Transport of active particles in complex environments

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DECLARATION

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration, except as declared in the Preface and specified in the text. It is not substantially the same as any work that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the preface and specified in the text. I further state that no substantial part of my thesis has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. This thesis does not exceed 60,000 words, including summary/abstract, tables, footnotes and appendices. Part of this work has been presented in the following publication:


Theresa Jakuszeit
Many biological systems are comprised of active particles, that is entities which move by consuming energy from their environment such as flocks of fish, cell tissues or bacterial swarms. The interaction of active particles with each other or complex environments, such as porous media and chemical fields, leads to behaviour unlike that of classical equilibrium systems. The present thesis is concerned with the theoretical and experimental investigation of the transport behaviour of active particles to elucidate how it results from their environmental interactions. To investigate the impact of different boundary conditions, I studied the diffusive transport of Active Brownian Particles (ABP) in an obstacle lattice using agent-based simulations. These show that boundary conditions which preserve the particle’s orientation can result in high diffusivities even at high obstacle densities, unlike classical specular reflection. The dynamics are well described by a model based on Run-and-Tumble particles (RTP) with microscopically derived parameters. The study was then extended to investigate the transport of RTP in structured environments in presence of chemical gradients, which bias the active particles in a process termed chemotaxis. Results of this model show how the reduction of chemotaxis in obstacle lattices depends on the boundary condition. To complement theoretical analysis, I investigated bacterial scattering experimentally by tracking wild-type and smooth-swimming mutants of the model bacterium *Escherichia coli* swimming in microfluidic channels with lattices of obstacles. Based on the microscopic analysis of scattering events, the diffusive transport was modelled and compared to the experimental measurements. In a final investigation, I considered how heterogeneities in the environment affect active particle transport. As non-classical surface interactions reduce the effective speed at obstacles, introducing a non-homogenous distribution of obstacles can introduce a spatial dependence of the swimming speed. Combining simulations and preliminary experiments, I show that this introduces a drift towards denser obstacle regions. A spatially varying speed can also be introduced directly by bacteria via chemokinesis, which is a change in swimming speed according to the absolute level of a chemical. I extend a Keller-Segel type model...
to include chemokinesis and apply it to predict the dynamics of bacterial populations in experimentally inspired chemical fields. I find that chemokinesis can not only enhance the chemotactic population response, but also modifies it qualitatively.
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When living things reach thermal equilibrium, they cease to be alive; or as Schrödinger puts it ‘Living matter evades the decay to equilibrium’ [1]. From flocks of sheep to cancer metastasis in the human body, from swarms of bacteria down to the cytoskeleton: the world, both within and around us, is brimming with systems made up of entities that convert free energy from their environment into systematic movement. The term ‘active particles’ is used to describe their perpetual non-equilibrium nature [2]. While this definition does not apply exclusively to living systems, the biological world offers countless examples of active matter. Indeed, a hallmark of life is movement, of which swimming is an important form.

1.1 Microswimmers: Life at low Reynolds numbers

Swimmers move autonomously in a fluid, commonly by periodic changes in their body shape. There are many different microswimmer types, such as bacteria, sperm cells, or synthetic particles, and their unifying characteristic is the size of a couple of microns [3]. The physics of swimming at the microscale, however, is markedly different from the physics of swimming at the macroscale, such as experienced by ourselves. We can gain some understanding of this different environment from the classical Navier-Stokes equation, which describes the flow of a Newtonian fluid with density \( \rho \) and viscosity \( \eta \) as

\[
\rho \left( \frac{\partial \mathbf{v}}{\partial t} + (\mathbf{v} \cdot \nabla)\mathbf{v} \right) = -\nabla p + \eta \nabla^2 \mathbf{v},
\]

(1.1)

where \( \mathbf{v} \) and \( p \) are the fluid velocity and pressure field, respectively [4]. Additionally, the incompressibility assumption requires that the pressure field is chosen such that
the condition
\[ \nabla \cdot \mathbf{v} = 0 \]  
(1.2)
is fulfilled. The flow regime of the Newtonian fluid can be characterised qualitatively by the Reynolds number $\text{Re}$ as the relative importance of inertial forces over viscous forces,
\[ \text{Re} = \frac{f_{\text{inertial}}}{f_{\text{viscous}}} \propto \frac{\rho v^2 L^2}{\eta v L} = \frac{\rho v L}{\eta}, \]  
(1.3)
where $v$ is the mean flow speed and $L$ is a characteristic length scale. For animals like fish or humans, $\text{Re} \gg 1$ and inertia dominates. On the other hand, for a typical microswimmer of length $2 \mu m$ swimming at $20 \mu m/s$ in water, we have $\text{Re} \approx 10^{-5}$. At low Reynolds numbers, the viscous forces dominate and inertia does not play a role. In other words, once a swimmer stops propelling (e.g. stops deforming its body), it does not continue to coast thanks to inertia but stops moving immediately (within $< 1 \mu s$).

When $\text{Re} \to 0$, Eq. (1.1) simplifies to the linear system [5]
\[ 0 = -\nabla p + \eta \nabla^2 \mathbf{v} \]  
(1.4)
\[ \nabla \cdot \mathbf{v} = 0. \]  
(1.5)

This so called Stokes regime does not include any dependence on time $t$ and the flow is, therefore, time reversible. As a direct consequence, Purcell postulated the scallop theorem [6]: any reciprocal motion - that is, any swimmer that changes shape periodically in a manner that is identical under a time-reversal transformation - cannot achieve net propulsion. A simple illustration is an (idealised) scallop. At the macroscopic scale, a scallop moves by first slowly opening and then quickly closing its shell to push out water. At low Reynolds numbers, on the other hand, time does not matter and a microscopic scallop does not accomplish any net propagation. Put differently, the displacement due to opening exactly reverses the displacement achieved by closing.

To circumvent the issue of kinematic reversibility, microswimmers have devised a variety of propulsion mechanisms that break time-reversibility. Bacteria propel themselves by rotating thin helical filaments that resemble a corkscrew and are termed ‘flagella’. While the structure of a flagellum is highly conserved, a broad variety of arrangements and number of flagella is known for different bacterial species [9]. A model organism for studying bacterial motility is the enteric bacterium *Escherichia coli*, whose cell body is about $2 \mu m$ long and $1 \mu m$ in diameter. Typically, cells can have up to 10 flagella that protrude all over their bodies and are approximately
1.1 Microswimmers: Life at low Reynolds numbers

Fig. 1.1 Microswimmer propagation mechanisms. (a) Bacteria are pushed by rotating helical filaments termed flagella. The cell body rotates in the opposite direction to balance the torque from the flagellar rotation. The black arrow indicates the propulsion direction. (b) Algae beat flagella in a breaststroke-like fashion with different power and recovery strokes. Adapted from [7]. (c) Synthetic particles such as Janus particles can propel via an asymmetric chemical reaction on their surface. Adapted from [8] with permission from the Royal Society of Chemistry.

10μm long [10, 11]. When all flagella turn counter-clockwise, the flagella form a bundle at one end of the cell body, which propels the cell. In order to balance the torque imposed by flagellar rotation, the bacterial cell body rotates clockwise during propulsion as illustrated in Fig. 1.1(a) [5]. Microalgae, on the other hand, employ a variety of propulsion mechanisms that differ according to the number of flagella [12]. The most commonly studied green alga Chalmydomonas reinhardti uses two flagella that beat in a breaststroke-like fashion and break time-reversibility by using different power and recovery strokes as shown in Fig. 1.1(b) [13]. Employing more flagella, the propulsion mechanisms of algae become more complicated and have been compared to, for instance, trot and gallop [12]. Beyond the biological world, the quest for man-made and controllable microswimmers has seen the design of a large number of synthetic microswimmers. To create self-propelling objects at low Reynolds numbers, time irreversibility has to be implemented in the propulsion mechanism. For example, Janus particles – aptly named after the double-faced Roman god – are spheres with a surface coating on only one hemisphere [8]. In a bath of ‘fuel’ (e.g. H₂O₂ for platinum coated polystyrene beads [14]) an asymmetric chemical reaction propels the particle via diffusiophoresis as illustrated in Fig. 1.1(c) [8].

The described propulsive mechanisms set up complicated hydrodynamic flows. The resulting hydrodynamic flow fields have been schematically classified into ‘pusher’ and ‘puller’ flow fields. A ‘pusher’ flow field describes active particles such as bacteria, which push the cell body via flagella from the back. The flow field depicted in Fig. 1.2(a) shows that fluid is sucked in from the sides, and is pushed out at the front and back. The flow field of a puller, on the other hand, draws in fluid from the front and back, and pushes it out at the sides. The Stokes equations (1.4) & (1.5) can be used to
describe the hydrodynamic flow theoretically. For example, a simple model is a singular point force in a Stokes flow. The fundamental solution to this problem is the Oseen tensor, also termed Stokeslet, and decays as the inverse of the distance [3]. As the microswimmers considered here are self-propelled, no external force is applied and the net force of the swimmer on the fluid should vanish. The lowest non-zero approximation of the force-free and torque-free swimmer is, therefore, the force dipole [17], which agrees with experimental measurements of flow fields [15]. The sign of the dipole then distinguishes between pusher and puller types. However, point dipoles are difficult to handle numerically. The squirmer model can be used instead to account for finite-size effects in the flow field [3]. In this case, the microswimmer is modelled as a hard sphere with an imposed tangential surface velocity that causes a directed propulsion. These are only two examples since further analytical and numerical approaches have been developed to examine the flow field surrounding microswimmers as discussed, for instance, in [3, 4].

1.2 Stochastic processes: random walks and diffusion

For living systems, movement is not a goal in itself but a means to an end: to escape predators, seek mates, or find food - in short, to explore one’s environment. So how do their propulsive mechanisms enable microswimmers to go about just that? Taking a step back, we can observe the motility of microswimmers from a broader perspective, which is concerned with the pattern rather than the mechanism of motion. At this
scale of abstraction, we realise that active particles perform a persistent random walk that may be biased by environmental factors. The study of random walks can be traced back to the famous work by Brown in 1828 on the erratic motion on pollen grains\textsuperscript{1} [19]. While it took until the early twentieth century to attract significant interest from the physics community, many important fields including random processes have since been developed [20]. Before turning to non-equilibrium systems, it is instructive to first revisit these foundations, in particular classical systems approaching thermodynamic equilibrium.

Let us consider a hard sphere immersed in a fluid, which is subject to a force caused by collisions with the surrounding fluid molecules. The forces acting on the sphere have two sources. There is a deterministic frictional force that is proportional to the velocity of the sphere, and a fluctuating stochastic force arising from fluid-particle collisions. The first systematic part of the force is the hydrodynamic drag, which resists the motion of the sphere, whereas the second stochastic component requires a probabilistic description. The motion of a particle of mass $m$ with instantaneous velocity $v$ can be described by a stochastic version of Newton’s second law of motion

$$\frac{d\mathbf{x}(t)}{dt} = \mathbf{v}(t)$$

$$m \frac{d\mathbf{v}(t)}{dt} = -\gamma \mathbf{v}(t) - \nabla V(\mathbf{x}) + \xi(t),$$

where $V(\mathbf{x})$ is a potential (e.g. gravity) which may be zero and $\xi(t)$ is the stochastic force. This stochastic differential equation (SDE) (1.7) is known as the Langevin equation. The friction coefficient $\gamma$ is given by Stokes law and is the force exerted on a spherical object of radius $R$ dragged through a fluid of viscosity $\eta$

$$\gamma = 6\pi \eta R.$$  

Due to its stochastic nature, each solution of Eq. (1.7) is a different random trajectory given a certain realisation of the noise $\xi(t)$. Yet, in reality, we are unlikely to know $\xi(t)$ in any specific case. We instead consider the averages of the possible realisations, and to this end make assumptions about the nature of the stochastic force. We may assume that the noise is fast and uncorrelated on the time-scale of interest. It is common to assume ‘white noise’, i.e. the fluctuating force has a Gaussian distribution with the

\textsuperscript{1}The phenomenon is known as Brownian motion although Jan Ingen-Housz made similar observations approximately 40 years earlier [18].
moments

\[
\langle \xi(t) \rangle = 0 \tag{1.9a}
\]

\[
\langle \xi_i(t)\xi_j(t') \rangle = \Gamma \delta_{ij} \delta(t-t'), \tag{1.9b}
\]

First, we assume that the noise vanishes on average as the deterministic average has already been extracted in (1.7). Next, Eq. (1.9b) corresponds to the assumption that there is no correlation between the noise at two different times, i.e. the impact experienced at the current time is not influenced by previous impacts. Thus, we assume that the correlation time scale between collisions is vanishingly small. At any given time \( t \), however, the strength of the force is well defined by \( \Gamma \).

To make the connection to diffusion, consider a random walk in 1D with no forcing, i.e. Eq. (1.7) reduces to

\[
m \frac{d^2 x}{dt^2} = -\gamma \frac{dx}{dt} + \xi. \tag{1.10}
\]

Multiplying by \( x \) and simplifying the derivatives yields

\[
\frac{m}{2} \frac{d^2 x^2}{dt^2} - mv^2 = -\frac{\gamma}{2} \frac{dx^2}{dt} + \xi x, \tag{1.11}
\]

where we used \( v = dx/dt \). In equilibrium, the kinetic energy of the Brownian particle should be

\[
\left\langle \frac{m}{2} v^2 \right\rangle = \frac{1}{2} k_B T, \tag{1.12}
\]

where \( k_B \) is Boltzmann’s constant and \( T \) is temperature. Thus, averaging Eq. (1.10) over a large number of particles and asserting that \( \langle \xi x \rangle = \langle \xi \rangle \langle x \rangle = 0 \), we obtain the general solution

\[
\frac{d\langle x^2 \rangle}{dt} = \frac{2k_B T}{\gamma} + C e^{-t/\tau_v}, \tag{1.13}
\]

where \( C \) is an arbitrary constant and \( \tau_v = m/\gamma \) is Einstein’s velocity relaxation time [21]. For time scales much larger than \( \tau_v \), integrating Eq. (1.13) yields

\[
\langle x^2 \rangle - \langle x^2_0 \rangle = \frac{2k_B T}{\gamma} t. \tag{1.14}
\]

A random walk in 1D is a series of independent random displacements, which should combine to a normal distribution, and thus we know that \( \langle (x(t)-x(0))^2 \rangle = \langle x^2 \rangle - \langle x_0^2 \rangle = 2Dt \), where \( D \) is the diffusion coefficient [22]. Using Eq. (1.14), we recover the Stokes-
Einstein equation of diffusion

\[ D = \frac{k_B T}{\gamma}. \]  \hspace{1cm} (1.15)

In Eq. (1.12) we assumed that the system is in thermal equilibrium. The system approaches equilibrium by balancing out the random force with the friction force. The hydrodynamic drag is a dissipative process that turns kinetic energy into heat. At the same time, the sphere moves around with a rapidly-changing velocity because of random collisions with fluid molecules which move faster in a hotter system, i.e. heat energy is turned into kinetic energy. The relation between the two processes can be derived via equipartition theorem as

\[ \Gamma = 2k_B T\gamma, \]  \hspace{1cm} (1.16)

where \( k_B \) is Boltzmann’s constant and \( T \) is absolute temperature (the derivation is beyond the scope of this work but may be found, e.g. in [23]). Eq. (1.16) is an example of the general fluctuation-dissipation theorem, which relates the friction that dampens the motion of the particle (the dissipative force) to the magnitude of the fluctuations caused by the kinetic energy of the system.

It is usually not possible to solve the SDE (1.7) exactly but we can solve the system computationally. For example, the general Langevin equation

\[ \frac{dx(t)}{dt} = f(x(t), t) + \sqrt{2D}\xi(t) \]  \hspace{1cm} (1.17)

may be solved numerically using a first-order finite difference scheme (Euler method)

\[ x(t + \Delta t) = x(t) + f(x(t), t)\Delta t + \sqrt{2D\Delta t}\xi'(t), \]  \hspace{1cm} (1.18)

where \( \xi'(t) \) is drawn from a Gaussian distribution with mean 0 and variance 1. However, this approach can be computationally expensive as we must simulate many trajectories to obtain reliable averages over all possible realisations of \( \xi'(t) \). Alternatively, we can switch to the equivalent description of a Fokker-Planck equation. While the Langevin equation is an SDE of a random variable, the Fokker-Planck equation is a deterministic partial differential equation (PDE) of the probability density function of the random variable: we do not know the exact position of the particle but we may ask what is the probability of finding the particle at position \( x \) at time \( t \) given the initial position \( x_0 \) at time \( t_0 \), \( P(x, t|x_0, t_0) \)? Due to the assumptions about the stochastic force (1.9), the process is both Gaussian and Markovian and, therefore, fully described
by the transition probability. The desired differential equation can be obtained directly from the underlying Langevin equation (1.7) by a Kramers-Moyal expansion, e.g. as discussed in [21],

\[ \frac{\partial P}{\partial t} = -\mathbf{v} \nabla P + \nabla V(x) \frac{\partial P}{\partial \mathbf{v}} + \frac{\partial}{\partial \mathbf{v}} \left[ \gamma \mathbf{v} P + D \frac{\partial P}{\partial \mathbf{v}} \right] \] (1.19)

We now have a deterministic PDE, which can be solved with standard techniques either numerically or analytically.

At the microscopic scale that we are interested in, the velocity relaxation time \( m/\gamma \) is of the order of magnitude of \( 10^{-4} \)s, which is much smaller than the typical experimental time scale. Therefore, inertial effects can be neglected and Eq. (1.7) can be simplified to the overdamped Langevin equation

\[ \frac{dx}{dt} = \mathbf{v} = -\frac{1}{\gamma} \nabla V(x) + \sqrt{2D} \xi(t). \] (1.20)

The corresponding Fokker-Planck equation is

\[ \frac{\partial P}{\partial t} = \frac{1}{\gamma} \nabla (P \nabla V) + D \nabla^2 P, \] (1.21)

which is also known as Smoluchowski equation [21]. If the potential is zero, Eq. (1.21) further simplifies to a special case of Fick’s law of diffusion

\[ \frac{\partial P}{\partial t} = D \nabla^2 P. \] (1.22)

In addition to the approach presented here, there are further ways of deriving a continuous diffusion equation by taking the limits of an uncorrelated, unbiased random walk as reviewed in [20]. In this simplest random walk, the absence of correlation means that the direction of motion only depends on the current location and the process is Markovian. Furthermore, the process is ‘unbiased’ as there is no preferred direction. Conversely, the non-equilibrium nature of active particles introduces correlations into the random walk. For instance, the microswimmers described in the previous section have a tendency to continue moving in the same direction, i.e. there is a correlation in the direction of motion which is termed ‘persistence’. While this introduces a local bias, the global orientation is distributed uniformly in the long term as the effect of the initial orientation subsides over time. However, a random walk is termed ‘biased’ if a global bias is introduced in the orientation, e.g., when animals are foraging in search
1.2 Stochastic processes: random walks and diffusion

Fig. 1.3 Persistent Random Walks. (a) Active Brownian Particle. A particle moves along a persistent direction, which is subjected to rotational noise. In addition, the location of the particle may undergo translational diffusion. This model has been used to describe, for example, the motion of Janus particles and smooth-swimming bacteria. (b) Run-and-Tumble Particle. A particle moves in a straight path, termed ‘run’. These are interspersed by sudden sharp reorientation events termed ‘tumbles’, which are assumed to be instantaneous. This model was first introduced to describe the motility pattern of \textit{E.coli} but some microalgae show similar behaviour [24].

of food. Active particles, thus, require modifications to the random walk model known from passive particles and in the following I will discuss two prominent examples which are central to this thesis. In order to study those non-equilibrium active systems, I will make use of the concepts of Langevin and Fokker-Planck equations as they are not restricted to equilibrium systems.

1.2.1 Active Brownian Particle

Consider a self-propelled sphere that moves with speed $v$ along direction $u$. Similar to the passive Brownian particle, this particle is subject to translational displacement by random collisions with the surrounding fluid molecules. In addition, those collisions also randomize the moving direction $u$ at long times. Thus, the dynamics of an Active Brownian Particle (ABP) follow the Langevin equation

\[
\begin{align*}
\frac{dx(t)}{dt} &= vu(t) + \sqrt{2D_T}\xi_T(t) \\
\frac{du(t)}{dt} &= \sqrt{2D_R}\xi_R(t) \times u(t),
\end{align*}
\]

where $D_T$ and $D_R$ are the translational and rotational diffusion coefficient, respectively. The translational and rotational stochastic forces, $\xi_T$ and $\xi_R$, have been rescaled such
that \( \langle \xi_{T,i}(t)\xi_{T,j}(t') \rangle = \langle \xi_{R,i}(t)\xi_{R,j}(t') \rangle = \delta_{ij} \delta(t - t') \). An ABP performs a persistent random walk with persistence time \( \tau_p = 1/D_R \) and persistence length \( l_p = v \tau_p \). The ABP model has been employed to describe the dynamics of synthetic active particles such as Janus particles as well as smooth-swimming bacteria whose swimming direction performs a random walk in orientation space. In 2D, the direction of movement can be written using the polar angle \( \varphi \) as \( \mathbf{u} = [\cos \varphi, \sin \varphi] \). The system of Eqs. (1.23) can then be transformed into the finite difference scheme

\[
\begin{align*}
    x(t + \Delta t) &= x(t) + v \cos \varphi(t) \Delta t + \sqrt{2D_T \Delta t} \xi_{T,x}(t) \quad (1.24a) \\
    y(t + \Delta t) &= y(t) + v \sin \varphi(t) \Delta t + \sqrt{2D_T \Delta t} \xi_{T,y}(t) \quad (1.24b) \\
    \varphi(t + \Delta t) &= \varphi(t) + \sqrt{2D_R \Delta t} \xi_R(t), \quad (1.24c)
\end{align*}
\]

where \( \xi_{T,x} \), \( \xi_{T,y} \) and \( \xi_R \) are independent realisations of white noise. An example trajectory is shown in Fig. 1.3(a). The corresponding Fokker Planck equation may be written as

\[
\dot{P}(x, u) = -\nabla[vuP] + \nabla_u[D_R \nabla_u P] + \nabla[D_T \nabla P],
\]

where \( \nabla_u \) is the angular gradient [25]. The first term on the right hand side is the advective current caused by the self-propulsion, while the second and last term account for rotational and translational diffusion, respectively.

