., Woodhouse, F. G., Dunkel, J., Kessler, J. O. & Goldstein, R. E. Confinement Stabilizes a Bacterial Suspension into a Spiral Vortex. *Physical Review Letters* **110**, 268102 (June 24, 2013).
90. Lushi, E., Wioland, H. & Goldstein, R. E. Fluid Flows Created by Swimming Bacteria Drive Self-Organization in Confined Suspensions. *Proceedings of the National Academy of Sciences* **111**, 9733–9738 (July 8, 2014).
91. Nishiguchi, D., Shiratani, S., Takeuchi, K. A. & Aranson, I. S. Vortex Reversal Is a Precursor of Confined Bacterial Turbulence. *Proceedings of the National Academy of Sciences* **122**, e2414446122 (Mar. 18, 2025).
92. Beppu, K. *et al.* Geometry-Driven Collective Ordering of Bacterial Vortices. *Soft Matter* **13**, 5038–5043 (2017).

93. Wioland, H., Lushi, E. & Goldstein, R. E. Directed Collective Motion of Bacteria under Channel Confinement. en. *New Journal of Physics* **18**, 075002 (July 1, 2016).
94. Kaiser, A. *et al.* Transport Powered by Bacterial Turbulence. *Physical Review Letters* **112**, 158101 (Apr. 17, 2014).
95. Kaiser, A., Sokolov, A., Aranson, I. S. & Löwen, H. Motion of Two Micro-Wedges in a Turbulent Bacterial Bath. *The European Physical Journal Special Topics* **224**, 1275–1286 (July 2015).
96. Wioland, H., Woodhouse, F. G., Dunkel, J. & Goldstein, R. E. Ferromagnetic and Antiferromagnetic Order in Bacterial Vortex Lattices. *Nature Physics* **12**, 341–345 (Apr. 2016).
97. Nishiguchi, D., Aranson, I. S., Snezhko, A. & Sokolov, A. Engineering Bacterial Vortex Lattice via Direct Laser Lithography. en. *Nature Communications* **9**, 4486 (Dec. 2018).
98. Reinken, H. *et al.* Organizing Bacterial Vortex Lattices by Periodic Obstacle Arrays. en. *Communications Physics* **3**, 76 (Dec. 2020).
99. Keber, F. C. *et al.* Topology and Dynamics of Active Nematic Vesicles. *Science* **345**, 1135–1139 (Sept. 5, 2014).
100. Opathalage, A. *et al.* Self-Organized Dynamics and the Transition to Turbulence of Confined Active Nematics. *Proceedings of the National Academy of Sciences* **116**, 4788–4797 (Mar. 12, 2019).
101. Wu, K.-T. *et al.* Transition from Turbulent to Coherent Flows in Confined Three-Dimensional Active Fluids. *Science* **355**, eaal1979 (Mar. 24, 2017).
102. Hardoüin, J. *et al.* Reconfigurable Flows and Defect Landscape of Confined Active Nematics. *Communications Physics* **2**, 121 (Oct. 4, 2019).
103. Vélez-Cerón, I., Guillamat, P., Sagués, F. & Ignés-Mullol, J. Probing Active Nematics with in Situ Microfabricated Elastic Inclusions. *Proceedings of the National Academy of Sciences* **121**, e2312494121 (Mar. 12, 2024).
104. Vélez-Cerón, I. *et al.* Active nematic pumps. *Proceedings of the National Academy of Sciences* **122**, e2427103122. eprint: <https://www.pnas.org/doi/pdf/10.1073/pnas.2427103122> (2025).
105. Araki, S., Beppu, K., Kabir, A. M. R., Kakugo, A. & Maeda, Y. T. Controlling Collective Motion of Kinesin-Driven Microtubules via Patterning of Topographic Landscapes. *Nano Letters* **21**, 10478–10485 (Dec. 22, 2021).
106. Norton, M. M., Grover, P., Hagan, M. F. & Fraden, S. Optimal Control of Active Nematics. *Physical Review Letters* **125**, 178005 (Oct. 23, 2020).
107. Kurjahn, M. *et al.* Collective Self-Caging of Active Filaments in Virtual Confinement. *Nature Communications* **15**, 9122 (Oct. 23, 2024).
108. Goodman, J. W. *Statistical Optics* 2nd. ISBN: 978-1-119-00945-0 (John Wiley & Sons, Hoboken, NJ, 2015).

109. Toner, J. & Tu, Y. Long-Range Order in a Two-Dimensional Dynamical XY Model: How Birds Fly Together. *Physical Review Letters* **75**, 4326–4329 (Dec. 4, 1995).