### 1.2.2 Run-and-Tumble Particle

While synthetic active particles are well captured by the ABP model, many wild-type bacteria show random large reorientation events that are not caused by rotational diffusion. In particular, the motility pattern of \( E. coli \) was the motivation to introduce the Run-and-Tumble Particle (RTP) model [20]. RTPs have two motility modes: a ‘run’ is terminated by a ‘tumble’. During a run, the particle moves at speed \( v \) with constant direction \( \varphi \). Tumbles, on the other hand, are large reorientations of the travelling direction, and are commonly assumed to be instantaneous. The time between tumbling events follows a Poisson distribution with constant rate \( \alpha \). Thus, the probability of a tumbling event is given by \( 1 - \exp(-\alpha t) \). In 2D, the RTP dynamics can then be modelled as
1.3 Interactions with boundaries: breaking time reversibility

\begin{align*}
x(t + \Delta t) &= x(t) + v \cos \varphi(t) \Delta t & \text{(1.26a)} \\
y(t + \Delta t) &= y(t) + v \sin \varphi(t) \Delta t & \text{(1.26b)} \\
\varphi(t + \Delta t) &= \varphi(t) + H(t) \Phi(t) & \text{(1.26c)} \\
H(t) &= \begin{cases} 1, & \text{with } P = 1 - e^{-\alpha \Delta t} \\ 0, & \text{otherwise} \end{cases} & \text{(1.26d)}
\end{align*}

where the random tumbling angle \( \Phi \) is drawn from a circular distribution defined on the domain \( \{0, 2\pi\} \). The distribution can either be assumed to be uniform in the domain or take into account the forward bias in tumbling angles observed in experiments, e.g., of \textit{E.coli}. For the latter type, common circular distributions include the von Mises distribution, the wrapped normal distribution and wrapped Cauchy distribution [20]. Fig. 1.3(b) shows an example trajectory with straight runs interspersed by sudden large reorientations due to tumbling events. For an RTP, the persistence time is given by the run time \( \tau_p = 1/\alpha \).

The corresponding Fokker-Planck equation to describe the RTP dynamics is given by

\[
\dot{P}(x, u) = -\nabla [vuP] - \alpha P(x, u) + \alpha \int W(u, u') P(x, u', t) du',
\]

where the second and third term on the right hand side are loss and gain due to out and in to direction \( u \) [26, 27]. The reorientation kernel \( W(u, u') \) defines the probability of a reorientation from direction \( u' \) to direction \( u \) during a tumble, and is normalised as \( \int W du' = 1 \).

1.3 Interactions with boundaries: breaking time reversibility

In the absence of any biasing environmental factors, both ABP and RTP perform an unbiased random walk at long time and length scales, which is equivalent to Brownian motion with a constant diffusivity \( D \). The diffusivity will be several orders of magnitude larger than achieved for a passive Brownian particle but it is nonetheless a diffusive process. Thus, the active particles dynamics can be mapped onto Brownian diffusion, which obeys detailed balance [28]. The principle of detailed balance states that the probability of the system transitioning from state \( x_1 \) to \( x_2 \) via any chosen path is equal
Fig. 1.4 Rectification. (a) Bacterial scattering at V shaped obstacles is asymmetric. Cells approaching from the open side (e.g. tracks labelled 3 and 4) are more likely to scatter off to the right. (b) Microfluidic channel with a column of V shaped obstacles. (c) As a consequence of the asymmetric surface interaction, bacteria accumulate on the right hand side of the channel as shown by fluorescent imaging, which is in stark contrast to equilibrium systems. Adapted with permission from [29]. Copyright (2007) American Society for Microbiology.

to the probability of the reverse process, i.e.

\[ P_0(x_1)\tilde{P}(x_1 \rightarrow x_2, t) = P_0(x_2)\tilde{P}(x_2 \rightarrow x_1, t), \]

where \( \tilde{P} \) is a path probability. However, environmental factors can introduce spatial variations in the motility parameters of active particles, which leads to biased diffusion without detailed balance. An example of how this can profoundly change results known from classical physics is the rectification of bacteria at asymmetric boundaries. Consider a V-shaped obstacle as illustrated in Fig. 1.4(a): bacteria colliding from the left will follow the boundary and leave again along a similar direction. Bacteria that encounter the obstacle from the other side are more likely to collide with a surface inside the wedge, which they will then glide along into the wedge, where they become reoriented. Following the neighbouring wing of the wedge they will leave in the direction they came from. Thus, at this obstacle the leaving angle does not depend on the incident angle. Bacteria in a chamber with a chain of these obstacles will thus accumulate on one side of this chain, whereas passive particles would have an equal distribution throughout the chamber.

The rectification phenomenon is based on the bacteria-surface interaction, which breaks microscopic reversibility. Accumulation at boundaries is indeed a trademark of active particles, and the characteristics of ‘persistence’ are sufficient to explain this
phenomenon [30, 31]. Take a suspension of active particles moving at speed $v$ with bulk density $\rho_b$. The flux of particles towards the boundary scales as $\rho_b v$. Once a particle hits the wall, it spends a finite time $\tau$ there before it escapes back to the bulk due to reorientation. This finite escape time leads to a build up of a surface density $\rho_s$. The rate of escape from the boundary then scales as $\rho_s/\tau$. In steady state, the flux towards the boundary has to equal the escape from it, i.e. $\rho_b v = \rho_s/\tau$. Thus, the surface density scales as $\rho_b v \tau$. Crucially, the surface density depends on the reorientation time scale $\tau$. If the boundary does not impact on the reorientation mechanism, the persistence length $l_p = v\tau$ determines the surface density. However, physical boundaries alter the propulsion of microswimmers, e.g. via steric hindrance or hydrodynamic effects, and can significantly increase $\tau$ [32, 33]. The specifics depend on the boundary and the propulsion mechanism of the microswimmer.

1.3.1 Flat wall

The flow fields surrounding the particle bodies and the different arrangement of propulsive appendages lead to distinct differences in boundary interactions. For puller-type particles swimming parallel to a wall, the passive hydrodynamic interaction is repulsive. Furthermore, it has been shown that the microalgae *C. reinhardtii* is reorientated at a boundary due to direct contact interactions of its flagella with the surface, see Fig. 1.5 [33]. This steric interaction indeed dominates as the scattering angle is determined by the length of the flagella.

Pusher-type particles such as bacteria, on the other hand, align their travelling direction upon hitting a wall and escape the surface only at long times [34]. Once they are trapped by the boundary, the bacteria swim in circles which is caused by the clockwise rotation of the cell body. Steric and hydrodynamic models, as well as combinations thereof, have been put forward to explain wall entrapment of bacteria [35–37]. Solutions of the far flow field predict that the passive hydrodynamic interaction...
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Fig. 1.6 Bacteria are trapped by surfaces. (a) Bacteria swim in circles at a glass cover as observed with holographic imaging in [31]. (b) Bacteria were released at a fixed distance from the glass cover and the cell was oriented at a certain angle $\theta$ with the surface. (c) When the cell approached the flat wall, there was no alignment of the swimming direction prior to impact. Upon impact, the angle $\theta$ quickly decreased to align the but the cell with the surface. During surface swimming, the body pointed into the surface and wobbled as shown by the oscillation in $\theta$. Taken from [31].

for pusher particles is attractive, which also predicts an alignment with the surface before impact [35]. However, pure steric effects can also lead to alignment with the surface upon impact as an elongated object pushing into a wall experiences a reorienting torque [31, 36].

To study the relative importance of hydrodynamic and steric effects, wall entrapment of smooth-swimming E. coli approaching a glass slide was imaged in 3D [31]. Using optical tweezers, the cells were released at a fixed distance and angle to the surface. The authors identified three phases: approach, reorientation and surface swimming. During the first phase, long-range hydrodynamic effects did not seem to affect the orientation as the angle with the surface only changed upon impact as illustrated in Fig. 1.6(b) and (c). However, the cell does not fully align but points into the surface, which is presumably caused by hydrodynamic effects. Further observations attributed to hydrodynamic effects are the long-term trapping and circle swimming, as well as the strong reduction in swimming speed while approaching the wall. During surface swimming, the angle with the surface, $\theta$, was found to oscillate around a positive value, which may be caused by two opposing hydrodynamic torques. A recent study using mesoscale hydrodynamics simulations attributed this wobbling of the cell to the misalignment of the flagellar bundle and the cell body axis [38]. Hydrodynamic effects might also prevent the unbundling of flagella required for tumbling, thereby reducing tumbling events by 50% within 20$\mu$m of the surface [39]. Despite this observed behaviour at flat walls, theoretical studies often consider tumbling as a means of escaping confinement [40, 41].
1.3 Interactions with boundaries: breaking time reversibility

Fig. 1.7 Bacteria at convex boundaries. (a) Example of a smooth-swimming E. coli tracing a microfluidic pillar. The scale bar is 100µm. Reprinted with permission from [42]. Copyright (2015) by the American Physical Society. (b) Pusher-type particles scatter at convex boundaries depending on the impact parameter $y_0$ with a deflection of $\Theta$. Adapted from [43] with permission from The Royal Society of Chemistry. (c) E. coli scatter at colloids depending on the angle of approach either with a small (forward scattering, top) or large angle (tumbling collision, bottom). The trajectories are colour coded according to instantaneous speed, and the scale bar corresponds to 5µm. Adapted from [46].

1.3.2 Convex boundaries

While rotational diffusion dominates the time a (smooth-swimming) bacterium spends at a flat wall, convex curvature can reduce the escape time. However, there is a trapping radius above which the particles are trapped, that is if the curvature of the surface is too small, the boundary resembles a flat wall for bacterium. For smooth-swimming E. coli, Sipos et al. [42] determined this trapping radius at 50µm at which point more than half the cells stayed at an obstacle for more than 3s after collision as illustrated in Fig. 1.7(a). This threshold agreed well with a prediction derived from hydrodynamic theory. The trapping threshold depends on bacterial properties, in particular its size and dipole strength as shown by [42] and [43]. Similar observations were made for Janus particles: the time they spend at a convex obstacle increased with the fuel concentration [44], which relates to the flow field created around the particle. However, the nature of propulsion of synthetic active particles can introduce further complications, e.g., via the interaction with a residual chemical field that introduces chemorepulsive torques for a microswimmer employing interfacial Marangoni gradients to propel, which leads to just a single orbit [45].

Below the trapping radius, pusher-type particles scatter at convex surfaces. Similar to the interaction at a flat wall [31], both steric and hydrodynamic effects may be involved in the scattering process (as a process distinct from hydrodynamic trapping).
Scattering rules for pusher-type particles have so far mainly been studied theoretically using analytical theory and squirmer simulations \cite{spagnolie2013, kuron2015}. Based on approximations of the flow field far away from the cell body, Spagnolie et al. \cite{spagnolie2013} derived a prediction for the critical trapping size of colloids and basins of attraction, as well as the distribution of trapping times. Below the critical trapping radius, the authors found that scattering at a spherical obstacle was influenced by the dipole strength of the swimmer and the angle of approach, as illustrated in Fig. 1.7(b). During contact with an obstacle below the trapping radius, hydrodynamic models predict that the angle the particle orientation makes with the obstacle surface will decrease until the particle can escape \cite{okubo1971, spagnolie2013}. Therefore, the leaving angle is approximately tangent to the obstacle surface; a small deviation due to hydrodynamic interactions is however possible \cite{okubo1971}. Recently, Kuron et al. \cite{kuron2015} combined far-field approximations and Lattice-Boltzmann simulations to further investigate the rich scattering interaction of squirmers at spherical obstacles as a function of the dipole strength of the swimmer, the incident angle and the obstacle radius. In addition to the results by Spagnolie et al \cite{spagnolie2013}, these authors identified a backward orbiting state for strong pushers in which swimmers reverse their direction upon collision compared to bulk swimming.

A recent experimental study identified two types of scattering events based on the angle of approach \cite{carlson2014}. The first type, termed ‘tumbling collision’, is observed for head-on collisions, which result in large reorientations (close to reversals) after spending a long time at the obstacle. On the other hand, the second type, termed ‘forward scattering’, occurs for swimmers that approach the obstacle almost tangentially, and this type does not influence the speed or direction of the bacterium to a large extent. Example trajectories are shown in Fig. 1.7(c). These experiments were performed for small colloids with a size comparable to the cell body of $E. \text{coli}$ (diameter of $\sim 3 \mu m$). However, an analysis of bacterial scattering at convex surfaces as a function of surface curvature and angle of approach is missing. Furthermore, it is unclear whether convex surfaces impact flagella unbundling and, thereby, impede a cell’s ability to tumble as has been observed at a flat wall \cite{weiss2013}.

### 1.4 Active particles in porous media

Macroscopic transport arises from the microscopic dynamics. Given the non-equilibrium boundary interactions, transport of active particles in porous media might differ from the expectations derived from classical systems, e.g. gas transport. Bacterial migration in porous media has been studied long before the field of active matter attracted interest.
Previously, research was motivated by ecological questions around bacteria-plant symbiosis and bioengineering applications such as bioremediation, which is a process to clean-up polluted soil using microorganisms. In this field, experimental studies on bacterial transport in porous media commonly did not include direct consideration of microscopic scattering events within porous matrices [48, 49]. For example, popular packed column experiments measuring breakthrough curves commonly lack the spatial and temporal resolution to observe the bacterial interaction with the porous medium [50]. A popular model based on these studies is a modified version of Knudsen diffusion [51, 52], which considers a gas with a characteristic mean free path $\lambda$ between molecule collisions. In a porous medium, a gas molecule is either reoriented by collisions with other molecules or by collisions with the physical boundaries of the porous medium.

If the average pore size $d_{\text{pore}}$ of the porous medium is smaller than the mean free path $\lambda$ of the gas, $d_{\text{pore}}$ becomes the effective mean free path between reorientations. Therefore, the diffusion coefficient of a gas in porous medium can be modelled as

$$D \sim v\lambda \rightarrow D \sim v d_{\text{pore}} \quad \text{if} \quad d_{\text{pore}} < \lambda$$

This gas-kinetic approach is adopted to describe bacterial populations by replacing the mean free path of the gas by the persistence length $l_p = v\tau_{\text{run}}$ of the bacterium [51, 52]. However, the model has the underlying assumption of elastic collisions of particles with the porous medium as illustrated in Fig. 1.8(a), which does not account for the non-equilibrium nature of bacteria-surface interactions.

Thanks to the recent interest in the field of active matter, the role of these surface interactions and, in general, active particles moving in complex environments has received more attention. The modelling of the transport of active particles such as bacteria typically follows one of two approaches: hydrodynamic models, or random walk models. With a full hydrodynamic approach, the particle-surface interactions can be studied directly, e.g., by modelling the active particle as a hard sphere with defined tangential surface velocity [3]. A recent study explored the migration of active particles through a body-centered cubic lattice of spheres of the same size as the particle [53]. Depending on the swimmer type (pusher vs. puller) and sphere packing density, the authors found trapped, random walk and straight trajectories. The computational demands of the simulations, however, prevented study of long-time behaviour. Random walk models, on the other hand, can be used to study the large-scale transport of active particles but require simplified rules for the interaction of particles with boundaries. Transport in complex media has been studied for several boundary interactions: for model particles that evade obstacles [54], particles that
Fig. 1.8 Active particles in porous media. (a) Models based on Knudsen diffusion assume an elastic scattering of bacteria at boundaries. Reprinted from [51], with permission from Elsevier. (b) Hydrodynamic study of a squirmer moving in a lattice of fixed spheres of the same size. The computational demand of the simulations prevented the study of macroscopic transport in porous media. Adapted from [53]. (c) Lattice model of a random walk of an RTP in a porous medium. When the RTP moves to a lattice site with an obstacle, it becomes trapped until either a direction change during a tumble occurs or the obstacle moves to a different lattice side. The direction after tumbling is chosen at random, thus discarding any effect that the surface interaction may have on the orientation of the particle. Reprinted with permission from [41]. Copyright (2017) by the American Physical Society.

are trapped before being randomly reorientated by tumbles [40, 41], and particles that interact with obstacles via an excluded volume potential [55]. In random porous media, theoretical studies found a rich behaviour ranging from subdiffusion [54, 55], superdiffusion [55], to localization or trapping [41, 54, 55]; but for active particles even diffusive transport in regular lattices is non-trivial [56].

However, it is unclear what the effect of different boundary conditions is on the observed transport properties, and how they compare to the established model of Knudsen diffusion based on specular reflection. Different assumptions of the underlying microscopic behaviour could lead to different macroscopic properties. For example, the non-equilibrium nature of scattering observed for bacteria could allow them to navigate a porous medium more efficiently [44, 57], while Licata et al. assumed that transport is reduced due to retention at boundaries [40]. Furthermore, although most studies have focussed on unbiased transport properties, biased transport is both prevalent and desirable. The motion of microswimmers in nature is biased by many environmental factors such as food and light sources. Similarly, synthetic active particles are often motivated as potential avenues for ‘micro-robots’ to navigate crowded environments like soil or the human body, where the ability to steer the motion is a main goal [58].
To date, experimental evidence for the transport of active particles in complex environments is scarce and existing studies vary widely in the choice of obstacle shape, size, and arrangement, or focus on advective transport via fluid flow [59]. In an interesting example of diffusive transport, Sosa-Hernández et al. [60] studied the motility of *E. coli* in a random porous medium of polystyrene beads that were of the size of the cell (radius $\sim 1.5\mu m$) and confined between two glass slides. The mean speed of the bacterial population decreased with increasing obstacle fraction, and the mean squared displacement of bacterial trajectories suggested subdiffusive behaviour at large packing fractions, which may have been caused by trapping. The authors report that cells were found to swim along colloids although a more in-depth analysis of those trajectories was not presented. It is important to note that the size of the obstacle relative to the active particle can alter the motility dramatically as was demonstrated in [61]. In a close-packed crystal of polystyrene colloids (radius 5$\mu m$), Janus particles orbited the colloids and hopped stochastically between colloids, whereas the motility of smooth-swimming *E. coli* was rectified from circle-swimming into long runs because the size of the cells (including flagella) limited their ability to change direction in the crystal geometry [61].

Instead of the size of the active particle, the persistence length of the random walker may be used as reference length scale to choose an obstacle size. A recent study investigated the diffusive transport of microalgae *C. reinhardtii* in microfluidic obstacle lattices [56]. Pillars that had a radius ($R = 200\mu m$) of the order of the persistence length of the swimmer were arranged in a square lattice. Decreasing the distance between the pillars increased the directional bias of swimming along the lattice channels, which was modelled as an anisotropic tumbling rate. Despite this potential channelling effect, the diffusive transport decreased significantly with decreasing distance between the pillars.

An intermediate range of obstacle size was used to observe the soil bacterium *Pseudomonas putida* swimming in a hexagonal lattice of microfluidic pillars (radius $12.5 - 50\mu m$) [57]. In free space, *P. putida* performs a run-reverse motility with turning angles biased towards 0° and 180°, but collisions in an obstacle lattice increased the frequency of intermediate turning angles (around 90°). Furthermore, the authors suggested that the particles were guided by the confining surfaces formed by the pillars. This conclusion was based on a comparison between the mean run length observed for swimmers with the mean free path expected for ballistic particles. However, it remains unclear how bacteria scatter at convex surfaces, and a model connecting microscopic behaviour to macroscopic transport is, therefore, missing as well.
Besides size, the shape of obstacles can also affect transport, e.g. by introducing a clear leaving point. While the aforementioned studies all involved obstacles with convex boundaries, a recent study used obstacles with a square outline to study bacterial diffusion in a lattice of such posts [62]. The corner of the posts are natural leaving points for *E. coli* cells and the interaction with the surface is like that of a flat wall. The posts were arranged in a square lattice so that channels were formed by the posts. The width of channels between posts was then varied. In this design, bacteria preferentially tumble at the intersection between channels - an effect that increases with decreasing channel width. As a result, the characteristics of the lattice topography were present in the dispersal distribution at early times, while at long times the population reached diffusive transport. The restriction in both effective tumbling frequency and tumbling angle increased the effective diffusivity for those narrow channels. Indeed, obstacles can be designed to direct the motion of active particles. For example, teardrop-shaped obstacles were used to guide synthetic rod-shaped active particles in [63]. Once a particle has made contact with an obstacle, the most likely point to leave the obstacle is the pointed end of the teardrop, which introduces a bias in the motion. Guiding by microfluidic obstacles can even allow synthetic microswimmers to steer perpendicular to an applied driving force and sort swimmers according to their motility properties [64].

1.5 Structure of this thesis

Both in the laboratory and the natural environment, active particles regularly encounter physical boundaries: synthetic microswimmers can be guided in microfluidic channels [64], sperm cells follow the female reproductive tract to reach the egg cell [65], and immune cells navigate the extracellular matrix to find the closest lymph node [66]. Many fascinating results have been achieved by applying the tools of statistical mechanics to the non-equilibrium systems of interacting active particles. In order to understand the properties of active matter in complex environments, however, we first require insight into the way its constituents interact with physical boundaries. This thesis explores the means by which microscopic boundary interactions and chemical fields shape the macroscopic transport of (non-interacting) active particles. At first, we may ask how different theoretically conceivable scattering rules on the microscopic scale can affect diffusive transport at the macroscopic scale. In chapter 2 I show that aligning the swimming orientation with the obstacle surface upon impact may allow active particles to traverse complex media faster than would be possible with a classical
specular reflection. As the search for food is a major reason for foraging in nature, in chapter 3 I look at the ability to bias transport in a porous medium in response to a gradient of chemical stimulus. The specifics of microscopic boundary interaction and gradient estimation affect the biased transport here beyond the expectation from diffusive transport.

Having established the importance of microscopic behaviour at boundaries theoretically in chapters 2 & 3, in chapter 4 I present experimental results for *E. coli* scattering at microfluidic pillars and the resulting diffusive transport in a lattice of pillars. The angle of approach as well as the presence of tumbling events strongly influence the scattering events at microfluidic pillars, and give some indication of the relative importance of hydrodynamic vs. steric effects. In chapter 5, I investigate both computationally and experimentally how a non-uniform density of the porous medium itself can bias the motion of active particles. By modifying the swimming speed, the non-classical boundary interaction of active particles can introduce a drift towards high obstacle densities in simulations of an ABP. Preliminary experiments carried out with the green alga *C. reinhardtii* agree with this prediction. Finally, in chapter 6 I consider the case where the swimming speed is a response variable itself in the motility pattern of bacteria. Chemokinesis is a change in swimming speed according to the absolute level of a chemoeffector, and I study the dynamics of bacterial populations capable of both chemokinesis and chemotaxis in varying chemoeffector fields. The model shows that chemokinesis may enhance the chemotactic population response, but also modifies it qualitatively.
Active Brownian Particles in structured environments

In this chapter, we study theoretically how different types of boundary scattering influence the diffusive transport of active particles in ordered arrays of obstacles. We consider particles specularly reflected from boundaries, as in the Lorentz gas model \cite{67}; particles that scatter by sliding around obstacles, like pushers \cite{42, 43}; and particles that interact with obstacles via a steric, torque-free interaction, which we refer to here as a “slide-off” condition \cite{55}. For these ‘pusher-like’ collisions, simulations and a run-and-tumble particle model predict, counterintuitively, that large diffusive transport is possible even at high obstacle densities. This result contrasts sharply with the expected low diffusivity of Lorentz gas particles at high densities. We show for the sliding boundary condition, using a simple deterministic model, how this large diffusion at high density is caused by particle guiding by the lattice. Simulations show that qualitatively the same effect occurs for the slide-off condition, but not for Lorentz gases. Overall, the results highlight the previously unexplored role of lattice geometry in active particle transport.

2.1 Equations of motion and scattering rules

We consider $N_P$ active particles in a two-dimensional space in which obstacles are placed in a hexagonal lattice. The centres of the obstacles are fixed with distance $d$, and the obstacle radius $R$ is varied. The equations of motion for the $i$-th particle are
Active Brownian Particles in structured environments

Fig. 2.1 Boundary conditions. Typical trajectory of a particle governed by Eqs. (2.1a) and (2.1b) with (a) a sliding, (b) a slide-off, or (c) a reflecting boundary condition. The particle collides with an obstacle at an angle $\beta$, which is the angle between the particle orientation $\mathbf{p}$ and the surface tangent at the collision point. For the sliding condition, the particle leaves at a tangent to the obstacle after traversing a fixed central angle $\alpha$. For the slide-off condition, the central angle $\hat{\alpha}$ depends on the incident angle, the obstacle radius, and the magnitude of rotational diffusion on the obstacle.

The equations governing the particle's motion are given by

$$\dot{x}_i = v \mathbf{p}(\varphi_i) \quad (2.1a)$$
$$\dot{\varphi}_i = \sqrt{2D_R} \xi_i(t), \quad (2.1b)$$

where dot denotes the time derivative, $v$ is the particle speed, $\mathbf{x}_i$ and $\varphi_i$ correspond to the position and moving direction of the $i$-th particle, respectively, and the unit vector $\mathbf{p} = [\cos \varphi, \sin \varphi]$. The white noise in Eq. (2.1b) obeys $\langle \xi(t) \rangle = 0$ and $\langle \xi_i(t)\xi_j(t') \rangle = \delta_{ij} \delta(t - t')$. Thus, the moving direction undergoes rotational diffusion with $\langle \varphi(t)^2 \rangle = 2D_R t$. As a result, the particle performs a persistent random walk with persistence length $l_p = v/D_R$ [68]. The simulation domain is governed by periodic boundary conditions.

Recent microfluidic experiments [42] and hydrodynamic models [42, 43] have shown that pillars with radii above a critical threshold strongly trap pushers, which escape at long times by rotational diffusion. We consider here only pillars with radii below this critical threshold (and so choose parameters motivated by experiment [57, 69]). In this case, swimmers collide with an obstacle at an angle $\beta$, defined as the angle between the tangent at the collision point and the orientation $\mathbf{p}$. If $\beta < \pi/2$, the particle travels clockwise around the obstacle; if $\beta \geq \pi/2$, the particle travels counterclockwise. After the collision, the angle between the particle and the obstacle surface tangent decreases until escape [43]. While it is acknowledged that the particle-obstacle
interaction includes both steric and hydrodynamic effects, no unifying theory has been developed.

2.1.1 Modelling scattering rules

In the following, we will study the effect of three different scattering rules on the effect of diffusive transport:

1. Sliding condition as in Fig. 2.1(a),
2. Slide-off condition as in Fig. 2.1(b),
3. Reflecting condition as in Fig. 2.1(c),

which are described in more detail below. For the sliding boundary condition, consider a collision with an obstacle: \( \beta \) is defined as the angle between the tangent at the collision point and the orientation \( \mathbf{p} \). The particle moves along the obstacle to traverse a central angle \( \alpha \) (Fig. 2.1(a) inset). A model of stochastic dynamics could determine, for a given incident \( \beta \), the resulting distribution of central angles \( \alpha \) (leaving times). However, such a model has yet to be developed. We know from modelling and experiments that after collision, the particle quickly rotates, through phoretic and/or hydrodynamic interactions with the surface, to align its orientation vector with the surface, regardless of the orientation of the particle upon collision [15, 70]. This rotation generally occurs on a much faster timescale than the trapping time of the particle [36]. Therefore, we choose to neglect the dependence of the sliding angle \( \alpha \) on \( \beta \), i.e., model the probability distribution of \( \alpha \) as \( P(\alpha) \) instead of as \( P(\alpha|\beta) \), with \( P(\alpha) \) peaked at some value \( \alpha_{\text{max}} \) determined by the competition between rotational diffusion and deterministic alignment with the surface. In this work we explore the effect of boundary conditions assuming a fixed central angle \( \alpha \) and further assume that, when a particle leaves an obstacle, its orientation \( \mathbf{p} \) is tangent to the obstacle surface. This is a necessary simplification of the hydrodynamic behaviour of pusher-type particles at convex obstacles. The model also neglects any potential impact of the chemical field surrounding synthetic active particles. The neglect of stochasticity in \( \alpha \) can be checked by simulations. Results with a fixed (mean) \( \alpha \) are qualitatively the same to those obtained with a distribution of \( \alpha \), provided the latter is peaked about its mean (e.g. a Gamma distribution).

As a comparison, we also consider a slide-off boundary condition, and a reflecting boundary condition. In the slide-off condition, when a particle collides with an obstacle, it retains its orientation vector, and advances around the obstacle depending on the component of its velocity parallel to the obstacle surface (initially \( \beta \)), i.e. \( v = v_0 \cos \beta \),
as shown in Fig. 1(b). The angle between particle orientation and obstacle tangent decreases as the particle moves around the obstacle’s surface. It will leave the obstacle when the orientation vector is parallel to (or pointing away from) the obstacle’s surface. In the absence of rotational diffusion, this means that the particle will traverse a central angle of \( \hat{\alpha} = \min(\beta, \pi - \beta) \), and so it bears some resemblance to the sliding condition. However, while sliding is motivated by hydrodynamic effects, the slide-off condition is motivated by steric effects, and has been used in various potential-based simulation studies to model Janus particles and active disks [55, 71]. Following previous studies [54, 55], this condition can be formally included in the Langevin equations as

\[
\dot{x}_i = v \mathbf{p}(\phi_i) + \mu \mathbf{F}, \\
\dot{\phi}_i = \sqrt{2D_R} \xi_i(t),
\]

(2.2a)  
(2.2b)

where \( \mu \) is a mobility coefficient and we assume that the particle is in contact with only a single obstacle at a given time. The interaction force \( \mathbf{F} \) between the particle and the obstacle is in this case:

\[
\mathbf{F} = \begin{cases} 
\frac{v}{\mu} (\mathbf{p} \cdot \mathbf{N}) \mathbf{N}, & |\Delta \mathbf{x}| \leq R \\
0, & \text{otherwise},
\end{cases}
\]

(2.3)

where \( \mathbf{N} \) is the unit normal vector to the obstacle surface, and \( |\Delta \mathbf{x}| \) is the distance between the particle and the obstacle centre.

For the reflecting condition, a particle is reflected with an angle equal to the incident angle, as illustrated in Fig. 2.1(c). This interaction type implies time-reversibility, which is an assumption underlying gas kinetic models derived for bacteria transport in porous media [51, 52]. By contrast, both the sliding and slide-off boundary condition are not time-reversible and violate detailed balance [28]. The system of Eqs. (2.1) and (2.2) with (2.3) is solved numerically as a parallelised agent-based model, and example particle tracks are shown in Fig. 2.1. We derive the diffusion coefficient from \( N_P \) simulated particle tracks by fitting the mean square displacement as \( \langle \delta x(t)^2 \rangle = 4D_{\text{eff}}t + 4D_{\text{eff}}\eta[\exp(-t/\eta) - 1] \) [72], where the time scale of ballistic motion, \( \eta \), is the second fitting parameter.
2.2 Diffusion of an active Lorentz gas

We first establish the diffusive properties of active particles with a reflective boundary condition. Here, we recognize an analogy to the Lorentz gas model, in which particles move ballistically between obstacles [67]. The Santalo formula is a well-known result for the mean-free path of a Lorentz gas [73] given by,

\[ \lambda = \frac{\pi A}{P} \]  

(2.4)

where \( A \) and \( P \) are the free area and obstacle perimeter in a unit cell (shown for a hexagonal lattice in Fig. 2.2(a)), respectively. Since the active particles move diffusively at large time scales, we derive an active version of Santalo’s formula with a circle of radius \( l_p \) as an additional boundary, as in Fig. 2.2(b). This yields the mean-free path of an active particle as

\[ \tilde{\lambda}_p = \frac{\pi NA}{(NP + 2\pi l_p)}, \]  

(2.5)

where \( N \) is the number of unit cells included in the circle of radius \( l_p \). For a hexagonal lattice of circular obstacles, we obtain \( A = \sqrt{3}d^2/2 - \pi R^2 \), \( P = 2\pi R \) and \( N = \pi l_p^2/(\sqrt{3}d^2/2) \). Note that in order to obtain the fit in Fig. 2.3(a), this mean free path
has to be scaled by \( \pi/2 \), i.e. \( \lambda_{lp} = 2\tilde{\lambda}_{lp}/\pi \), which may be due to the choice of averaging conditions made in earlier work [73].

**Comparison with simulations**

As shown in Fig. 2.3(a), applying this adjusted mean-free path in \( D = \lambda_{lp}v/2 \) matches the simulations. The inset plots the theoretical prediction and the diffusion coefficient fitted from simulations on a lin-log scale, showing that at large \( R/d \) the diffusion coefficient scales as \( \ln(1/\rho) \), where obstacle density \( \rho = 2\pi/\sqrt{3}(R/d)^2 \). If we vary the obstacle separation \( d \) instead of the obstacle radius \( R \), we see in Fig. 2.3, as expected, that the diffusion coefficient approaches \( D_0 \) when \( d \gg l_p \). We can understand the reduction in diffusion coefficient qualitatively: as the obstacle density increases, particles spend most of their time in the wells between triplets of obstacles in the hexagonal lattice, and their motion becomes a jump-diffusion process from well to well, as described by Machta and Zwanzig [67] and illustrated by the particle track in Fig. 2.1(c).

### 2.3 Run-and-tumble framework

While the active Santalo formula matches the reflective simulations well in Fig. 2.3(a), it cannot account for the effective persistence introduced by the sliding and slide-off boundary conditions, and a different approach is required. Considering an example particle track in Fig. 2.4 and ignoring the obstacles, we are left with a track that consists of several long tracks of high persistence interspersed with sudden sharp reorientations. Thus, this track resembles a run-and-tumble trajectory with additional rotational diffusion during the runs. The ‘tumbles’ can be identified as obstacle-induced reorientation with reorientation angle \( \psi \), while the distance covered during a ‘run’ between obstacle collisions is \( v\tau \), where \( \tau \) is the time between obstacle collisions. We will derive a theoretical description of diffusion in obstacle lattices based on the model of run-and-tumble particles (RTP) [26, 27]. The diffusion coefficient for an RTP also undergoing rotational diffusion is known to be

\[
D = \frac{\psi^2}{2[D_R + (1 - \langle \cos \psi \rangle)/\tau]},
\label{eq:2.6}
\]

where \( \tau \) is the mean run time and \( \psi = \psi(\alpha, P(\beta)) \) is the reorientation angle during a tumble [74, 75]. In the following, we will derive expressions for the parameters in Eq. (2.6): i) the reorientation function \( \langle \cos \psi \rangle \), ii) the mean run time \( \tau \), and iii) the
2.3 Run-and-tumble framework

Fig. 2.3 Diffusion with reflecting and slide-off boundary condition. (a) Diffusion with reflecting boundary condition, \( D_{\text{ref}} \), is scaled by diffusion coefficient in the absence of any obstacles, \( D_0 = \nu^2 / 2D_R \). Simulations agree with Santalo’s formula that was adjusted for rotational diffusion, \( \lambda_{\lambda} \) (green dotted curve). The run-and-tumble model introduced in section 2.3 in Eq. (2.6) with Santalo mean free path \( \lambda \) in \( \tau_c = \lambda / \nu \) (orange dashed) is compared to RTP model with \( \tau_c^B = 1 / \rho \) [41] (purple dashed-dotted) and \( \langle \cos \psi \rangle = -1 / 3 \). (b) The diffusion coefficient \( D_{\text{ref}} \) approaches the free diffusion coefficient \( D_0 \) as the distance between obstacle surfaces \( (d' = d - 2R) \) increases well above the persistence length of the particles. Parameters: \( N_p = 10^3 \), \( D_R = 0.1 \text{ s}^{-1} \), \( v = 20 \text{ \mu m s}^{-1} \), \( d = 60 \text{ \mu m} \), unless otherwise stated.

effective speed \( \nu \) based on the microscopic details of the sliding, the reflecting and the slide-off boundary conditions. We will then compare the RTP model with these parameters to simulation results of the agent-based model introduced in section 2.1.

2.3.1 Sliding boundary condition

Reorientation function

The reorientation angle is a combination of alignment upon collision with the obstacle at an angle, \( \beta \), and sliding according to the central angle, \( \alpha \), which can be see from the schematic drawing in Fig. 2.5(a). The sliding particle travels along the path ABCD. By construction, angle FCH must be a right angle. Since the particle leaves at a tangent, OCD is also a right angle. By the alternate angle theorem, \( OCF = \alpha \), and so \( DCF = \pi / 2 - \alpha \). Therefore,

\[ \psi = \pi / 2 - \beta - (\pi / 2 - \alpha) = \alpha - \beta \] (2.7)
Fig. 2.4 Obstacle reorientation as a tumbling process. (a) A particle with a sliding boundary condition is reorientated by rotational diffusion and obstacle interactions while moving through a hexagonal lattice of obstacles. (b) The same track as in (a) but without the obstacles displayed illustrates the analogy to a run-and-tumble particle track. The distance covered between obstacle collisions is $v\tau$, where $\tau$ is the time between obstacle collisions, while $\psi$ is the reorientation angle.

The average $\langle \cos \psi \rangle$ in Eq. (2.6) is performed over the collision angle $\beta$, with probability distribution $P(\beta)$.

To derive this distribution, let us consider a single circular obstacle of radius $R$. A particle can start at any distance $x$ from the centre of the obstacle, with its initial direction $\phi$ uniformly distributed, as illustrated in Fig. 2.5(b). We assume that the particle moves in a straight line, and may or may not collide with the obstacle. Given a uniform distribution of starting directions, what is the observed collision angle distribution $P(\beta)$? For this, we need only consider a truncated distribution

$$P(\phi) = \frac{1}{2 \cos^{-1}(R/x)}$$

(2.8)

between the angles $-\cos^{-1}(R/x) \leq \phi \leq \cos^{-1}(R/x)$, where the particle will only graze the obstacle at a tangent to its surface. Beyond this range of angles, the particle will not hit the obstacle. From the sine rule, we can see that

$$\frac{x}{\sin(\pi/2 + \beta)} = \frac{R}{\sin \phi},$$

(2.9)
2.3 Run-and-tumble framework

Fig. 2.5 (a) Schematic of the reorientation angle $\psi$ as a function of $\alpha$ and $\beta$. The sliding particle travels along the path ABCD. Lines OB and CF are parallel, as are lines AB and CG, and lines EB and CH. (b) Schematic of the set-up to determine the incoming angle distribution. A particle starts a distance $x$ from the centre of the pillar, and then travels at an orientation $\phi$ relative to the line joining the centre of the circle to the particle’s origin, hitting the circle at an angle $\beta$ to the tangent of the circle.

and so $\phi(\beta, x) = \sin^{-1} \left( \frac{R}{x} \cos \beta \right)$. The transformation between the uniform initial angle distribution $P(\phi)$ and the collision angle distribution $P_x(\beta)$ is given by

$$P_x(\beta) = P(\phi) \left| \frac{d\phi}{d\beta} \right| = P(\phi) \frac{R \sin(\beta)}{x \sqrt{1 - (R^2 \cos^2 \beta)/x^2}},$$

(2.10)

where $|d\phi/d\beta|$ is the Jacobian of the transformation. We can write the collision angle distribution averaged over all space as

$$P(\beta) = \frac{\int_0^{L} dx \int_0^{\pi} \frac{2\pi x}{P_x(\beta)}}{\int_0^{\pi} d\beta \int_0^{L} dx \int_0^{\pi} \frac{2\pi x}{P_x(\beta)}} = \frac{\sin \beta}{2},$$

(2.11)

where $L$ is an arbitrary system size. Here, the factors of $2\pi x$ arise from summing over annular regions of starting points. The denominator is a normalisation factor. Despite using deterministic trajectories to calculate this distribution, simulations at low densities agreed with this prediction. Using Eq. (2.11), the reorientation function is then given by the average:

$$\langle \cos \psi \rangle = 2 \int_0^{\pi/2} \cos(\alpha - \beta) P(\beta) d\beta$$

$$= \frac{1}{4} \left( 2 \cos \alpha + \pi \sin \alpha \right),$$

(2.12)
noting that $\cos \psi$ is even about $\beta = \pi/2$. This framework is general, and so can be adapted for specific boundary conditions, as long as the reorientation function $\langle \cos \psi \rangle$ can be determined. For the reflecting boundary condition, $\psi = 2\beta$, and $\langle \cos \psi \rangle = -1/3$.

**Residence time**

The second parameter in the RTP model (2.6) is the mean run time $\tau$, which corresponds to the time between obstacle collisions. It has been shown that the characteristic time between collisions is independent of the details of a diffusive random walk and depends purely on confinement [76], which has recently been confirmed experimentally for E.coli bacteria [77]. Therefore, we can use the mean collision time

$$\tau_c = \frac{\lambda}{v}$$  \hspace{1cm} (2.13)

for all boundary conditions, where $\lambda$ is the mean free path given by Santalo’s formula. However, the mean run time between obstacle reorientations needs to be adjusted by the time spent on an obstacle

$$\tau = \tau_c + \tau_R.$$  \hspace{1cm} (2.14)

To obtain the residence time $\tau_R$, we use the definition of the arc length traversed by the particle $l_{\text{arc}} = v\tau_R = \alpha R$ such that the residence time for the sliding boundary condition is given as

$$\tau_R = \frac{R\alpha}{v}.$$  \hspace{1cm} (2.15)

**Effective speed**

A final consideration is that travelling on the obstacle causes an effective reduction in velocity. When the particle traces along the pillar, it travels a distance $l < v\tau_R$, which gives $v_{\text{obs}} = l/\tau_R$. By the cosine rule, $l = R\sqrt{2 - 2\cos \alpha}$. The effective speed in Eq. (2.6) is then

$$v_{\text{eff}} = v\frac{\tau_c}{\tau} + v_{\text{obs}}\frac{\tau_R}{\tau} = v\frac{\tau_c}{\tau} + \frac{l}{\tau}.$$  \hspace{1cm} (2.16)

**Comparison with simulation**

To check how the model performs in a simple case, we first compare the RTP theory with simulations using the reflecting boundary condition, using $\langle \cos \psi \rangle = -1/3$ and $\tau_R = 0$. As shown in Fig. 2.3(a), the RTP model with $\tau = \tau_c$ yields a good approximation of the simulation results. As a comparison, we test the RTP model with a different
2.3 Run-and-tumble framework

Fig. 2.6 Diffusion with sliding boundary condition. (a) Simulations reveal dependence on both obstacle density and central angle. (b) Theoretical prediction Eq. (2.6) with $\tau_c = \lambda/v$ in $\tau = \tau_c + \tau_R$ and $\langle \cos \psi \rangle$ given by Eq. (2.12). Parameters: $N_p = 1000$, $D_R = 0.1$ s$^{-1}$, $v = 20$ $\mu$m s$^{-1}$, $d = 60$ $\mu$m.

The RTP framework reproduces the main features of the simulations, compare Figs. 2.6(a) and 2.6(b): it maintains a large diffusion coefficient for small to intermediate $\alpha$. Since $\tau_c$ is independent of the boundary condition, this must stem from the reorientation function $\langle \cos \psi \rangle$ in Eq. (2.12), which has a maximum at $\alpha \approx \pi/3$ and a minimum at $\alpha \approx 4\pi/3$. These extrema coincide with the predicted maximum derived mean collision time [41], where $\tau_c = 1/\rho$. Using this collision time, the model approximates the simulations at low densities but diverges in the high density regime. Therefore, we use the mean collision time $t_c$ based on the Santalo formula in the remainder.
and minimum of the diffusion coefficient observed for small to intermediate $R/d$ in Fig. 2.6(b). Beyond $\alpha = 4\pi/3$, the reorientation introduced by the obstacles decreases again, which leads to a small increase in the diffusion coefficient at small $R/d$. However, at large $R$ and $\alpha$ any increase in the diffusion coefficient is suppressed by the increase in residence time since $\tau_R \sim R\alpha$ in Eq. (2.15).