110. Toner, J. *The Physics of Flocking: Birth, Death, and Flight in Active Matter* 1st ed. (Cambridge University Press, May 23, 2024).
111. Dunkel, J., Heidenreich, S., Bär, M. & Goldstein, R. E. Minimal Continuum Theories of Structure Formation in Dense Active Fluids. *New Journal of Physics* **15**, 045016. arXiv: [1208.4464 \[cond-mat, physics:physics\]](#) (Apr. 23, 2013).
112. Swift, J. & Hohenberg, P. C. Hydrodynamic Fluctuations at the Convective Instability. en. *Physical Review A* **15**, 319–328 (Jan. 1, 1977).
113. Aditi Simha, R. & Ramaswamy, S. Hydrodynamic Fluctuations and Instabilities in Ordered Suspensions of Self-Propelled Particles. *Physical Review Letters* **89**, 058101 (July 15, 2002).
114. Bratanov, V., Jenko, F. & Frey, E. New Class of Turbulence in Active Fluids. *Proceedings of the National Academy of Sciences* **112**, 15048–15053 (Dec. 8, 2015).
115. Mukherjee, S., Singh, R. K., James, M. & Ray, S. S. Intermittency, Fluctuations and Maximal Chaos in an Emergent Universal State of Active Turbulence. en. *Nature Physics* **19**, 891–897 (June 2023).
116. Mukherjee, S., Kumar, K. & Ray, S. S. *Turbulence-Induced Fluctuating Interfaces in Heterogeneously-Active Suspensions* arXiv: [2502.16443 \[cond-mat\]](#).
117. Worlitzer, V. M. *et al.* Turbulence-Induced Clustering in Compressible Active Fluids. *Soft Matter* **17**, 10447–10457 (2021).
118. Saintillan, D. & Shelley, M. J. Instabilities, Pattern Formation, and Mixing in Active Suspensions. en. *Physics of Fluids* **20**, 123304 (Dec. 2008).
119. Saintillan, D. & Shelley, M. J. Instabilities and Pattern Formation in Active Particle Suspensions: Kinetic Theory and Continuum Simulations. en. *Physical Review Letters* **100**, 178103 (Apr. 29, 2008).
120. Saintillan, D. & Shelley, M. J. Active Suspensions and Their Nonlinear Models. *Comptes Rendus. Physique* **14**, 497–517 (May 15, 2013).
121. Saintillan, D. & Shelley, M. J. en. in *Complex Fluids in Biological Systems* (ed Spagnolie, S. E.) 319–355 (Springer New York, New York, NY, 2015). ISBN: 978-1-4939-2064-8 978-1-4939-2065-5.
122. Saintillan, D. & Shelley, M. J. Orientational Order and Instabilities in Suspensions of Self-Locomoting Rods. en. *Physical Review Letters* **99**, 058102 (July 30, 2007).
123. Škultéty, V., Nardini, C., Stenhammar, J., Marenduzzo, D. & Morozov, A. Swimming Suppresses Correlations in Dilute Suspensions of Pusher Microorganisms. en. *Physical Review X* **10**, 031059 (Sept. 15, 2020).
124. Weady, S., Stein, D. B. & Shelley, M. J. *Thermodynamically Consistent Coarse-Graining of Polar Active Fluids* arXiv: [2203.05869 \[cond-mat, physics:physics\]](#).

125. Jeffery, G. B. The motion of ellipsoidal particles immersed in a viscous fluid. *Proceedings of the Royal Society of London. Series A, Containing Papers of a Mathematical and Physical Character* **102**, 161–179. eprint: <https://royalsocietypublishing.org/doi/pdf/10.1098/rspa.1922.0078> (1922).
126. Talbot, J., Antoine, C., Claudin, P., Somfai, E. & Börzsönyi, T. *Jeffery Orbits with Noise Revisited* arXiv: [2403.06795](https://arxiv.org/abs/2403.06795) [[cond-mat](#), [physics:physics](#)].
127. Wei, D. *et al.* Scaling Transition of Active Turbulence from Two to Three Dimensions. *Advanced Science* **11**, 2402643 (Oct. 2024).
128. Perez-Estay, B. *et al.* *Bacteria Collective Motion Is Scale-Free* arXiv: [2509.15918](https://arxiv.org/abs/2509.15918) [[cond-mat](#)]. Pre-published.
129. Martinez, V. A. *et al.* A Combined Rheometry and Imaging Study of Viscosity Reduction in Bacterial Suspensions. en. *Proceedings of the National Academy of Sciences* **117**, 2326–2331 (Feb. 4, 2020).