At very large $R/d$, the simulations predict a double peak around $\pi/3$, which is not present in the RTP model. As the RTP model is oblivious to the obstacle arrangement, this discrepancy might be caused by geometrical guiding effects. At very large $R/d$, the obstacles are closely packed with channels of free area given by the lattice geometry. If a particle travels along one of these channels, a certain set of reorientation angles $\alpha$ may reinforce the travelling direction along these channels upon repeated obstacle collision: a particle may effectively bounce between two rows of obstacles along a channel, which may increase its persistence compared to free space. We will analyse this potential geometrical guiding effect in more detail in section 2.4.

2.3.2 Slide-off boundary condition

The slide-off boundary condition preserves the particle orientation $\mathbf{p}$ at the point of collision. We consider two different cases: i) when rotational diffusion is fully suppressed while on the obstacle, here called the deterministic slide-off condition, and ii) when rotational diffusion remains the same as in free space while on the obstacle, here called the stochastic slide-off condition.

Reorientation function

For the deterministic slide-off condition, there is by definition no effect on the orientation of the particle, and so $\langle \cos \psi \rangle = 1$. In the case of the stochastic slide-off condition, the particle is moving around the obstacle while the orientation changes due to rotational diffusion and, thus, $\langle \cos \psi \rangle \leq 1$. The relative importance of diffusion to movement around the obstacle on the particle’s orientation depends on the size of the obstacle compared to the persistence length of the particle, $R/l_p = RD_R/v$. As discussed in chapter 1, if the obstacle is extremely large ($R \gg l_p$), it resembles a flat wall for the particle. A change in the particle’s surface angle due to movement around the obstacle will be very small, as the curvature is low. Therefore, diffusion will dominate the reorientation, and $\langle \cos \psi \rangle < 1$. At a very small obstacle ($R \ll l_p$), on the other hand, the particle will quickly move around the obstacle and leave before it is reoriented significantly by rotational diffusion so drift $(v/R)$ will dominate.
Both processes can be captured by the evolution of the angle the particle makes with the obstacle surface, $\Theta$. According to the alternate angle theorem we may use $\delta \alpha = \delta \Theta$. From the definition of the arc length, we know that $\delta \alpha = (v/R) \delta t$. Furthermore, the speed of the particle along the obstacle surface is $v \cos \Theta$. The angle with the surface, therefore, changes by $(v/R) \cos \Theta \delta t$ due to the movement along the surface. As the orientation is subject to noise, $\Theta$ follows a Langevin equation:

$$\dot{\Theta} = -\frac{v}{R} \cos \Theta + \sqrt{2D_R} \xi(t).$$  \hspace{1cm} (2.17)

The particle collides with a certain incident angle $\Theta(0) = \beta$, which then evolves due to a combination of deterministic drift and diffusion. The particle leaves the obstacle when either $\Theta = 0$ or $\Theta = \pi$, which are both possible solutions due to the white noise $\xi(t)$.

To derive the reorientation function, we want to know the average distance traversed around the pillar for a given incident angle, $\alpha(\beta)$. This is given by adding up all the contributions of the particle’s movement over the surface during its interaction with the pillar:

$$\hat{\alpha}(\beta) = \langle \int_0^T dt \frac{v}{R} \cos \Theta(t) \rangle_{\Theta(0) = \beta},$$  \hspace{1cm} (2.18)

where $T$ is the time at which the particle angle $\Theta$ first reaches 0 or $\pi$ and leaves the obstacle. The average is, thus, only over paths that leave the domain $\{0, \pi\}$. We can substitute using the Langevin equation (2.17):

$$\hat{\alpha}(\beta) = \langle \int_0^T dt \left(-\dot{\Theta} + \sqrt{2D_R} \xi(t) \right) \rangle_{\Theta(0) = \beta}$$

$$= \beta - \langle \Theta(T) \rangle_{\Theta(0) = \beta}. \hspace{1cm} (2.19)$$

Here we have made the assumption that the stochastic integral vanishes, which is not obviously true, as we are not averaging over all trajectories at the time $T$, only those ones that reach the boundaries for the first time. However, this seems reasonable, given we may exit the region on both sides.

As the particle can leave either at $\Theta(T) = 0$ or $\Theta(T) = \pi$, the average in Eq. (2.19) is not straightforward but we can deduce it based on the probability of leaving at 0, $p_0(\beta)$, or $\pi$, $p_\pi(\beta)$:

$$\langle \Theta(T) \rangle = \pi p_\pi(\beta) + (0) p_0(\beta) = \pi p_\pi(\beta). \hspace{1cm} (2.20)$$

For a one-dimensional diffusive process governed by Eq. (2.17) between absorbing boundaries $a = 0$ and $b = \pi$, it can be shown that the probability of exiting at $b$ before
Fig. 2.7 Numerical solution for the reorientation function in Eq. (2.22) (solid curves) vs. the analytical approximation (dashed curves) in Eq. (2.23) for $D_R = 0.1\, s^{-1}$ (blue curves) and $D_R = 0.5\, s^{-1}$ (red curves). Parameters: $v = 20\, \mu m/s$, $d = 60\, \mu m$.

encountering $a$ is given by the splitting probability $[21]$

$$p_\pi(\beta) = \frac{\int_0^{\pi} \exp\left(\frac{v \sin(z)}{RD_R}\right) dz}{\int_0^{\pi/2} \exp\left(\frac{v \sin(z)}{RD_R}\right) dz}, \quad (2.21)$$

where again, we consider $\beta \leq \pi/2$ and make use of the symmetry for $\pi/2 \leq \beta \leq \pi$. To get the reorientation, we then integrate over the incident angle:

$$\langle \cos \psi \rangle = 2 \int_0^{\pi/2} \cos(\hat{\lambda}(\beta) - \beta) P(\beta) d\beta \nonumber = \int_0^{\pi/2} d\beta \sin \beta \cos(\Theta(T))_{\Theta(0)=\beta}. \quad (2.22)$$

Expressions (2.21) and (2.22) cannot be solved analytically but can be evaluated numerically. A very good approximation for small to medium $R/l_p$ (see Fig. 2.7) is given by:

$$\langle \cos \psi \rangle \approx 1 - \frac{1}{2} \sqrt{\frac{R}{l_p}}. \quad (2.23)$$

Thus, the reorientation function depends on the obstacle radius compared to the persistence length, $R/l_p = RD_R/v$ as expected from Eq. (2.21).

**Residence time**

Again, we first consider the easier deterministic slide-off condition. For the residence time, we must take into account the speed reduction on the obstacle. Accordingly, we
may write the residence time as an integral:

\[
\tau_R(\beta) = \int_0^\beta \frac{Rd\Theta}{v \cos \Theta} = \frac{R}{v} \ln \left[ 1 + \frac{2}{\cos(\beta/2) - 1} \right]
\] (2.24)

The residence time averaged over the incident angle distribution is then

\[
\tau_R = \int_0^{\pi/2} d\beta \tau_R(\beta) \sin \beta = \frac{\pi R}{2v}.
\] (2.25)

Addition of rotational diffusion on the obstacles complicates the analysis. Now, the residence time can be characterised by the mean hitting time of the Langevin equation (2.17) on the exiting boundaries. This has a known expression [78]:

\[
\tau_R(\beta) = \frac{1}{D_R} \int_0^{\beta} dy e^{l_p \sin y/R} \int_y^{\pi/2} d\Theta e^{-l_p \sin \Theta/R},
\] (2.26)

where we assumed a reflecting boundary at \( \Theta = \pi/2 \) and an absorbing boundary at \( \Theta = 0 \), and we recall that \( l_p = v/D_R \) is the persistence length of the swimmer. While it is possible to derive asymptotic solutions to this integral, a more intuitive interpretation similar to the one introduced for the reorientation function offers more insight into the processes at play. When the persistence length is much larger than the radius of the obstacle \( (l_p \gg R) \), the drift time along the surface \( \tau_{\text{drift}} = \pi R/2v \) dominates the mean first passage time. However, when the persistence length is much smaller than the radius \( (l_p \ll R, \text{i.e. approaching the flat wall case}) \), we may expect that rotational diffusion time, \( \tau_{\text{diff}} = 1/D_R = v/l_p \) – calculated as the mean first passage time out of a flat potential for the incident angle distribution \( P(\beta) \) – dominates. A very reasonable fit to numerical solutions of the integral (2.26) is indeed given by

\[
\tau_R = \frac{\tau_{\text{drift}} \tau_{\text{diff}}}{\tau_{\text{drift}} + \tau_{\text{diff}}} = \frac{\pi R}{2v} \frac{1}{1 + \pi R/2l_p},
\] (2.27)

as shown in Fig. 2.8.

**Effective speed**

As we know from the sliding condition \( v_{\text{obs}} = l/\tau_R \) with \( l = R\sqrt{2 - 2\cos \alpha} \). In order to compute the effective speed, we require the average \( \hat{\alpha} \). For the deterministic slide-off, there is by definition no reorientation and \( \psi = 0 \), i.e. \( \alpha(\beta) = \beta \). Using the (normalized)
Fig. 2.8 Numerical solution of Eq. (2.26) (solid curves) compared to the analytical approximation in Eq. (2.27) (dashed curves). These are plotted for $D_R = 0.1s^{-1}$ (blue curves) and $D_R = 0.5s^{-1}$ (red curves). Parameters: $v = 20\mu m/s$, $d = 60\mu m$.

Fig. 2.9 Numerical solution of $\hat{\alpha}$ (solid curves) vs. the analytical approximation in Eq. (2.29) (dashed curves), for $D_R = 0.1s^{-1}$ (blue curves) and $D_R = 0.5s^{-1}$ (red curves). Parameters: $v = 20\mu m/s$, $d = 60\mu m$.

distribution $P(\beta)$ in Eq. (2.11) to average over the collision angle $\beta$ gives unity. Thus, it follows that $l = R\sqrt{2 - 2\cos(1)}$ for the deterministic slide-off condition. For the stochastic slide-off, we also require the average angle travelled over the pillar, $\hat{\alpha}$. Using that

$$\hat{\alpha} = \int_0^{\pi/2} d\beta \, \hat{\alpha}(\beta) \sin \beta,$$

and the definition of $\hat{\alpha}(\beta)$ in Eq. (2.19), we can solve for $\hat{\alpha}$ numerically, and find a reasonable analytical approximation:

$$\hat{\alpha} \approx 1 - \sqrt{\frac{\pi R}{2l_p}} + \left(\frac{R}{2l_p}\right)^{3/2},$$

as shown in Fig. 2.9. The average distance travelled is then $l = R\sqrt{2 - 2\cos\hat{\alpha}}$. As for the sliding condition, the effective speed in Eq. (2.6) for deterministic and stochastic slide-off follows as $v_{\text{eff}} = v\tau_c/\tau + v_{\text{obs}}\tau_R/\tau = v\tau_c/\tau + l/\tau$. 
Fig. 2.10 Diffusion with slide-off boundary condition, $D_{SO}$, is scaled by the free diffusion coefficient. If the particle orientation is fixed on the obstacle (i.e. $D_R = 0$ on obstacle), diffusion is enhanced at large obstacle densities (purple markers). With stochastic slide-off boundary condition (i.e. $D_R \neq 0$), the diffusion coefficient decreases with increasing obstacle density. If $D_R$ is increased (both free space and on obstacle), the relative decrease in diffusion coefficient, $D_{SO}/D_0$, due to obstacle collisions is smaller (orange vs. green markers). Note that the absolute value of $D_{SO}$ is smaller for larger $D_R$. The dashed lines are the respective theoretical approximations to the simulations (details given in section 2.3). Parameters: $N_P = 1000$, $D_R = 0.1$ s$^{-1}$, $v = 20$ µm s$^{-1}$, $d = 60$ µm, unless otherwise stated.

**Comparison with simulations**

Simulation results for the agent-based model with deterministic and stochastic slide-off boundary conditions are given in Fig. 2.10, where the role of the persistence length is studied by varying $D_R$. That is, in Fig. 2.10, $D_R = 0$ refers to the deterministic slide-off case, where the particle orientation is fixed at the obstacle but the particle is still subject to the usual rotational diffusion in free space ($D_R = 0.1$ as studied before). For $D_R \neq 0$ in Fig. 2.10, the rotational diffusion acting on the particle orientation is the same in free space and on the obstacle. We can then see that in all simulated cases, the diffusion coefficient $D_{SO}$ is equal to the free diffusion coefficient $D_0$ in the limit of small (or far apart) obstacles. However, at higher obstacle densities, the deterministic slide-off diffusion coefficient increases significantly compared to the free space case (see the purple markers in Fig. 2.10). This increase appears despite the decrease in speed on the obstacle. As the speed on the obstacle is given by $v = v_0 \cos \beta$, the particle will
propagate very slowly when it is oriented at right angles to the surface. However, we note that in addition the rotational diffusion is suppressed during the time the particle interacts with an obstacle. At higher obstacle densities, the time that the particle spends on these obstacles without being reorientated increases so that the effective persistence increases. This could cause an increase in diffusion coefficient, and, thus, we use an effective rotational diffusion $D_{\text{eff}}^{R} = D_{R} \tau_{c}/\tau$ in Eq. (2.6). With this correction, the RTP model is a good approximation of the simulation results with deterministic slide-off condition, see dashed purple line.

Restoring rotational diffusion on the obstacle surface dramatically changes the dependence of $D_{\text{SO}}$ on the obstacle density. The stochastic slide-off diffusion coefficient decreases monotonically, but this is still much higher than the Lorentz gas result (compare the green markers in Fig. 2.10 to the markers in Fig. 2.3(a)). If the rotational diffusion is increased both in free space and on the obstacle, the effect of the obstacle lattice on the relative diffusion is reduced (green vs. orange markers in Fig. 2.10).

The RTP model in Fig. 2.10 reproduces the main trends seen in the simulation results: (i) the diffusion coefficient decreases as the obstacle density increases, and (ii) the relative decrease in the diffusion coefficient to that of free space is smaller as the persistence length decreases (i.e. $D_{R}$ increases). If the particle is more prone to reorientation in free space, the relative importance of reorientation due to the obstacles is smaller, as can be seen by comparing the green and orange dashed curves in Fig. 2.10. However, the RTP model predicts a much sharper decline in the diffusion coefficient at larger values of $R$. This discrepancy could be caused by the approximations we made to derive expressions for the parameters in the RTP model. But comparison with numerical solutions showed particularly good agreement of the approximations in the case of $D_{R} = 0.1$, even at large $R/d$. Therefore, this could be another hint that the geometry of the obstacle lattice may be playing a role at high densities, which we will address in the next section.

### 2.4 High-density geometrical effects

While the RTP model accounts for the diffusion coefficient $D_{\text{slid}}$ of the sliding boundary condition at low to intermediate obstacle densities, it fails to completely describe the simulations at high density. Figure 2.11(a) shows fixed $R/d = 0.47$ (the largest value) cross-sections of the surfaces in Figures 2.6(a) and 2.6(b). At this high density, the diffusion coefficient for the hexagonal lattice simulations has peaks that exceed the RTP model. There are two of these peaks at low $\alpha$ as well as smaller overshoots
Fig. 2.11 Geometric effects for sliding boundary condition. (a) The discrepancy between the RTP model and the hexagonal lattice simulation results at high density ($R/d = 0.47$) is centred around the deterministic stable regions [shaded as in (c)], revealing influence of geometry. Inset: Diffusion coefficient for a square lattice. (b) Schematic of a 1-D system, considering a flight along one channel in the lattice. The leaving angle at each pillar is given by $\theta_n$. The lower schematic shows possible termination of flights in a horizontal channel. (c) Iterative map of the leaving angle as a function of the previous leaving angle for different central angles $\alpha$, $\theta_{n+1} = f(\theta_n) + \alpha$. The shaded regions correspond to regions of stable flights. Stable fixed points cross the dashed line with a gradient between -1 and 1 (a mapping with a stable fixed point is shown in the lower shaded region, with an example trajectory in pink). An example of a bounded mapping of leaving angles is shown as orange trajectory.

at higher $\alpha$. However, if we instead perform the simulations in a square lattice (see Fig. 2.11(a) inset), we get a different peak structure, with a single peak at low $\alpha$. We will show that this is due to the geometry of the lattice, and its guiding effect on the self-propelled particles.

For the geometry of the lattice to influence the particle paths, there must be a correlation between successive collisions with pillars. This means that the particle must not lose the memory of its orientation between collisions, i.e. the obstacle separation must be much smaller than the persistence length, $d - 2R \ll l_p$. In this case, a purely deterministic model ($D_R = 0$) provides a good approximation to explore correlations between collisions for the sliding boundary condition. In such a model, the particle travels in a straight line between pillars, and is reoriented by $\alpha$ by sliding scattering. We consider a ‘channel’ defined by two rows of pillars within the lattice (Fig. 2.11(b)). A particle traverses the channel by skirting around pillars, leaving the surface of the $n$th pillar with a polar angle $\theta_n$. For deterministic (ballistic) dynamics between collisions, we can completely specify a trajectory by the ‘flight’ $\{\theta_n\}_{n=1}^N$, the sequence of leaving angles from successive collisions, as in Fig. 2.11(b). The sequence size $N$ defines the
flights length. Successive leaving angles are determined by the recurrence relation:

\[ \theta_{n+1} = g_\alpha(\theta_n) = f(\theta_n) + \alpha, \quad (2.30) \]

where, in this deterministic model, \( f(\theta_n) \) is a function determined solely by the geometry.

When the radius \( R > \sqrt{3}d/4 \), the hexagonal obstacle lattice has a nearest neighbour horizon; that is any straight line drawn from the surface of a pillar must hit one of its nearest neighbours. As we are considering ballistic particle trajectories here, this close packed limit of overlapping pillars is required to avoid an infinite mean free path. The function \( f(\theta_n) \) can be determined through geometry, and takes the form

\[
f(\theta_n) = \begin{cases} 
\cos^{-1}\left(1 + \frac{d}{R}\sin\left(\frac{\pi}{6} - \theta_n\right)\right) - \theta_n, & \theta_n < \theta^* \\
\theta_n - \cos^{-1}\left(1 + \frac{d}{R}\cos\theta_n\right), & \theta_n \geq \theta^* 
\end{cases} \quad (2.31)
\]

As the particle moves along a channel during a flight, it can transition between pillars on the opposite (e.g. \( \theta_{n-1} \to \theta_n \)) or same (e.g. \( \theta_{n+2} \to \theta_{n+3} \)) side of the channel, as shown in Fig. 2.11(b). The angle \( \theta^* = 2\pi/3 - \cos^{-1}(R/d) \) is a transition angle that determines on which side of the channel a particle will next hit. If \( \theta_n < \theta^* \), the particle will hit a pillar on the opposite side of the channel, whereas for \( \theta \geq \theta^* \) the particle will hit on the same side of the channel. This means the map \( g_\alpha(\theta_n) \) is discontinuous at \( \theta_n = \theta^* \), as in Fig. 2.11(c).

The flights considered in the deterministic model correspond well to what we observe in the simulations. At high densities, these show particle trajectories made up of long flights along lattice channels, interrupted by ‘tumbles’ into the next long flight. The deterministic model allows to establish if the flights are geometrical in origin. In this model, a flight terminates when the leaving angle \( \theta_n \) becomes too small (\( \theta_n < \theta^\text{min} \)) or too large (\( \theta_n > \theta^\text{max} \)) as it will be deflected out of the channel on its next collision, illustrated in Fig. 2.11(b). Stable flights are trajectories that remain in the region \( \theta^\text{min} \leq \theta_n \leq \theta^\text{max} \) indefinitely. This can happen in two ways:

1. A stable fixed point may exist (a point \( \theta \) such that \( g_\alpha(\theta) = \theta \), and \( |g'_\alpha(\theta)| < 1 \)), so that long trajectories have a single repeated leaving angle;

2. The map \( g_\alpha(\theta_n) \) is bounded within the allowed region of leaving angles: \( \theta^\text{min} \leq g_\alpha(\theta_n) \leq \theta^\text{max} \) for all \( \theta^\text{min} \leq \theta_n \leq \theta^\text{max} \), so that no trajectory may leave the allowed region.
As \( f(\theta_n) \) is fixed by a given geometry, the polar angle \( \alpha \) in Eq. (2.30) determines whether stable solutions are possible.

The iterative map \( \theta_{n+1} = g_{\alpha}(\theta_n) \) is plotted for \( R/d = 0.47 \) in Fig. 2.11(c), where lines correspond to solutions of Eq. (2.30) for different values of \( \alpha \). Two stable ranges (shaded regions) are seen to emerge corresponding to ranges of \( \alpha \), which controls stability. For \( \theta_n < \theta^* \), increasing \( \alpha \) causes a stable fixed point to develop. An example trajectory is shown in purple Fig. 2.11(c). Increasing \( \alpha \) further, in the range that defines the lower region (shaded in blue), provides a map bounded in the interval \([\theta_{\text{min}}, \theta_{\text{max}}]\), which is illustrated by the orange trajectory. Flights in this lower shaded region bounce from one side of the channel to the other. If \( \alpha \) is increased further, the map again becomes unbounded \( (g_{\alpha}(\theta_n) > \theta_{\text{max}}) \) and stability is lost. For \( \theta_n > \theta^* \), the upper region (shaded in pink) has a stable fixed point, so that particles perform stable flights by running along only one side of the channel in this region.

Stable trajectories from the deterministic model cannot give rise to diffusive behaviour. But, any rotational diffusion, however small, will eventually cause a deviation of trajectory large enough to take the particle out of the stable interval \([\theta_{\text{min}}, \theta_{\text{max}}]\). This will cause flights to terminate, and observed transport is, thus, diffusive, not superdiffusive. In view of the large persistence length of flights for stable values of \( \alpha \), the diffusion coefficient for such flights is expected to be large compared to that corresponding to other values of \( \alpha \). By plotting the stable regions of \( \alpha \) predicted by the deterministic model against the simulation results at high density in Fig. 2.11(a), we see that this is indeed the case: the spikes in diffusion coefficient for the simulations correspond well to the stable regions in the deterministic model. It is important to note that the obstacle sizes we are considering here are below the critical trapping radii typically found for microswimmers such as *E. coli* [42, 43]. It is possible to reach a high density state where the obstacle separation is larger than the persistence length, where the presented results would not hold. However, in this regime, the obstacles would be much larger than the trapping radius, and so particles would be trapped for long periods on obstacles [42, 43], making diffusion very slow. Finally, the stochastic slide-off collision rule also exhibits an increase over the bare RTP model as \( R/d \) gets very large.

Although we do not try to model this here, it is clear from particle trajectories in the simulations (see e.g. Fig. 2.1(b)) that in this case there is also a geometrical guiding effect (this is also the case for the deterministic slide-off rule).


2.5 Discussion

We have shown that non-classical surface interactions significantly impact the active diffusive transport in complex environments, such as ordered obstacle arrays. Compared to a high-density Lorentz gas model, where particles get trapped in the wells of lattices, and the behaviour is jump diffusive, the sliding and slide-off boundary conditions allow particles to escape these wells and traverse the lattice efficiently. These boundary conditions share certain general features and differences to the classical specular reflection boundary condition. The most striking difference is that they are not invertible; given an outgoing orientation and leaving point, we cannot infer both the incoming angle and collision point. The sliding condition maps particles with different orientations on collision to the same leaving point, and so information on the incident angle is lost. Similarly, the deterministic slide-off condition maps particles with the same incident angles but different collision points onto the same leaving point. The stochastic slide-off condition loses both pieces of information. We believe this noninvertibility provides a stabilizing effect on trajectories due to geometrical guiding at high obstacle densities.

These results highlight the importance of choosing realistic microscopic boundary conditions to obtain realistic macroscopic dynamics. In particular, models employing reflective boundary conditions, e.g. those used in [51, 52] to describe bacteria in porous media, should not give realistic results for active particles. The RTP model presented here is general in the sense that it can be applied for different lattices and particles. A different lattice of obstacles requires the re-calculation of the reorientation function $\langle \cos \psi \rangle$ and the collision time $\tau_c$. A change in the scattering interaction, on the other hand, requires specification of the reorientation function $\langle \cos \psi \rangle$, the residence time $\tau_R$, and potentially the speed at an obstacle $v_{\text{obs}}$, which may also be inferred using experimental measurements. While the slide-off condition based on steric effects is a convenient choice for numerical studies, the sliding boundary condition offers a more straightforward way of connecting the model to experimental measurements through parameter fits of $\langle \cos \psi \rangle$, $\tau_R$, and potentially $v_{\text{obs}}$. In chapter 4, we will apply this approach to gain understanding of the bacterial scattering process at convex surfaces and the impact on diffusive transport in microfluidic obstacle lattices using smooth-swimming *E. coli* mutants as well as the run-and-tumble wild-type. To understand the properties of the run-and-tumble wild-type, we will, however, first continue the theoretical analysis and study how addition of ‘biologically’ induced tumbles can affect the diffusive and directed transport in obstacle lattices in the following chapter.
Chapter 3

Run-and-Tumble particles in structured environments

3.1 Chemotaxis: sensing chemical gradients

The motility pattern of bacteria has been studied long before the interest in ‘active matter’ arose. For example, the model of Run-and-Tumble particles was introduced in the 1970s to describe the swimming of *E.coli*. Howard Berg recognised that this bacterium swims in a roughly straight line by rotating its flagella in a bundle for some time, which he termed a ‘run’ [79]. Runs are interrupted by sudden large reorientation events termed ‘tumbles’ (albeit originally referred to as ‘twiddles’). These reorientation events are introduced when at least one flagellum in the bundle switches its rotation from counter-clockwise (CCW) to clockwise (CW) and all flagella unbundle.

If the bacterium did not tumble but was only reorientated due to (unavoidable) rotational diffusion, cell populations could achieve a much larger dispersal and explore space more efficiently [68]. Why then does the bacterial motility pattern commonly include a reorientation event like the tumbles observed for *E.coli*? The induced strong reorientation offers the cell a degree of freedom to bias its random walk based on the environmental conditions. By modulating its tumbling rate $\mu$, *E.coli* can follow spatial gradients of attracting or repelling chemoeffectors\(^1\) in a process termed chemotaxis. If the cell is moving up the gradient of a chemoattractant, it on average reduces its tumbling rate $\mu$, whereas the rate is unchanged if it moves down the gradient. For many environmental situations, this biased random walk is a successful strategy of closing in on a source of chemoattractant as illustrated in Fig. 3.1.

\(^1\)In the following, we will restrict the discussion to chemoattractants for the sake of clarity.
Fig. 3.1 Chemotaxis as a biased random walk. The bacterium swims in a straight line during a ‘run’, which is terminated by a sudden reorientation event during a ‘tumble’ with frequency $\mu$. In a gradient of chemoattractant, $\mu$ is reduced when the cell is moving up the gradient and, thereby, runs are extended. As a result, the random walk is biased up the gradient.

While larger eukaryotic cells can detect a chemical gradient by sensing along their cell body, bacteria are generally thought too small to efficiently measure gradients common to their environment along their cell body of a few micrometers. They instead achieve the biased random walk by an intricate system to compare past measurements of chemoattractant via an internal memory. The internal chemotactic signalling pathway, as described for *E.coli* [11] and illustrated in Fig. 3.2, has two main components: i) a signal transduction pathway (a phosphorelay cascade) to respond to changes in attractant concentration; and ii) an adaptation mechanism to adapt to a new level of chemoattractant over time. In the default state, the flagellar motor rotates counter-clockwise, i.e. the flagella bundle together for a run.

Chemoattractants are detected by receptors, which are transmembrane protein complexes that accept methyl-groups. Receptors with (relatively) high methylation levels activate the kinase CheA, which autophosphorylates and passes on the phosphor-group to another kinase, the response regulator CheY, that diffuses and binds to the flagellar motor. Increasing the level of the phosphorylated form, CheY-P, in the system then increases the likelihood of flagella to switch from CCW to CW rotation, i.e. increases the likelihood of tumbling. On the other hand, excitation (i.e. ligand binding) inactivates CheA while CheY-P is constantly hydrolyzed, thereby reducing the likelihood of CW rotation and extending runs. The methylation level of the receptor is controlled by the interplay of the methyltransferase CheR and the methylesterase CheB. The structural change in receptor configuration upon ligand binding induces an increase in methylation level (via CheR), which is a relatively slow process. Demethylation, on the other hand, is mediated via CheB and occurs fast. Adaptation to constant concentration levels is achieved when methylation and demethylation balance.

The temporal comparison between chemical concentrations required for gradient detection is mediated by a combination of signal detection and methylation. The
3.1 Chemotaxis: sensing chemical gradients

Fig. 3.2 Chemotaxis signalling pathway. The chemotaxis pathway is an internal signalling cascade with chemoattractant concentration as input and CCW/CW flagellar rotation as output. The likelihood of CW rotation is increased by binding of the response regulator CheY-P. CheY receives its phospho-group from the kinase CheA. The kinase CheA is bound to receptors, which are transmembrane protein complexes that accept methyl-groups, and active at high methylation level. Upon binding of ligands to the receptor, CheA is inactivated. The chemotactic memory is encoded in the methylation level of the receptor. Adaptation is achieved by the interplay of the methyltransferase CheR and the methylesterase CheB. Receptor occupation activates CheR and increases the receptor methylation, which is a slow process. Demethylation is a fast process and mediated by CheB, which is activated by the active kinase CheA.

receptor occupation with ligands encodes the current attractant concentration and changes rapidly. The methylation level, on the other hand, changes more slowly and thereby encodes the recent levels of attractant concentration. This time lag between the regulating mechanisms leads to a receptor with relatively low methylation level when the cell is moving up gradients, which results in extended runs.

Chemotaxis based on a temporal comparison between concentration measurements relies on control over the swimming direction. The spatial gradient can only be estimated by temporal measurements if the travelling direction is as straight as possible such that $\partial_t c \approx \mathbf{v} \cdot \nabla c$, where $c$ is the field of chemoattractor and $\mathbf{v}$ the velocity vector of the swimmer. Therefore, the persistence time sets an upper bound for the time during which a chemical gradient can be estimated accurately by temporal comparison. If the cell has been reoriented significantly, e.g. by rotational diffusion, this approach does not lead to reliable estimates of the spatial gradient any more. Hence, it is generally assumed that the characteristic duration of a run is limited by the rotational diffusion experienced by the cell body [68]. This raises the question of how obstacle collisions affect the cell’s ability to perform chemotaxis. Obstacle scatterings reduce the effective persistence time and introduce reorientations that cannot be controlled by the cell. A stark example is a reflecting boundary condition (see chapter 2): if a bacterium that is travelling up the gradient collides with an obstacle head-on, its orientation is
Run-and-Tumble particles in structured environments changed significantly and then directed down the chemical gradient. While a tumble is an ‘output’ of the internal chemotaxis pathway, the bacterium has no control over reorientations due to obstacle collisions, and there is (up to date) no report about a potential mechanism to incorporate those events into the internal signalling pathway. Therefore, the specifics of the scattering rules and the time scale of the chemotactic memory might influence how well bacteria can respond chemotactically in an obstacle lattice.

3.1.1 Theoretical approaches to bacterial chemotaxis

A plethora of chemotaxis models can be found in the literature, both for single cells and populations as reviewed, for instance, in [80] and [81], respectively. On the macroscopic scale, continuum models such as generalised Keller-Segel models have been very successful [82]. In a generalised form, the response of a bacterial population $b$ to a chemoattractant field $c$ can be described by a drift-diffusion equation

$$\frac{\partial b}{\partial t} = \nabla(D(c)\nabla b) - \nabla(\chi(c)b\nabla c) + g(b,c),$$  

(3.1)

where $D(c)$ is the diffusivity of the bacterial population, $\chi$ is the chemotactic sensitivity and $g(b,c)$ is a combined growth-death function. Here, the chemotactic sensitivity $\chi$ quantifies the observed drift in the bacterial population. Originally, the model was derived phenomenologically to describe chemotactic travelling bands of bacteria observed in capillary experiments [82], but there now exist several approaches to connect it to single cell dynamics. The more detailed approaches try to incorporate insights into internal signal-transduction, e.g. by directly modelling receptor states [83, 84]. A variety of forms have been proposed for the chemotactic sensitivity based on estimated parameter values and kinetics for binding/unbinding of ligands at the transmembrane receptors [85–87]. An alternative approach does not directly require information about receptor kinetics but considers the change in CCW/CW rotation observed experimentally upon stimulation. For this approach, de Gennes introduced a response kernel $K(t)$ that described how the memory of the bacterium compares past and present attractant concentrations [88]. Using this response kernel, the microscopic run and tumble parameters can be connected to the macroscopic chemotactic sensitivity $\chi$.

The above studies have considered bacteria swimming unobstructed in ‘free’ liquid. Theoretical work on chemotaxis in porous media, on the other hand, has been scarce. Similar to diffusive transport, there are several studies suggesting a scaling based on Knudsen diffusion [51, 52]. As Knudsen diffusion has been derived for classical
gas kinetic models, those models implicitly assume a reflecting scattering rule for the bacterial interaction with obstacles. By contrast, experiments on migrating waves of bacteria in soft agar assays found that chemotaxis breaks down before diffusive transport, which cannot be explained by a model based on gas kinetics [89]. Instead a modified Keller-Segel model was proposed based on de Gennes [88] derivation of the chemotactic sensitivity, and was able to reproduce the stark break down of the chemotactic response at agar concentrations > 0.3\%(w/v). The model assumed that interaction with the agar structure increased the effective tumbling rate by introducing additional reorientation events. However, direct insight into the interaction of bacteria with the agar structure at microscopic level was missing. Therefore, it was necessary to guess the functional relationship between the tumble rate and the agar concentration.

In this chapter, we will first add biologically motivated tumbles to the agent-based model and theoretical framework introduced in chapter 2, and study their effect on diffusive transport. Subsequently, we will investigate how a chemotactically biased tumbling rate can bias the random walk up a chemical gradient through an obstacle lattice. To this end, we will introduce a single-cell model for the chemotactic bias in the tumbling rate, which can then be coarse grained to a population-level model. Combining the population model with simulation results, we will address some of the open questions regarding chemotaxis in porous media.

### 3.2 Run and Tumble motility

#### 3.2.1 Tumble frequency

Since the tumbling frequency $\mu$ follows an exponential distribution\(^2\), we can model tumbles as independent events of a Poisson process with rate

$$\mu(t) = \mu_0 e^{\Delta(t)},$$

where $\mu_0$ is the tumbling frequency in the absence of chemical stimulus and $\Delta(t)$ is the chemotactic bias. In the case of a small chemotactic bias, i.e. $\Delta(t) \ll 1$, Eq.(3.2) may be linearised to

$$\mu(t) \approx \mu_0 [1 - \Delta(t)].$$

\(^2\)Although some recent studies suggest that the run time distribution of *E.coli* is broader and rather follows a power law [90, 91], an exponential distribution is generally assumed so far.
While the tumbling frequency has been reported many times for the model organism *E. coli* in free liquid, it is unclear how obstacle interactions may affect the ability of the cell to tumble. Very dense porous media with only small pore spaces likely prevent the flagellar bundle from unbundling [61, 92]. However, even in the case of less restriction in space, hydrodynamic interaction with a boundary might affect unbundling. A study of *E. coli* motility close to a flat surface showed that tumbling is reduced by about 50%, which impedes escape from surfaces [39]. However, this study considered a flat wall, while the hydrodynamic effects may be different at a curved surface. Therefore, we compare the effect of tumbling being suppressed at the surface to tumbling at the surface as in free liquid. When the particle is at an obstacle Eq. (3.2) is modified to

$$\mu_0(t) = \varepsilon \mu_0 e^{\Delta(t)}, \quad (3.4)$$

where $\varepsilon \in [0, 1]$ is the fraction of the tumbling rate at the obstacle with $\varepsilon < 1$ indicating a reduction in tumbling. As long as $\varepsilon > 0$, tumbling can occur close to a surface and may act as a means of escape from surface interaction if the reorientation is large enough.

### 3.2.2 Tumble angle

The distribution of reorientation angles is confined to the range $[-\pi, \pi]$. For *E. coli*, a forward bias has been reported in the seminal work by Berg [79]. Following Pohl et al. [93] we model the reorientation angle $\psi$ as a gamma distribution restricted to $[0, \pi]$

$$P(|\psi|) = \gamma(a, b) = \frac{|\psi|^{b-1} \exp(-|\psi|/a)}{a^b \Gamma_L(b, \pi/a)}, \quad (3.5)$$

where $\Gamma_L(b, x) = \int_0^x t^{b-1} \exp(-t)dt$ is the lower, incomplete gamma function. To sample this probability distribution numerically, Eq. (3.5) can be integrated to arrive at the cumulative distribution function (CDF)

$$F_X(x) = \int_0^x P(\psi) d\psi = \frac{\Gamma(b) - \Gamma_U(b, x/a)}{\Gamma_L(b, \pi/a)}, \quad (3.6)$$

where $\Gamma_U(b, x) = \int_x^\infty t^{b-1} \exp(-t)dt$ is the upper incomplete gamma function. A standard practice to generate random numbers from an arbitrary probability distribution involves drawing random numbers $Y$ that are uniformly distributed on the interval $[0, 1]$ and then using the inverted CDF for $F_X^{-1}(Y) = x$. However, Eq. (3.6) cannot be inverted so that we apply a root finding algorithm to $F_X(x) - Y$ on the interval $[0, \pi]$.
3.2 Run and Tumble motility

each time a random number has to be generated from Eq. (3.5). The sign of the angle change is then also determined randomly.

3.2.3 Numerical implementation

Based on the model introduced in the previous chapter, we will include tumble events in the agent-based model of active particles. Thus, the run-and-tumble particles considered here are reoriented by rotational diffusion during a run both in free space and on an obstacle. Reorientations due to tumbling in free space are defined by the distributions of frequency and angle in the previous sections. Whenever the particle is at an obstacle, tumbling may occur if \( \varepsilon > 0 \) in Eq. (3.4), which can but does not have to result in an escape from the obstacle. For the stochastic slide-off boundary condition (see section 2.3.2), the particle leaves the obstacle after a tumble only if the tumble reorientates the particle sufficiently such that the angle with the obstacle surface \( \Theta \) leaves the domain \( \{0, \pi\} \). A similar implementation could be made for the sliding boundary condition (see 2.3.1), which assumed that the particle’s direction of motion is aligned with the surface tangent of the obstacle. However, for the sake of simplicity, we will focus on the slide-off condition in the following.

3.2.4 Effect on diffusion in obstacle lattices

Without obstacles, the diffusion coefficient of an RTP (in 2D) is

\[
D = \frac{v^2}{2[D_R + (1 - \langle \cos \psi_T \rangle)\mu]},
\]

(3.7)

where \( \langle \cos \psi_T \rangle \) is the mean cosine of the tumbling angle, and \( \mu \) is the tumbling frequency [75]. Reorientation due to tumbling and due to obstacles can, in the first instance, be assumed to be independent of each other. For independent Poisson processes, we may write

\[
D = \frac{v^2}{2[D_R + (1 - \langle \cos \psi \rangle)/\tau + (1 - \langle \cos \psi_T \rangle)\mu]},
\]

(3.8)

where \( \langle \cos \psi \rangle \) and \( \tau \) are the obstacle-induced reorientation and run time between obstacle collisions introduced in the previous chapter. Eq. (3.8) is indeed the case if we assume there is no suppression of tumbling on the obstacles. However, there is some evidence to suggest that the presence of obstacles suppresses the physical act of tumbling due to the hydrodynamic interactions between the swimmer and the
Fig. 3.3 The role of tumbling. Diffusion in a hexagonal lattice results with slide-off condition and tumbling. The diffusion coefficient for particles which do not tumble on obstacles ($\varepsilon = 0$) stays nearly constant with increasing obstacle density: while obstacles introduce a reorientation process, they here also reduce another reorientation process (biologically induced tumbles). If tumbles occur with the same frequency as in free space ($\varepsilon = 1$), the diffusion coefficient reduces steadily. The diffusion coefficient expected for active Brownian particles ($D_{ABP}$) with a slide-off boundary condition and no biologically induced tumbles is shown as a comparison.

trapping surface [39]. Let us first examine the case of total suppression of tumbling at the obstacle ($\varepsilon = 0$). It is possible to calculate this by considering the time spent on obstacles compared to the total time. In time $t$, there is an average of $t/\tau$ collisions, where $\tau = \tau_c + \tau_R$ is the sum of the collision time and the residence time on the pillar. The fraction of time spent off of pillars is therefore $\tau_c/\tau$, and we must multiply the tumble rate by this factor to get the effective tumble rate,

$$\tilde{\mu} = \mu \frac{\tau_c}{\tau}.$$

Then, the diffusion coefficient is modified to

$$D = \frac{\psi^2}{2[D_R + (1 - \langle \cos \psi \rangle)/\tau + (1 - \langle \cos \psi_T \rangle)\tilde{\mu}]}.$$

In Fig. 3.3 we plot the diffusion coefficient from the agent-based model with stochastic slide-off condition for no tumbling on obstacles ($\varepsilon = 0$, blue markers) vs.
same tumbling rate in free space and on obstacles ($\varepsilon = 1$, purple markers). As expected, both types have a diffusion coefficient close to the free diffusion coefficient $D_0$ at low $R/d$. With increasing obstacle density, the relative diffusion coefficient decreases significantly if the tumbling process is the same on the obstacle as in free space, $D_{\varepsilon=1}$. If the tumbles are suppressed on the obstacle, on the other hand, the diffusion coefficient $D_{\varepsilon=0}$ decreases very little, staying nearly constant with increasing $R/d$. While obstacle collisions introduce a new reorientation process, they also reduce the reorientation from tumbling. The theoretical prediction in Eq. (3.10) agrees well with the simulations. Even for $\varepsilon = 1$ not all tumbles at an obstacle result in an escape from the boundary as the reorientation may not be large enough. Therefore, $D_{ABP}$ is smaller than $D_{\varepsilon=1}^{RTT}$ as well.

### 3.3 Chemotaxis model

#### 3.3.1 Single cell response

Let us now turn to modelling the chemotactic bias in the tumbling rate. Fig. 3.4(a) shows the experimentally measured bias to CCW rotation (i.e. runs) upon stimulation with an attractant at $t = 0$ [94]. For the theoretical analysis to follow, several properties deserve attention: i) The kernel is bilobed: a strong bias towards CCW rotation immediately after stimulation for about $\sim 1$s is followed by a smaller but broader bias to CW rotation. Thus, the cell effectively compares the measurements of the previous $\sim 3$s with the current concentration. ii) The area of both peaks is almost identical, i.e. $\int_0^\infty K(t) = 0$, which means that the cell adapts perfectly at long times. iii) Before stimulation, $K(t) = 0$ as the zero level is defined by the steady-state CCW probability before stimulation. Introducing this response function $K(t)$ and applying perturbation theory, de Gennes connected the microscopic tumbling frequency and swimming speed with the macroscopic chemotactic drift [88]. An alternative approach introduced Markovian internal variables based on the Linear Chain Trick [95], and derived a Fokker-Planck equation [96]. In the following, we will use the latter approach as it facilitates the connection to numerical simulations for varying lengths of the chemotactic memory.