130. Chandrakar, P. *et al.* Confinement Controls the Bend Instability of Three-Dimensional Active Liquid Crystals. *Physical Review Letters* **125**, 257801 (Dec. 18, 2020).
131. Lama, H., Yamamoto, M. J., Furuta, Y., Shimaya, T. & Takeuchi, K. A. Emergence of Bacterial Glass. *PNAS Nexus* **3** (ed Nizet, V.) pgae238 (June 28, 2024).
132. Maliet, M., Fix-Boulier, N., Berthier, L. & Deforet, M. *Bacterial Glass Transition* arXiv: [2504.04205](https://arxiv.org/abs/2504.04205) [[cond-mat](#)].
133. Kallas, P. *et al.* Adhesion of Escherichia Coli to Nanostructured Surfaces and the Role of Type 1 Fimbriae. *Nanomaterials* **10** (2020).
134. Pratt, L. A. & Kolter, R. Genetic analysis of Escherichia coli biofilm formation: roles of flagella, motility, chemotaxis and type I pili. *Molecular Microbiology* **30**, 285–293 (1998).
135. Vogeleeer, P., Tremblay, Y. D. N., Mafu, A. A., Jacques, M. & Harel, J. Life on the Outside: Role of Biofilms in Environmental Persistence of Shiga-toxin Producing Escherichia Coli. *Frontiers in Microbiology* **5** (July 1, 2014).
136. Bianchi, S., Donini, G., Cannarsa, M. C., Frangipane, G. & Di Leonardo, R. Dynamic Velocity Response of E. Coli Powered by Proteorhodopsin. en. *Biophysical Reports* **6**, 100254 (June 2026).
137. Ito, K. I., Nakamura, S. & Toyabe, S. Cooperative Stator Assembly of Bacterial Flagellar Motor Mediated by Rotation. *Nature Communications* **12**, 3218 (May 28, 2021).
138. James, M. & Wilczek, M. Vortex Dynamics and Lagrangian Statistics in a Model for Active Turbulence. en. *The European Physical Journal E* **41**, 21 (Feb. 2018).
139. Singh, R. K., Mukherjee, S. & Ray, S. S. Lagrangian Manifestation of Anomalies in Active Turbulence. en. *Physical Review Fluids* **7**, 033101 (Mar. 29, 2022).

140. Forgia, G. L., Cavaliere, D., Espa, S., Falcini, F. & Lacorata, G. Numerical and Experimental Analysis of Lagrangian Dispersion in Two-Dimensional Chaotic Flows. *Scientific Reports* **12**, 7461 (May 6, 2022).
141. Serra, M., Lemma, L., Giomi, L., Dogic, Z. & Mahadevan, L. Defect-Mediated Dynamics of Coherent Structures in Active Nematics. *Nature Physics* **19**, 1355–1361 (Sept. 2023).
142. Wilson, L. G. *et al.* Differential Dynamic Microscopy of Bacterial Motility. en. *Physical Review Letters* **106**, 018101 (Jan. 5, 2011).
143. Martinez, V. *et al.* Differential Dynamic Microscopy: A High-Throughput Method for Characterizing the Motility of Microorganisms. en. *Biophysical Journal* **103**, 1637–1647 (Oct. 2012).
144. Gachelin, J., Rousselet, A., Lindner, A. & Clement, E. Collective Motion in an Active Suspension of *Escherichia Coli* Bacteria. *New Journal of Physics* **16**, 025003 (Feb. 13, 2014).
145. Colin, R., Drescher, K. & Sourjik, V. Chemotactic Behaviour of *Escherichia Coli* at High Cell Density. *Nature Communications* **10**, 5329 (Dec. 2019).
146. Datsenko, K. A. & Wanner, B. L. One-Step Inactivation of Chromosomal Genes in *Escherichia Coli* K-12 Using PCR Products. *Proceedings of the National Academy of Sciences* **97**, 6640–6645 (June 6, 2000).
147. Cherepanov, P. P. & Wackernagel, W. Gene Disruption in *Escherichia Coli*: TcR and KmR Cassettes with the Option of FLP-catalyzed Excision of the Antibiotic-Resistance Determinant. en. *Gene* **158**, 9–14 (Jan. 1995).
148. Tipping, M. J., Steel, B. C., Delalez, N. J., Berry, R. M. & Armitage, J. P. Quantification of Flagellar Motor Stator Dynamics through *in Vivo* Proton-Motive Force Control: PMF-dependent Motor Dynamics. *Molecular Microbiology* **87**, 338–347 (Jan. 2013).
149. Raffel, M. *et al.* *Particle Image Velocimetry* en. ISBN: 978-3-319-68851-0 978-3-319-68852-7 (Springer International Publishing, Cham, 2018).