Following Celani & Vergassola [96], we start by approximating the general memory kernel as a Laguerre polynomial

$$K(t) = \Lambda e^{-\Lambda t} \sum_{k=1}^{k_M} \beta_k (\Lambda t)^k,$$

(3.11)
Fig. 3.4 (a) Based on the work by [94], the bias measured experimentally (circles) as the probability that flagella rotate CCW upon aspartate stimulation, where the zero level is defined by the pre-stimulus value. The solid line is a prediction of the optimum response. Adapted from [96]. (b) The internal variables \( \mathbf{m} = (m_0, m_1, m_2) \) that are introduced by the Linear Chain Trick may be interpreted as an abstract signal transduction and adaptation, which regulates the tumble rate \( \mu \).

where \( k > 0 \) ensures a finite delay time. The degree of the polynomial, defined by \( k_M \), must be chosen appropriately to approximate the response function. Since we know from Fig. 3.4(a) that \( K(t) \) is bilobed, we require \( k_M > 1 \), but it is sufficient to restrict the polynomial to \( k_M = 2 \). Thanks to the memoryless property of the exponential, Eq. (3.11) conserves the Markov property. The chemotactic bias \( \Delta(t) \) can then be determined as a convolution of the memory kernel \( K(t-s) \) with the chemical concentration perceived by the bacterium during its run

\[
\Delta(t) = \int_{-\infty}^{t} K(t-s)c(x(s), s)ds. \tag{3.12}
\]

Linearising the tumble rate as in Eq. (3.3) gives

\[
\mu = \mu_0 \left( 1 - \int_{-\infty}^{t} K(t-s)c(x(s), s)ds \right) = \mu_0 (1 - \Delta(t)), \tag{3.13}
\]

where \( \mu_0 \) is the tumble rate in the absence of a gradient. Using Eq. (3.11) for \( k_M = 2 \), we can write the chemotactic bias as

\[
\Delta(t) = \int_{-\infty}^{t} \Lambda e^{-\Lambda(t-s)} \beta_1 \Lambda(t-s)c(s) + \Lambda e^{-\Lambda(t-s)} \beta_2 \Lambda^2(t-s)^2 c(s)ds. \tag{3.14}
\]

The temporal change of the chemotactic bias, \( d\Delta(t)/dt \), then generates an integro-differential system. We may assume that the observed delay is caused by a lacking insight into internal dynamics: if we had full knowledge of the underlying dynamics causing the observed response, we could describe the time evolution of each involved player by an Ordinary Differential Equation (ODE) [95]. The dynamics of the observed
state would then be described by a chain of ODEs without the need of a delay term. Using the Linear Chain Trick, the integro-differential system \( d\Delta(t)/dt \) can be transformed into a linear chain of ODEs by specifying a set of ‘hidden’ variables as illustrated in Fig. 3.4(b) [95]. Following [96], let us define the internal state of a bacterium using the internal variables

\[
m_k(t) = \int_{-\infty}^{t} e^{-\Lambda(t-t')} (t-t')^k c(X_t, t) \, dt'
\]  

(3.15)

for \( k \geq 0 \). Now, we can repeatedly apply the Leibniz rule to derive a linear system of ODEs

\[
\dot{m}_0 = -\Lambda m_0 + c(X_t, t) \tag{3.16a}
\]

\[
\dot{m}_1 = -\Lambda m_1 + m_0 \tag{3.16b}
\]

\[
\dot{m}_2 = -\Lambda m_2 + 2m_1, \tag{3.16c}
\]

where \( 1/\Lambda \) is the time scale of the chemotactic memory. Thus, the internal state \( m_k \) evolves according to a relaxation term and a forcing term. Using the internal variables, we can re-write Eq. (3.14) as

\[
\Delta(t) = \sum_{k=1}^{M=2} \Lambda^{k+1} \beta_k m_k(t).
\]  

(3.17)

Furthermore, we know \( \int_{0}^{\infty} K(t) \, dt = 0 \) has been measured for bacteria [94]. It can also be shown from Eq. (3.11) that \( \beta_1 + 2\beta_2 = \int_{0}^{\infty} K(t) \, dt \), so that \( \beta_2 = -\beta_1/2 \), which is the optimal chemotactic strategy according to [96]. In the following we will, therefore, drop the subscript and refer to \( \beta_1 \) as \( \beta \). Finally, the chemotactic bias is given by

\[
\Delta(t) = \beta \Lambda^2 \left( m_1 - \frac{\Lambda}{2} m_2 \right).
\]  

(3.18)

Note, \( \Delta(t) \) can take on negative values when the cell is moving down the gradient, which corresponds to an increase in the tumbling rate. Using Eq. (3.11), we obtain the kernel

\[
K(t) = \beta \Lambda^2 e^{-\Lambda t} \left( t - \frac{\Lambda}{2} t^2 \right),
\]  

(3.19)

which is a common form used. As shown in Fig. 3.5, the time at which the kernel crosses from the positive lobe to the negative lobe depends on the memory rate \( \Lambda \) and is
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Fig. 3.5 The response kernel given by Eq. (3.19). This models the chemotactic response to a step-change in chemoattractant concentration. The cross-over time from positive to negative weights occurs at $t = 2/\Lambda$, where $\Lambda$ is the rate of the chemotactic memory.

given by $t = 2/\Lambda$. Furthermore, the kernel has a maximum of $K_{\text{max}} = \beta \Lambda (\sqrt{2} - 1)e^{\sqrt{2} - 2}$ at $t = (2 - \sqrt{2})/\Lambda$.

3.3.2 Population response

Assuming a small chemotactic bias $\Delta$, the forward Fokker-Planck equation for the probability $P(x, t, u, m)$ of finding the bacterium at position $x$ at time $t$ moving in direction $u$ with the internal state $m$ is [96]

$$
\frac{\partial P}{\partial t} + v \nabla \cdot (uP) + MP = D_R \nabla^2 u P - \mu_0(1 - \Delta) \left[ P - \int W(u \cdot u')P(x, t, u', m)u' \right],
$$

(3.20)

where $\mu_0$ is the tumble rate in the absence of chemotactic bias and $\Delta$ is given by Eq. (3.17). The second term on the left-hand side is an advective term caused by the persistence swimming and the third term describes the dynamics of the internal variables based on the system of Eqs. (3.16) as

$$
MP \equiv \sum_{k=0} \partial m_k [(\delta_{k,0}c(x, t) + km_{k-1} - \Lambda m_k)P],
$$

(3.21)

where $\delta$ is the Kronecker delta. The right hand side of Eq. (3.20) accounts for the reorientation due to rotational diffusion with $\nabla^2 u$ is the angular Laplacian (first term) and tumbling in and out of direction $u$ (second term). $W(u \cdot u')$ is the normalized transition probability for a particle tumbling from direction $u'$ into direction $u$. To proceed, we can first average $P$ over internal states $m$ and swimming directions $u$. 
Then, homogenization methods can be applied to the statistical moments to obtain approximations of the dynamics at large scales and at long times. The detailed derivation is beyond the scope of this work but can be found in [96]. Following [96], the probability distribution of the bacterial position for time and space scales much larger than tumbling events evolves according to

$$\frac{\partial b(x,t)}{\partial t} = D \nabla^2 b(x,t) - \nabla [\chi b(x,t) \nabla c(x,t)]$$  \hspace{1cm} (3.22)

where the chemotactic sensitivity $\chi$ is:

$$\chi = D \beta \Psi \frac{\Lambda^2}{(\Lambda + \sigma)^3},$$  \hspace{1cm} (3.23)

and

$$D = \frac{v^2}{2(D_R + \Psi)}$$  \hspace{1cm} (3.24)

$$\Psi = (1 - \langle \cos \psi_T \rangle) \mu_0$$  \hspace{1cm} (3.25)

$$\sigma = D_R + \Psi.$$  \hspace{1cm} (3.26)

The chemotactic sensitivity $\chi$ depends on the length scale of the chemotactic memory as illustrated in Fig. 3.6(a). The maximum chemotactic sensitivity is achieved for $\Lambda_{\text{max}} = 2D_R + 2\mu_0(1 - \langle \cos \psi_T \rangle) = 2\sigma$. Indeed, the chemotactic memory time has likely been optimised in relation to the persistence time $1/\sigma$ [96]. If the memory time scale is too long, the cell gives weight to past measurements that are irrelevant and cannot respond quickly to a new stimulus, e.g. when it is reoriented [97]. If the memory time is too short, on the other hand, the accuracy of the temporal gradient measurement is reduced because the bacterium ‘forgets’ previous concentrations too quickly. Finally, the drift velocity in shallow gradients can be approximated at long times as

$$v_0^0 \approx \chi \nabla c,$$  \hspace{1cm} (3.27)

with the chemotactic sensitivity given by Eq. (3.23).

Up to this point, the approach in section 3.3 followed the derivation given in [96] adjusted for $k_M = 2$ and has been outlined here for completeness. Now, we would like to adapt this approach for chemotaxis in obstacle lattices. Keeping in line with the approach developed in the previous chapter, we can define effective parameters for particles performing chemotaxis in an obstacle lattice. Specifically, as in section 3.2.4,
Fig. 3.6 Chemotactic drift. (a) Chemotactic sensitivity given by Eq. (3.23) depends on the chemotactic memory rate $\Lambda$. (b) Relative decrease in diffusion coefficient vs. relative decrease in drift velocity, $\xi$. For small memory rates $\Lambda$, drift decreases more strongly than diffusion in obstacle lattices as a function of distance between obstacles. When the distance between obstacles exceeds the persistence length of the particles, $l_p$, the ratio $\xi$ approaches unity. Parameters: $\beta = 0.67$, $v = 20\mu m/s$, $D_R = 0.1rad^2/s$, $\psi_T = 1.4931$, $\mu_0 = 1s^{-1}$.

we assume that tumbling and obstacle-induced reorientations are independent Poisson processes so that

$$\tilde{\Psi} = (1 - \langle \cos \psi_T \rangle)\mu_0 + (1 - \langle \cos \psi \rangle)/\tau,$$

which also introduces effective parameters $\tilde{D} = v^2/2(D_R + \tilde{\Psi})$ and $\tilde{\sigma} = D_R + \tilde{\Psi}$. Finally, the relative chemotactic sensitivity can be written as

$$\frac{\tilde{\chi}}{\chi} = \frac{\tilde{D} \tilde{\Psi}}{D \Psi} \left( \frac{\Lambda + \sigma}{\Lambda + \tilde{\sigma}} \right)^3. $$

The ability to move in a specific direction depends on the ability to move in any direction. Thus, as dispersion decreases in an obstacle lattice, chemotactic drift decreases as well. However, for active systems the magnitude of the decrease need not be the same for diffusion and chemotaxis, or be constant with the obstacle density. We can quantify the relative importance as

$$\xi = \frac{\tilde{v}_d/v_d^0}{D/D} = \frac{\tilde{\chi}/\chi}{\tilde{D}/D} = \frac{\tilde{\Psi}}{\Psi} \left( \frac{\Lambda + \sigma}{\Lambda + \tilde{\sigma}} \right)^3.$$

Thus, $\xi < 1$ corresponds to the drift velocity decreasing more strongly than the diffusion, whereas $\xi > 1$ corresponds to the diffusion coefficient decreasing more strongly than the drift. This ratio depends on both the chemotactic memory length and the specifics of the particle-obstacle interaction. The ratio $\xi$ can, therefore, also change with the
obstacle density as illustrated in Fig. 3.6(b) but the change is rather small. By contrast, previous work on chemotaxis in porous media assuming detailed balance derived that both diffusion and chemotactic drift were reduced by the same factor, that is $\xi = 1$ [51, 52].

### 3.3.3 Numerical implementation

Chemotaxis is implemented in the agent-based model (see chapter 2) via the internal variables given by system (3.16) using a first order Euler scheme and the modulation of the tumbling rate is determined using Eq. (3.18). We consider a linear gradient in the chemical concentration given by

$$c(x(t)) = \rho u_{c} \parallel u_{c} \parallel \cdot x(t),$$

where $\rho$ is the strength of the gradient and $\varphi \in \{0, \pi/2\}$ is the angle that the gradient direction makes with the x-axis. In a lattice of obstacles, the free space along the direction $\varphi$ will vary with lattice geometry. For a deterministic fixed gradient, we can integrate Eq. (3.12) using Eq. (3.19) to obtain the expected chemotactic bias. For example, for a linear gradient along the x-direction, we can write

$$c(x(t) - u) = \rho x(t) - \rho u_{c},$$

Thus, the expected chemotactic bias is

$$\Delta(t) = \int_{0}^{\infty} \beta \Lambda^{2}(\Lambda u - \Lambda^{2}u^{2}/2)\rho v(t - u)du = \frac{\rho v\beta}{\Lambda},$$

where we have approximated $x(t - u) \approx v \cdot (t - u)$. For the linearisation in Eq. (3.3) and, thus, for the model to be valid, $\rho$ has to be chosen such that $\Delta(t) \ll 1$.

The bacteria are initialised with random initial position drawn from a Gaussian distribution $N(\bar{x}, s)$, where $\bar{x} = (x_{\text{max}}/2, y_{\text{max}}/2)$ and $s = 0.5R$, with $R$ being the obstacle radius. Initial positions within obstacles are re-sampled. The initial orientation of particles is distributed uniformly. For the initial internal variables we assume steady state, which implies

$$m(0) = \begin{pmatrix} c(x_{0})/\Lambda \\ c(x_{0})/\Lambda^{2} \\ 2c(x_{0})/\Lambda^{3} \end{pmatrix},$$

where $x_{0} = x(0)$ is the initial position of the particle. While it is possible that bacteria modulate their migratory behaviour via mechanosensing, it has (to the best of my
knowledge) not been reported in the context of the swimming motility of *E. coli*. We assume that obstacle interactions do not affect the internal variables $m$ as these may be interpreted as representations of the biochemical network in the cell. However, there may be a hydrodynamic effect on the ability of the flagella to unbundle [39]. We will consider this as a change in the tumbling frequency in the absence of any chemical stimulus, $\mu_0$, as in Eq. (3.4).

The parameters used in the simulations are $R = 20 \mu m$, $\beta = 0.67$, $\varrho = 0.0075$, $\mu_0 = 1 s^{-1}$, $v = 20 \mu m/s$, $N_P = 10^4$. For those parameters, the chemotactic sensitivity is maximum at $\Lambda_{\text{max}} = 2.045 s^{-1}$. This value is close to the range of previously reported values for *E. coli* from $1.2 s^{-1}$ [96] to $2.0 s^{-1}$ [98]. The cross-over time for the kernel with $\Lambda_{\text{max}}$ is at about $t = 1 s$ and the cell recovers from a step-stimulus within 4s. The ratio $\Lambda/\sigma$ quantifies the length of chemotactic memory rate compared to the reorientation rate. In free space, $\sigma$ depends on rotational diffusion and tumbling. While rotational diffusion is beyond the control of a bacterium, the tumbling rate and chemotactic memory rate might have been optimised together for *E. coli* [96]. To study the effect of the chemotactic memory rate, $\Lambda$ will be varied from 0.5 to 4.0 while the unbiased tumbling rate is kept constant. As $\mu_0 = 1 s^{-1}$ and the kernel crossover is at $t = 2/\Lambda$, $\Lambda < 2$ means that measurements over a longer period than the mean run time are weighed positively, while for $\Lambda > 2$ measurements with a shorter duration than the mean run time have a positive weight.

### 3.4 Chemotaxis in an obstacle lattice

An illustration of a particle moving in a chemical gradient in $y$ direction ($\varphi = \pi/2$ in Eq. (3.32)) is shown in Fig. 3.7(a). The example particle is able to follow the chemical gradient despite scattering at obstacles. The mean position of a population of particles as a function of time along the $y$ axis is shown in Fig. 3.7(b) for different obstacle separations $R/d$. The chemotactic drift velocity is determined as

$$v_d = \langle v \cos \theta \rangle$$

(3.35)

where $\theta$ is the angle between swimming orientation and orientation of the chemical gradient and the average is taken over the ensemble of trajectories. We rescale the drift velocity obtained in obstacle lattices for different chemotactic memory rates $\Lambda$ by the expected drift velocity in free space, $v_d^0(\Lambda)$, which is given by Eq. (3.23) and Eq. (3.27). Thus, we consider the relative change in the drift velocity, $v_d/v_d^0$. 
3.4 Chemotaxis in an obstacle lattice

Fig. 3.7 (a) Illustration of a chemotactic cell moving in an obstacle lattice up a gradient. The track is colour coded according to time along the track. The chemoattractant $c(x)$ is given by Eq. (3.32) with an angle $\varphi = \pi/2$. The level of chemoattractant is illustrated by the shaded blue background. (b) Mean position of the population in $y$ direction over time relative to the maximum domain size $y_{\text{max}}$. The dashed line corresponds to a linear fit with the slope corresponding to the drift velocity, $v_d$. There is a clear dependence of the drift on the obstacle spacing $R/d$.

This approach facilitates the comparison of the effect of the obstacle lattice on the chemotactic drift between different memory rates $\Lambda$, but it is important to bear in mind that the absolute drift velocity additionally depends on $\Lambda$. If the relative drift $v_d/v_0d$ is the same for different $\Lambda$ values, one $\Lambda$ still leads to a larger absolute chemotactic drift $v_d$ as illustrated in Fig. 3.6(a). The drift velocity is then averaged over at least three independent simulations with the same parameters.

3.4.1 Effect of boundary conditions

Scattering rule

We first investigate the effect of chemotactic memory on the drift velocity at increasing obstacle densities with a slide-off scattering rule, as shown in Fig. 3.8(a). The drift velocity is scaled by the expected drift velocity in free space, $v_0d(\Lambda)$. This gives curves that roughly follow a similar trend, regardless of the memory rate $\Lambda$. Despite a steady decline, the chemotactic drift is close to its obstacle-free value up to medium $R/d$, and even at very obstacle densities there is still considerable chemotactic drift. The theoretical prediction based on Eq. (3.29) is shown as dashed line for each $\Lambda$. The agreement between simulations and theory is good for most obstacle densities but
Fig. 3.8 Chemotaxis in an obstacle lattice with slide-off surface scattering for varying chemotactic memory rate $\Lambda$. (a) Chemotactic drift velocity $v_0^d$ decreases in obstacle lattice compared to free medium. Theoretical prediction (dashed lines) in Eq. (3.29) agrees well with simulation results. Same tumbling rate on obstacle as in free space, i.e. $\varepsilon = 1$ in Eq. (3.4). Inset: The model assumption of small response (i.e. $\Delta \ll 1$) is met reasonably well for all simulations. (b) Relative change in chemotactic drift velocity compared to relative change in diffusion coefficient. For all simulations $\xi \sim 1$, and the decrease in chemotactic drift velocity is, thus, mainly caused by the decrease in diffusive transport. The theoretical prediction of Eq. (3.30) (dashed lines) is within the range of the simulation variation.

The theoretical prediction underestimates the chemotactic drift at very high obstacle densities. As a control, the maximum chemotactic bias $\Delta(t) = \beta\Lambda(\Lambda m_1 - \Lambda^2 m_2/2)$ is shown in the inset of Fig. 3.8(a). The maximum bias clearly depends on the memory rate $\Lambda$. For the derivation in section 3.3 to be valid, we require $\Delta(t) \ll 1$, which is reasonably met for all simulations. The ability to follow the gradient is related to diffusive transport, see Eq. (3.30). Comparing the relative change in drift to the relative change in diffusion in Fig 3.8(b), the ratio is close to 1 for most obstacle densities and chemotactic memory lengths. Thus, the observed decrease in drift velocity is mainly caused by a decrease in diffusive transport. The theory predicted a small change in $\xi$ for increasing obstacle density, but the variation in simulation data is likely too large to detect it here (see dashed lines in Fig. 3.8(b)).

While the effect of increasing the obstacle lattice density does not change results significantly for different chemotactic memory rates when using a stochastic slide-off condition, varying the obstacle density significantly modifies the chemotactic drift of a particle with a reflecting boundary condition for different memory rates, as shown in
Chemotaxis in an obstacle lattice

Fig. 3.9 Chemotaxis in an obstacle lattice with reflecting surface scattering for varying chemotactic memory rate $\Lambda$. (a) Chemotactic drift velocity $v_0^d$ decreases strongly with increasing obstacle density. A smaller chemotactic memory rate results in a stronger decrease. Theoretical prediction (dashed lines) in Eq. (3.29) overestimates the chemotactic drift at increasing obstacle density. (b) Relative change in chemotactic drift velocity compared to relative change in diffusion coefficient. For smaller $\Lambda$, chemotactic drift decreases more strongly than diffusive transport for increasing $R/d$. The theoretical ratio in Eq. (3.30) (dashed lines) predicts that the chemotactic drift should decrease compared to the diffusive transport than observed in simulations, in particular for large chemotactic memory rates $\Lambda$.

Fig. 3.9(a). There is a strong decrease of the drift velocity compared to free space for all values of $\Lambda$. Indeed, at the highest obstacle density studied, there is approximately no chemotactic response observed. The reduction in $v_d$ is strongest for very small chemotactic memory rates, that is those that are smaller or close to the tumbling rate $\mu_0$ ($\Lambda < 2$). As shown in the previous chapter, the diffusion coefficient for a particle with reflecting boundary condition decreases strongly. For large chemotactic memory rates, much of the drop in chemotactic drift can indeed be explained by the decrease in diffusion coefficient as the ratio $\xi$ in Fig. 3.9(b) remains close to 1 up to the highest obstacle density. For small chemotactic memory rates $\Lambda$, on the other hand, the ratio $\xi$ quickly drops below 1 and the drift velocity decreases more strongly than the diffusion coefficient for most obstacle densities. In free space, the likelihood of tumbles is reduced when swimming up the gradient such that the particle keeps moving in the favourable direction for longer. Upon specular reflection in an obstacle lattice, however, the particle is strongly reoriented. At high densities, the particle may get trapped in lattice wells, which not only decreases diffusion but also makes it nearly impossible to respond to a chemical bias. Larger chemotactic memory rates correspond to averages of concentration measurements taken over a shorter time period.
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Fig. 3.10 Suppressing tumbles at obstacles. (a) The effect of an obstacle lattice on relative drift velocity strongly depends on the memory rate $\Lambda$ for particles with slide-off condition and suppression of tumbles at obstacles (i.e. $\varepsilon = 0$ in Eq. (3.4)). (b) Relative change in chemotactic drift velocity compared to relative change in diffusion coefficient. For smaller $\Lambda$, chemotactic drift decreases less than diffusive transport for increasing $R/d$, whereas for larger $\Lambda$ it decreases more strongly than diffusive transport. The theoretical prediction of Eq. (3.30) (dashed lines) captures the observed relative decrease well for most $\Lambda$.

As the effective run time is strongly reduced by obstacle collisions, the assumption of $\partial_t c \approx v \nabla c$ is only met over a shorter averaging time. Thus, in addition to a strong decrease in diffusive transport, directed transport is hindered by a breakdown of gradient estimation in addition to the large reorientation, which might explain the discrepancy between the theoretical prediction and simulation results in Fig. 3.9(a) and (b). The slide-off boundary condition, on the other hand, statistically preserves the main velocity component such that the particle is able to respond to the chemical gradient. Furthermore, the unbiased tumbling frequency $\mu_0$ is assumed the same in free space and on the obstacle. Tumbles can reduce the time spent close to the surface even if not all tumbles reorient the particle sufficiently to escape the obstacle.

**Suppression of tumbles at obstacles**

Tumbles might be suppressed or at least reduced at a convex surface, similarly to what has been observed at a flat wall [39]. To study the effect that a potential suppression could have on the chemotactic drift, we can set $\varepsilon = 0$ in Eq. (3.4) for a particle with slide-off boundary interaction. As can be seen from Fig. 3.10, suppression improves the chemotactic drift at large obstacle densities. Part of the increase compared to Fig. 3.8 may be explained by the increase in relative diffusion coefficient $\tilde{D}/D$. As
shown in Fig. 3.3, the diffusion coefficient remains approximately constant for this scattering rule. Thus, the relative change in drift velocity vs. the relative change in diffusion coefficient shows a very similar trend in Fig. 3.10(b) as the drift velocity in Fig. 3.10(a). However, the variation between different chemotactic memory rates $\Lambda$ is large in both Fig. 3.10(a) and (b), and must be caused by variations in the chemotactic drift itself. As we argued for the diffusion coefficient, a suppression of tumbles at the obstacle can reduce the reorientation rate $\tilde{\sigma}$ below its free space value $\sigma$ despite the addition of obstacle-induced reorientations. However, if $\sigma > \tilde{\sigma}$, the cubed part in Eq. (3.30) is larger than 1, and indeed increases with decreasing $\Lambda$. Thus, the relative chemotactic drift increases for $\Lambda = 0.5s^{-1}$ (black data in Fig. 3.10(b)) and remains approximately constant for $\Lambda = 1.0s^{-1}$ (purple data in Fig. 3.10(b)). On the single cell level, this trend might be explained by an effective increase in the memory rate $\Lambda$. Assuming the internal variables $m_k$ are unaffected, a suppression of tumbles at the obstacle means that the cell cannot tumble regardless of its internal memory. At the same time, the particle keeps updating the memory with new measurements while on the obstacle. Thus, measurements taken long ago are effectively ‘discarded’ without affecting the tumble status, which is similar to shorter memory (i.e. increasing $\Lambda$). This effect is stronger, the more time the particle spends at an obstacle. Since the chemotactic sensitivity increases with increasing $\Lambda$ for $\Lambda < \Lambda_{\text{max}}$ (e.g. see 3.6(a)), the relative chemotactic drift then increases with increasing obstacle density.

### 3.4.2 Effect of lattice geometry

Based on their pattern, periodic lattices define ‘channels’ in which particles can travel freely or may be guided, as described in chapter 2. By the same token, there are also directions that are more obstructed. Consequently, the direction of the gradient in relation to the channels of the lattice geometry may influence the ability of particles to follow chemical gradients using chemotaxis. This effect is expected to be stronger at higher obstacle densities. In the previous chapter, we saw strong guiding at these densities in hexagonal lattices, which could further influence the observed response. To avoid this complication, I will turn to a square lattice to study the effect of the relative gradient orientation. In such lattices, the guiding effect was reduced and any difference may, thus, be caused mainly by the obstruction of a direction itself. The chemical gradient is aligned with the lattice channels for $\varphi = 0$ and $\varphi = \pi/2$. Furthermore, tumbling again occurs as in free space, i.e. $\varepsilon = 1$ in Eq. (3.4).

Comparing results for $\varphi = 0$ and $\varphi = \pi/4$ in Fig. 3.11(a) and 3.12(a) shows that the chemotactic drift decreases less for the aligned chemical gradient. When the chemical
Fig. 3.11 Chemotaxis in a square obstacle lattice with aligned chemical gradient. (a) Chemotactic drift velocity $v_0^d$ decreases in an obstacle lattice compared to free medium. Theoretical prediction (dashed lines) in Eq. (3.29) agrees well with simulation results but at large densities underestimates the drift. The direction of the chemical gradient ($\varphi = 0$) is illustrated in the inset. (b) Relative change in chemotactic drift velocity compared to relative change in diffusion coefficient. At large obstacle density, the chemotactic drift decreases less than expected from the diffusion coefficient and, thus, $\xi > 1$. The theoretical ratio in Eq. (3.30) (dashed lines), on the other hand, predicts an approximately constant $\xi$.

Fig. 3.12 Chemotaxis in a square obstacle lattice with tilted chemical gradient. (a) Chemotactic drift velocity $v_0^d$ decreases in obstacle lattice compared to free medium. Theoretical prediction (dashed lines) in Eq. (3.29) agrees well with simulation results. The direction of the chemical gradient ($\varphi = \pi/4$) is illustrated in the inset. (b) Relative change in chemotactic drift velocity compared to relative change in diffusion coefficient. The chemotactic drift decreases approximately as much as the diffusion coefficient with increasing obstacle density and, thus, $\xi \approx 1$. 
3.5 Discussion

The model studied here showed that the chemotactic response in porous media may depend on the structure of the medium, as well as the specifics of the microscopic scattering at surfaces. The results presented here predict a decrease in chemotactic drift velocity in line with the decrease in diffusion coefficient for a slide-off scattering rule. However, experiments with *E. coli* populations in soft agar showed that chemotaxis breaks down before diffusive transport [89]. While the bacterial population still propagated at medium agar concentrations, the characteristic chemotactic wave did not develop at those densities. The structure of an agar gel differs significantly from the regular lattice considered here. In particular, agar gels at high concentrations can generate traps with channels that are smaller than the size of the bacterium. While the bacterium might still escape by tumbling and, thereby, diffuse, the chemotactic response might become impossible. Furthermore, the free pathways available to the cells might be misaligned with the direction of the chemical gradient, thus further reducing the ability to respond chemotactically. Future work should consider the chemotactic response in random porous media and the necessary adaptations of the model developed in this thesis to account for traps in these media. Traps with infinite mean trapping time lead to subdiffusive transport [99]. While we expect that infinite mean trapping times will strongly reduce the chemotactic drift, it would be interesting to study the effect that finite trapping times may already have on the chemotactic response.

Even in the absence of chemotaxis, a possible direction of future research concerns random porous media rather than the structured lattices considered here. In this case, the position of circular obstacles may follow a uniform random distribution, where the gradient is aligned with a lattice channel, as in Fig. 3.11(b), the ratio $\xi$ slightly increases above 1, i.e. the cells can follow the chemical gradient more easily than expected from the diffusive transport, while the theoretical ratio in Eq. (3.30) predicted a decrease in line with the diffusion coefficient. The model in section 3.3.2 does not take into account the channel geometry of the obstacle lattice. As the gradient is aligned with the lattice channel, a bacterium travelling up the gradient may be less reoriented by the lattice than expected from the obstacle density. On the other hand, if the chemical gradient is not aligned with one of the lattice channels, the chemotactic drift velocity decreases in line with the diffusion coefficient as $\xi$ in 3.12(b) remains close to 1, but there seems to be only a small effect of the gradient orientation.
Fig. 3.13 RTP with a slide-off boundary condition in a random porous medium. The positions of the obstacles are drawn from a uniform distribution, with obstacles allowed to penetrate. While the particle can still explore the space at low obstacle density in (a), it can get trapped at higher obstacle density in (b), where the inset shows a magnification of the track at long-times.

obstacles are allowed to overlap as illustrated in Fig 3.13. Percolation theory predicts that there exists a density of overlapping obstacles after which there is an infinite cluster of overlapping disks and the percolating free space in 2D [100]. Close to the percolation threshold, passive Brownian particles display a subdiffusive behaviour, while they become localised and trapped at even larger obstacle densities [100]. Zeitz et al. showed that ABPs followed an extended subdiffusive regime compared to the passive particles but that the characteristics of the random medium ultimately determine the crossover from long-range transport to trapping [55]. On the other hand, the decrease in diffusion coefficient at low obstacle densities was observed to depend on the specifics of the ABP motion and the time they spent at obstacle surfaces. Future research could thus investigate whether specific tumble strategies allow RTPs to escape traps more easily in the diffusive regime and, thereby, increase effective transport. For example, it has been suggested that the run-reverse motility of the soil bacterium P. putida is an adaptation to avoid trapping in the porous regime [101]. Similarly, the extend of the subdiffusive regime could be affected by tumbling reorientation. While simulating this model sufficiently long to reach stationary state and observe trapping can be computationally demanding, a main theoretical challenge is the treatment of the escape of traps. The latter point is particularly relevant for the study of the long-term transport, where an analytical derivation of the MSD could distinguish the diffusive, subdiffusive and localised regimes based on the characteristics of the RTP.
The chemotactic response in porous media depends strongly on the interaction with obstacles. Depending on the type of surface scattering, the effective reorientation rate can increase significantly, while the chemotactic memory rate is fixed. Outside the lab, *E. coli* is a common gut bacterium, but when it leaves the intestine it might inhabit very different environments such as soil. Could bacteria adapt their chemotactic response strategy to the physical environment they find themselves in? To explicitly adjust for external reorientation due to surface scattering, the bacterium would require a way of measuring the new reorientation rate and changing either $\mu_0$ or $\Lambda$ accordingly. A non-specific adjustment of the chemotactic response could be achieved by a reduced ability to tumble close to a surface. The model showed that the suppression of tumbles can sustain diffusion and chemotactic drift at higher levels than possible with tumbling unaffected by the presence of a surface. The suppression facilitates sustained diffusion as it reduces a reorientation process while obstacle collisions introduce another process. Indeed, thanks to the suppression the effective reorientation stays close to the free space level, which may have been used to optimise the chemotactic memory rate $\Lambda$.

A recent study reported that *E. coli* is able to sense the obstacle density in regular microfluidic lattices [102]. In particular, the authors suggest that bacteria can adjust their tumbling rates to improve chemotaxis at increasing obstacle densities. However, it is unclear how *E. coli* should be able to detect the obstacle density, in particular as the obstacles were spaced such that the bacterium was not in contact with more than one obstacle. The conclusion might be based on the experimental procedure and the way the trajectories were analysed. Example particle trajectories in this study showed that in the obstacle-free case many tracks are circular (as expected close to a boundary), while circular trajectories are less common at higher obstacle densities. However, it has been shown both theoretically [54] and recently experimentally for *E. coli* [46] that obstacles enhance the diffusive transport for circular swimmers since their presence introduces reorientation events that allow bacteria to break free from the restricting circular trajectory. Furthermore, the tumbling frequency in free space might be overestimated in [102] due to the heuristic tumbling recognizer that was employed: when the cumulated angular movement of a cell reaches a threshold, a tumble is detected. This definition contrasts with the classical definition of the heuristic tumbling recognizer, which detects a tumble if the summed angular deviation around a maximum significantly exceeds the angular deviation expected from rotational diffusion. This crucial difference in definition could lead to stark differences in the results, in particular for circle swimmers: due to continuous angular movement, the cumulated movement will regularly exceed a threshold. Thus, further experimental work is required.
to understand how motility parameters might change close to (convex) surfaces and how they are incorporated into the chemotactic response.

Besides the question whether a single species of bacteria is able to modify its motility pattern in response to a change in its environment, different species may have adapted to different environments. While \textit{E. coli} may spend only a small fraction of time in soil, porous media are the typical environment for soil bacteria. The motility pattern and chemotactic response of several soil bacteria differs from \textit{E. coli} \cite{103, 104}. Thus, future research could test whether these modifications can improve chemotaxis in porous media.

In the model developed here, the chemotactic bias $\Delta(t)$ can be negative for cells moving down the gradient, while experimental results for \textit{E.coli} generally suggest that the tumbling rate is only modified when moving up the gradient \cite{105}. If an obstacle-induced reorientation directs the cell down the chemical gradient, adaptation could be faster than in the case of a constant tumbling rate. Thus, future work should investigate the effect of a one-sided response in $\Delta(t)$. Furthermore, the results here are based on Markovian dynamics. While this approximation seems to be reasonable for bacterial dynamics \cite{96}, it would be interesting to see how non-Markovian dynamics might change the chemotactic response in an obstacle lattice. Considering the computational model, it should also be noted that the variation in chemotactic drift between different simulations is considerable (as can be seen from the standard deviation in Figs. 3.8-3.12), whereas the variation in the diffusion coefficient was substantially smaller in simulations here and in the previous chapter. Increasing the number of simulated particles might reduce the variation in future studies.

The ability to follow the specific direction of the chemical gradient depends on the lattice geometry as well. If there is no ‘channel’ aligned to follow the chemical gradient directly, the chemotactic response might be further reduced. Such ‘misalignment’ of chemical gradient and porous media channels might be particularly relevant for porous media, in which bacterial transport becomes dominated by the topology of the medium \cite{106}. The results presented here can also have importance for the design and interpretation of experiments. Regular obstacle lattices are a popular choice for studying transport in porous media \cite{56, 57, 62}. It might seem convenient and straightforward to align the direction of chemical gradient with channels of obstacle lattices. However, as shown in this study conclusions drawn for the transport at high densities might depend on the direction of chemical gradient relative to the lattice channel.
This chapter and the previous one presented theoretical predictions for the transport of active particles in obstacle lattices based on assumptions about their behaviour at convex boundaries. The results showed that the scattering rules imposed on the particles can have distinct effects on the transport properties, and underlined the importance of good models of the microscopic behaviour at boundaries. The next chapter attempts to provide experimental insight, and scrutinize the previously made assumptions by investigating the interaction of bacteria at convex surfaces and the resulting diffusive transport in a microfluidic obstacle lattice.
Chapter 4

Scattering and Diffusive Transport of *Escherichia coli* in Microfluidic Lattices

To understand how the macroscopic transport arises in environments with physical boundaries, we first need a better understanding of the underlying process of scattering at those boundaries. As discussed in chapter 1, to date the rules governing bacterial scattering at convex surfaces have mainly been studied theoretically [42, 43, 47]. To recap, during contact with an obstacle (pillar) that has a radius below the critical trapping radius, hydrodynamic models predict that the angle the particle orientation makes with the obstacle surface will decrease until the particle can escape [42, 43]. Therefore, the leaving angle is approximately tangent to the obstacle surface and ‘orbiting’ of bacteria trapped at larger pillars does not occur. Spagnolie et al. [43] found that both the scattering angle and the time it takes a pusher to escape the obstacle depend on the initial collision angle with the obstacle, the dipole strength as well as the size of the obstacle. Their approximation of the scattering angle for small incident angles included a long-range hydrodynamic effect that reorients the body already before impact. Furthermore, for small incident angles, the authors predicted that hydrodynamic interaction upon collision can lead to ‘backscattering’, during which the particle is deflected with a negative angle, i.e. towards the obstacle due to hydrodynamic attraction. A recent study using far-field approximations and Lattice-Boltzmann simulations identified further possible variations in the scattering behaviour [47]. In addition to the results presented in Spagnolie, Kuron et al. identified a backward orbiting state for strong pushers in which swimmers reverse their direction upon collision compared to bulk swimming [47].

By contrast, most computational studies of active particles assume a mainly steric interaction with convex surfaces [55, 107, 108]. Although this interaction type also predicts a dependence of the deflection angle on the incident angle, as well as the radius
of the obstacle, it does not allow for the variety of trajectories observed in the hydrodynamic studies. Instead, the interaction predicts a monotonic decrease of the deflection angle with decreasing incident angle as discussed in section 2.3.2. In an extreme case, when the particle approaches the obstacle at a tangent to the surface, it should not be reoriented but escape immediately. Besides similar dependencies, hydrodynamic and steric mechanisms thus differ in their predicted scattering interactions. As the comparison between slide-off and sliding in chapter 2 showed, microscopic difference can affect the macroscopic transport.

A first experimental indication that the scattering process might depend on the angle of approach can be found in a study of run-and-tumble *E. coli* interacting with colloids on a glass surface (i.e. the cells predominantly followed circular trajectories) [46]. A head-on collision typically resulted in a slow down of the bacteria at the obstacle and a large change in direction of motion. On the other hand, bacteria approaching the obstacle almost tangentially only showed very small changes in speed and direction. However, the obstacles were on the size of the cell (diameter \(\sim 3\mu m\)), and a detailed analysis of the influence of the angle of approach was not performed.

Due to the lack of experimental studies, the relative importance of steric and hydrodynamic effects during scattering events and their impact on diffusive motion remains an open question. Moreover, the relationship between (biologically induced) tumbling and obstacle collisions has been studied neither theoretically nor experimentally. Hydrodynamic interaction at a convex surface could suppress tumbling, similar to the effects observed at a flat wall [39]. On the other hand, tumbling could also be a means of leaving the surface and thereby influencing both time spent at the obstacle as well as deflection angle. As shown in the previous chapter, this can have a substantial impact on the macroscopic transport.

To address some of the open questions, in this chapter I will study bacterial scattering and diffusion in microfluidic channels with pillars as obstacles. Microfluidic studies offer the advantage of tracking microscopic interactions with surfaces while at the same time studying macroscopic transport properties, which facilitates comparison with theoretical predictions. In addition, they provide the freedom to design different environments with a variety of complexity. As the radius of the pillars is expected to influence the scattering interaction while the lattice density should affect diffusion, radius and distance of pillars will both be varied. After describing the methods, I will analyse the obstacle interaction first for a smooth-swimming mutant *E.coli* \(\Delta\text{cheY}\), which displays runs only, and then for the wild-type *E.coli*, which shows the classical run-and-tumble motility. Finally, I will connect the microscopic analysis to
the macroscopic transport coefficients by applying the theory that was derived in the previous chapters.

4.1 Methods

4.1.1 Bacterial culturing

Experiments were performed using *E. coli* strains AD52 (AB1157 motility wild-type [109] with plasmid expressing eGFP pWR21) and AD83 (an AB1157 ΔcheY smooth-swimming mutant, JSL1 [110], with plasmid expressing eGFP pWR21), which were kindly provided by Angela Dawson and Dr. Jana Schwarz-Linek at the University of Edinburgh. The preparation of the bacterial cultures followed standard protocol developed by H.C. Berg for motility studies using *E. coli* as outlined, e.g., in [110], where growth temperature and media as well as buffer have been optimised for bacterial motility. Cultures were grown from frozen stocks on Luria Broth agar plates (LB 10g/L tryptone, 5g/L yeast extract, 10g/L NaCl, 1.5g/100mL agar) overnight at 30°C 1 (New Brunswick Scientific, Inova 42 R). A single culture was transferred from plates to liquid LB medium and grown overnight in LB at 30°C and 200rpm for ∼ 16h. Cultures were then diluted 1:100 in Tryptone broth (TB 10g/L tryptone, 10g/L NaCl) and incubated at 30°C and 200rpm for approximately 4h (up to OD₆₀₀ ∼ 0.4 – 0.5). The growth media were supplemented with 100µg/ml ampicillin and 30µg/ml kanamycin, where needed.

At the end of the second growth phase, 1mL of culture was washed three times by centrifuging at 8000g at 20°C for 2min, discarding the supernatant and resuspending the pellet gently before adding 1mL of Berg’s motility buffer (BMB: 6.2mM K₂HPO₄, 3.8mM KH₂PO₄, 67mM NaCl, 0.1mM EDTA). After the final centrifugation, BMB +4% of bovine serum albumin (BSA) was used to prevent the surface attachment of bacteria. The final bacterial density used for imaging corresponds to OD₆₀₀ ∼ 0.1.

The protocol was slightly adjusted for the smooth-swimming mutant as this swimmer accumulates more at the upper and lower boundaries of the channel: after the final centrifugation, the culture is resuspended in a 2:3 Percoll:BMB, where buffer and BSA were concentrated to reach the same final concentration as for the wild-type. The final bacterial density used for imaging corresponds to OD₆₀₀ ∼ 0.05.

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1While growth is optimal at 37°C, motility is facilitated at lower temperatures [111].
4.1.2 Microfluidic channels

A 4 inch silicon wafer that was provided by Will Arter (Keyser group, University of Cambridge) was used to prepare the microfluidic channels. PDMS was prepared in a 1:10 mixture of elastomer:silicone (SYLGARD 184). After mixing elastomer and silicone, the PDMS was degassed in a desiccator for approximately 30 min, then cast on the silicon wafer and cured for 1.5–2 h in a 60°C oven. The resulting channels had a height of 50 µm and were filled with hexagonal lattices of pillars with varying radius and distance. The pillars have a radius of either $R = 16 \mu m$ or $R = 36 \mu m$, which is well below the critical trapping limit $R \sim 50 \mu m$ [42]. An example channel is shown in Fig. 4.1(a). Both inlet and outlet had a diameter of 1 mm.

The PDMS channel and a glass slide were cleaned using Ethanol and scotch tape. Subsequently, both were subjected to a plasma treatment using air at 25 bar for 10 s (diener Femto plasma system). Immediately after treatment, the activated surfaces were bonded to create the microfluidic channel. Next, the channel was stored at 60°C for 15 min to improve bonding. Due to the large surface area of the channel and the hydrophobic nature of PDMS at this point, the channel was loaded first with ethanol at 100 µl/min using a syringe pump (KD Scientific Legato 110). Then, the ethanol was replaced by BMB (without BSA) at 100 µl/min to avoid cell death. Finally, the microfluidic channel was loaded with the prepared bacterial culture at 50 µl/min. The inlet and outlet tubes were both closed by clamps, which reduced the remaining drift to two orders of magnitude below the swimming speed. In total, each 26 experiments were performed for smooth-swimming mutant and wild-type $E. coli$. Using 9 combinations of obstacle radius $R$ and distance $d$, at least two experiments were performed for each combination.

4.1.3 Imaging and Data analysis

The microfluidic channel was imaged on an Olympus IX73 Inverted Microscope using fluorescence imaging (Prior Lumen 200 illumination) at 10x magnification. Images were captured at 20 fps for 2 min. The contrast was enhanced in matlab by stretching the pixel values based on the standard deviation of the image. Finally, a bandpass filter was applied to enhance edges and reduce low-frequency noise. Particle tracking was based on the algorithm developed by Crocker and Grier [112], and the obtained trajectories were smoothed using a Gaussian-weighted moving average.

To detect the position of pillars, an image of the channel in brightfield was taken as a reference. First, a median filter was applied to remove small dirt particles. Then,
the background was subtracted and the contrast enhanced by saturating 0.2% of pixel values. If necessary, a binary threshold was applied as well. Pillars were then identified using a circular Hough transform implemented in matlab’s `imfindcircles` function.

**Circle swimmers**

There were some circular tracks present, which correspond to cells swimming close to the upper and lower boundary of the channel. The fraction of these circle swimmers was a problem in particular for the smooth-swimming mutant as it relies entirely on rotational diffusion to escape the boundary. The circular trajectories would interfere with the estimation of the diffusion coefficient as they suggest a very limited transport. Therefore, these tracks were removed from the analysis. Several measures may be applied to identify the circular trajectories, e.g. considering the correlation of the trajectory or radius of gyration of particle tracks. The best results were achieved by setting a threshold for the mean curvature (here $>0.8$ for circular swimmers), and a threshold each for the mean ($<0.3$) and standard deviation ($>0.96$) of the relative distance covered $|x - \bar{x}|/L$, where $\bar{x}$ is the centre of mass of the trajectory and $L$ its total length. This process identified non-motile cells as well.

**Mean-squared displacement**

The remaining particle tracks were then used to calculate the mean squared displacement (MSD) of the ensemble of swimming bacteria, $\langle r(t)^2 \rangle$. For a persistent random walk with velocity $v$ and persistent time $\tau$, an analytical expression for the MSD can be derived as [75]

\[
\langle r(t)^2 \rangle = 2v^2 \tau^2 \left( \frac{t}{\tau} - 1 + e^{-t/\tau} \right)
\]

\[
= 2v^2 \tau t + 2v^2 \tau^2 \left( e^{-t/\tau} - 1 \right),
\]

where the first term on the right hand side corresponds to the long term diffusive behaviour, while the second term is caused by the ballistic motion at short times. The cross-over from ballistic to diffusive behaviour is characterised by $\tau$. The results for the smooth-swimming *E.coli* \(\Delta\text{cheY}\) are shown in Fig. 4.1. The diffusion coefficient characterising the macroscopic transport can then be obtained as

\[
D = \frac{v^2 \tau}{2}.
\]
Scattering and diffusive transport of *Escherichia coli* in microfluidic lattices

Fig. 4.1 Microfluidic set-up and Mean Squared Displacement. (a) Brightfield image of a microfluidic channel with obstacles of radius $R = 36 \mu m$ and distance $d = 100 \mu m$. This image is used to identify the pillars as circles (b) Fluorescence image of the same channel with the pillars identified as circles added for illustration purposes, and bacteria are the bright spots. (c) Example MSD of different experiments using the smooth-swimming mutant *E.coli* ΔcheY (strain AD83). Inset: Log-log plot of the MSD. At short times, the MSD is ballistic, whereas the long-time behaviour is diffusive.

For wild-type *E.coli*, a run-and-tumble particle with a forward bias during tumbles, the value of $\tilde{\tau}$ obtained from fitting Eq. 4.2 is the effective run time

$$\tilde{\tau} = \tau \left( 1 - \langle \cos \psi_T \rangle \right),$$

where $\langle \cos \psi_T \rangle$ is the mean cosine of the tumbling angle.

**Instantaneous swimming speed and heuristic tumble recognizer**

The instantaneous velocity of a bacterium was obtained as

$$v = \frac{r(t + \Delta t) - r(t)}{\Delta t},$$

where $\Delta t = 0.05s$ is the imaging time step. The mean speed is obtained as the average over the entire trajectory. Taking the mean over all populations used in the experiments yielded $\bar{v} = 19.9 \mu m$, which is in the range of values found in the literature ($15 \mu m - 30 \mu m$ [11, 79, 113]).
The tumbles of wild-type *E. coli* were identified using an heuristic tumble recognizer as described in [114]. The heuristic tumble recognizer is based on the observation that during a tumble the speed of a cell is reduced while the angular velocity increases. Based on the first observation, local minima were identified in the speed $v(t) = |v(t)|$ at time points $t_{\text{min}}$. Next, two adjacent maxima were found at time $t_1$ and $t_2$, respectively. The depth of the minimum was calculated as

$$\Delta v = \max[v(t_1) - v(t_{\text{min}}), v(t_2) - v(t_{\text{min}})],$$

and the minimum was identified as a tumble if the relative depth of the minimum exceeded a threshold

$$\frac{\Delta v}{v(t_{\text{min}})} > \delta_v.$$

The cell was considered to be in tumbling state during the subinterval where $v(t) \leq v(t_{\text{min}}) + \delta_t \Delta v$. The parameters $\delta_v = 3.0$ and $\delta_t = 0.2$ were chosen empirically to produce reasonable values for low obstacle density experiments. In [114], a second criterion considered variations in the turn rate

$$\omega(t) = \frac{\varphi(t) - \varphi(t + \Delta t)}{\Delta t},$$

where $\varphi$ is the direction of motion. First, local maxima in the turn rate were determined at $t_{\text{max}}$, and then their surrounding minima at $t_1$ and $t_2$ were found. If the total change in interval $[t_1, t_2]$ was sufficiently larger than expected from rotational diffusion, the maximum is identified as a tumble. This criterion was quantified as

$$\sum_t |\Delta \varphi| \geq \gamma \sqrt{D_{\text{rot}}(t_2 - t_1)},$$

and tumbling occurred during a subinterval of $[t_1, t_2]$ where $|\omega(t_{\text{max}}) - \omega(t)| \leq \varepsilon \Delta \omega$ with $\Delta \omega = \max[\omega(t_{\text{max}}) - \omega(t_1), \omega(t_{\text{max}}) - \omega(t_2)]$. However, as noted in [114], it is generally sufficient to consider only one criterion, and in the following we will restrict the analysis to the speed criterion.

**Microscopic analysis**

The microscopic behaviour can be analysed further by studying the single-cell interactions with pillars. To this end, I will introduce the impact parameter $b$: First, those parts of trajectories were identified which were in contact with an obstacle, i.e. the
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Fig. 4.2 Microscopic analysis. (a) Parts of trajectories that are interacting with an obstacle are identified according to Eq. (4.10). Partial trajectories before and after collision are retained. The trajectories are shifted such that the centre of the pillar is at the origin. The trajectories are then rotated clock-wise according to the orientation before collision such that the incoming trajectory is aligned with the $x-$axis as shown in (b). (b) The impact parameter $b$ is the $y$-component of the rotated trajectory before collision, which relates to the collision angle $\beta$ via $\cos(\beta) = b/R$. The reorientation angle due to obstacle collision is the orientation of the rotated track after collision. As derived in section 2.3, it is a combination of the collision angle $\beta$ and polar angle $\alpha$, $\psi = \beta - \alpha$.

distance between an obstacle centre and particle position $r$ satisfied

$$|O - r| < R + \epsilon,$$  \hspace{1cm} (4.10)

where $O$ and $R$ are the obstacle centre vector and radius, respectively. The threshold $\epsilon$, which constitutes a layer around the obstacle, was chosen as 1.0\(\mu\)m unless discussed otherwise. Once the interactions were identified, I determined the captured image frames that correspond to 0.4s before and after each interaction, or shorter durations where particles were not tracked for the full 0.4s before or after collision. Trajectories that were tracked less than 0.1s before or after collision were discarded as reorientation could not be identified reliably for those tracks. Subsequently, each identified interacting trajectory was shifted such that the centre of the obstacle was at the origin. Note that the same particle might interact with multiple obstacles and therefore each part of the
4.2 Particle scattering at microfluidic pillars

trajectory was shifted separately. Next, making use of the symmetry of the pillars, particle tracks were rotated clockwise based on their orientation before impact such that their incoming direction was aligned with the $x$-direction. The impact parameter $b$ is then the $y$-component of the rotated track before collision. This process is illustrated in Fig. 4.2(a). For example, by virtue of the above definition, an impact parameter $b = 0$ corresponds to a head-on collision. The relationship between the impact parameter $b$ and the angle between the particle orientation and the surface tangent at collision point, $\beta$, is given by the alternate angle theorem as $\cos(\beta) = b/R$, see Fig. 4.2(b). Hence, a small collision angle $\beta$ corresponds to a large absolute impact parameter $|b|$ and vice versa. In the following, both parameter will be used interchangeably.

The reorientation angle due to the obstacle interaction, $\psi$, was determined as the orientation of the track after the particle leaves the obstacle, see Fig. 4.2(b). If trajectories were sufficiently long before collision, but lost soon after collision (e.g. via tumble out of the focus plane), the track was used to calculate the impact parameter but disregarded for the calculation of the reorientation angle, $\psi$. The residence time follows individually for each trajectory simply as the number of frames in which Eq. (4.10) is satisfied divided by 20fps.

4.2 Particle scattering at microfluidic pillars

4.2.1 Smooth-swimming mutant $E.\text{coli} \Delta \text{cheY}$

Based on previous studies we may expect that the impact parameter $b$ affects the interaction of the particle with the obstacle. To illustrate this point, particle trajectories were binned according to their impact parameter $b$. We can then obtain the average trajectory given a certain impact parameter as shown in Fig. 4.3, where the trajectories are colour-coded according to their impact parameter. As can be seen from Fig. 4.3, the magnitude of reorientation varies depending on the impact parameter. Furthermore, the travelling direction around the obstacle is mainly dictated by the sign of the impact parameter. However, the average trajectory of impact parameters close to zero often appears to point into the obstacle, as is the case in Fig. 4.3. This is an artefact caused by a similar proportion of trajectories going either direction upon collision, and therefore ‘cancelling’ out in the average. This could be explained by either steric or hydrodynamic effects. For example, at small $b$ (i.e. large collision angle $\beta$), rotational diffusion can reorient the cell far enough to seemingly reverse direction, a behaviour also observed in the simulations of stochastic slide-off in chapter 2. Those simulated
Fig. 4.3 Average trajectory upon obstacle collision. Particle trajectories from the smooth-swimming mutant are rotated such that their incoming direction is aligned with the x-direction. The impact parameter $b$ is the y-component of the rotated track. Trajectories are binned according to impact parameter $b$ and the average trajectory upon obstacle collision is shown given a certain $b$. The collision-induced reorientation, $\psi$, corresponds to the orientation of the rotated track after the obstacle interaction. The trajectories are colour coded based on impact parameter.
4.2 Particle scattering at microfluidic pillars

Fig. 4.4 Reorientation depends on impact parameter for *E.coli* ΔcheY. (a) The reorientation angle $\psi$ decreases with increasing impact parameter. Individual experiments for different $R$ and $d$ combinations are shown in grey. The samples for $R = 16\mu$m (in total 50,241 tracks) and $R = 36\mu$m (27,307 tracks) are combined to produce the green and purple curve, respectively. The error bars correspond to the standard error of the sample. While the mean shows a clear trend, the distribution can be broad, see Appendix A for examples. (b) Following the picture in Fig. 4.2, the reorientation angle $\psi$ is a combination of reorientation due to collision angle $\beta$ and polar angle $\alpha$. The polar angle $\alpha$ is calculated for individual trajectories according to Eq. (4.12). There is only a weak dependence of $\alpha$ on either impact parameter $|b|$ or radius $R$. Individual experiments for different $R$ and $d$ combinations are shown in grey. The samples for the $R = 16\mu$m and $R = 36\mu$m are combined to produce the green and purple curve, respectively. The error bars correspond to the standard error of the sample.

tracks usually ‘hover’ a long time before moving but this is rarely observed for the experimental trajectories. Hydrodynamic simulations of squirmers have reported the possibility of ‘mobility reversals’ [47], which do not include time spent ‘hovering’.

Due to the (mostly) symmetrical nature of the interaction, it is sufficient to consider only the dependence on the absolute value of the impact parameter $|b|$ in the following analysis. First, Fig. 4.4(a) shows the reorientation angle $\psi$ as a function of the (absolute) impact parameter, which has been scaled by the pillar radius (including the circle layer $\epsilon$)\(^2\). The reorientation $\psi$ decreases with increasing impact parameter, and a larger pillar radius $R$ leads to a larger reorientation. When a particle collides with an obstacle, it needs to be reoriented at least by the collision angle $\beta$ in order to point away from the surface and escape. Since $\beta$ relates to $b$ and $R$ via $\cos(\beta) = b/R$, $\psi$ has

\(^2\)We note that in spite of a broad distribution of values (see Appendix A for examples), the average $\psi$ of the samples shows a clear trend, and we may therefore restrict the following analysis to the mean of the parameters.
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to depend on $b$ and $R$ as observed in Fig. 4.4(a) if $\beta$ is the minimum reorientation. However, $\psi$ might exceed the expected minimum reorientation since rotational noise or hydrodynamic attraction might increase the reorientation, and could, thereby, introduce further dependence on $b$ and/or $R$. We can quantify the reorientation that goes beyond the minimum $\beta$ by using the polar angle $\alpha$.

As described in chapter 2, the reorientation angle $\psi$ can be defined as a combination of the collision angle $\beta$ and the polar angle $\alpha$ if we assume that the particle leaves at a tangent to the obstacle surface $^3$,

$$\psi = \beta - \alpha = \cos^{-1}\left(\frac{b}{R}\right) - \alpha. \quad (4.11)$$

Furthermore, we note that in chapter 2 we assumed for the sliding condition that $P(\alpha \mid \beta) \approx P(\alpha)$ and that $\alpha$ is independent of $R$, whereas the RTP theory for the slide-off condition showed a dependence of $\alpha$ on $\beta$ and thereby for the average $\hat{\alpha}$ on $R/l_p$ (see section 2.3.2). The polar angle $\alpha$ can, hence, give an indication of the reorientation that exceeds the expected reorientation due to the collision angle $\beta$. As both incident and leaving point on the obstacle are known, we can calculate the polar angle independently as the angle between two points of distance $c$ on a circle of radius $R$,

$$\alpha = 2 \sin^{-1}\left(\frac{c}{2R}\right). \quad (4.12)$$

The polar angle $\alpha$ as a function of $b$ is shown in Fig. 4.4(b). The polar angle remains constant at approximately 0.45 over a large range of impact parameters but decreases for the largest impact parameters $b$. Thus, the strong $|b|$-dependence of $\psi$ can mainly be explained via the collision angle $\beta$ in Eq. (4.11). Furthermore, the $\alpha$ value is slightly larger for the smaller radius but the values are close for both radii tested. The lack of dependence on the impact parameter is puzzling as a dependence was expected for both hydrodynamic and steric interactions. As discussed in section 2.3.2, the slide-off condition entails a decrease in $\alpha(\beta)$ with decreasing $\beta$ (increasing $b$) up to $\alpha \to 0$ when $\beta \to 0$. Yet there is a clear non-zero $\alpha$-value even for the largest impact parameter (smallest $\beta$). Similarly, the existing hydrodynamic models do not seem to be sufficient to explain the observed behaviour. While there has not been any hydrodynamic study including a parameter such as $\alpha$, hydrodynamic simulations and analytical theory in [43] predicted a non-monotonic dependence of $\psi$ on the impact parameter, where $\psi$ decreases continuously with increasing $b/R$ followed by an increase.

$^3$The derivation in chapter 2 defined $\psi$ clockwise but the common definition of a polar angle is counter-clockwise. Therefore, we here use the opposite sign to that in Eq. 2.7.
4.2 Particle scattering at microfluidic pillars

Fig. 4.5 Obstacle lattice effects on reorientation of *E.coli* ΔcheY. (a) The average impact parameter slightly increases for denser lattices. (b) The mean cosine of the reorientation angle $\psi$ is mainly constant for both pillar radii after an initial drop for both $R = 16\mu m$. A larger value of $\langle \cos(\psi) \rangle$ corresponds to a higher diffusivity. The error bars correspond to the standard deviation of different repeats.

close to $b/R \sim 1.0$ [43]. Thus, I would expect this non-monotonic dependence to be reflected in $\alpha$ as well. On the other hand, in the hydrodynamic model in [42], the angle with the surface decreased steadily until escape for obstacles below the trapping radius. The rate of change in the angle with the surface depended on both the current angle and the radius of the obstacle. It seems intuitive that a bacterium with a smaller collision angle would then traverse a smaller distance along the obstacle, i.e. cover a smaller $\alpha$. Furthermore, as with the other models, a dependence of $\alpha$ on the radius would be expected.

Next, I analysed how average reorientation parameters changed with the lattice density, where $\rho = 2\pi/\sqrt{3}(R/d)^2$. Fig. 4.5(a) shows that the average impact parameter increases slightly for smaller obstacle separations, i.e. increasing $R/d$. For all obstacle lattices, the distribution of impact parameters has a peak at large impact parameters, as illustrated in Fig. 4.6(a). The average impact parameter increases with increasing lattice density because the distribution shifts further towards the larger impact parameters. A shift towards larger impact parameter (i.e. smaller $\beta$) was also observed in the agent-based simulations performed for chapter 2 when $R/d$ was increased. In simulations, collisions with small collision angle are more common in dense lattices because particles travel along a lattice channel and repeatedly collide with neighbouring obstacles, which can eventually lead to the geometrical guiding described in chapter 2. However, both theory and simulations predicted that the collision angle at low obstacle densities follows $P(\beta) = \sin(\beta)/2$ with a maximum at $\beta = \pi/2$, and the distribution of the
Fig. 4.6 Distribution of impact parameters for *E. coli* ΔcheY. (a) The distribution depends on the lattice density, as here shown for pillar radius $R = 36\mu m$. The increase in the average impact parameter observed in Fig. 4.5(a) is caused by a shift towards larger impact parameters for denser lattices. (b) Increasing the circle layer $\epsilon$ in Eq. (4.10) for $R/d = 0.2$ shifts the peak to smaller impact parameters. The impact parameter is rescaled by the effective radius, $R + \epsilon$. To improve the representation, the histogram values were smoothed using a moving average filter.

impact parameter was thus expected to peak at $b \approx 0$. We may hypothesise that hydrodynamic effects are causing the deviation from this expected distribution since neither theory nor simulations include any long-range hydrodynamics. In fact, we can check for this possibility by increasing the circle layer $\epsilon$ in Eq. (4.10): the theoretical prediction $P(\beta) = \sin(\beta)/2$ applies to any arbitrary circular boundary in space. Once $\epsilon$ exceeds the hydrodynamic interaction range, the distribution $P(\beta)$ should approach the prediction. Fig. 4.6(b) shows that with increasing $\epsilon$ small impact parameters are recorded more often and the peak itself shifts towards smaller values as well. However, even for a circle layer as large as $\epsilon = 60\mu m$ the peak can still be identified.

The obstacle-induced reorientation feeds into the estimation of the diffusion coefficient via the mean cosine of $\psi$ in $1 - \langle \cos(\psi) \rangle$, see Eq. (2.6) in chapter 2. Therefore, everything else being equal, a larger value of $\langle \cos(\psi) \rangle$ corresponds to a higher diffusivity. As shown in Fig. 4.5(b), after a drop of about 0.1, $\langle \cos(\psi) \rangle$ remains mainly constant with increasing obstacle density at $\sim 0.65$. The average trend is the same for both pillar radii studied.
4.2 Particle scattering at microfluidic pillars

4.2.1 Reorientation depends on impact parameter for wild-type E.coli

Fig. 4.7 Reorientation depends on impact parameter for wild-type E.coli. (a) The reorientation angle $\psi$ decreases with increasing impact parameter $|b|$. Individual experiments for different $R$ and $d$ combinations are shown in grey. The samples for $R = 16\mu\text{m}$ (80, 410 tracks) and $R = 36\mu\text{m}$ (44, 581 tracks) are combined to produce the green and purple curve, respectively. The error bars correspond to the standard error of the sample. (b) Following the picture in Fig. 4.2, the reorientation angle $\psi$ is a combination of reorientation due collision angle $\beta$ and polar angle $\alpha$. The polar angle $\alpha$ is calculated for individual trajectories according to Eq. (4.12). There is only a weak dependence of $\alpha$ on $|b|$ but a smaller radius increases $\alpha$. Individual experiments for different $R$ and $d$ combinations are shown in grey. The samples for the $R = 16\mu\text{m}$ and $R = 36\mu\text{m}$ are combined to produce the green and purple curve, respectively. The error bars correspond to the standard error of the sample.

4.2.2 Run-and-Tumble wild-type E.coli

We now consider the scattering interactions for the wild-type E.coli. Unlike the smooth-swimming mutant, the random walk pattern of this microswimmer includes ‘tumbles’ as sudden reorientation changes.

Similarly to E.coli $\Delta$cheY, the reorientation angle $\psi$ decreases with increasing impact parameter as shown in Fig. 4.7(a). However, the reorientation is on average larger for this bacterium as the mean $\psi$ of all samples is $0.2 \pm 0.4\text{rad}$ above the values for the smooth-swimming mutant reported in Fig. 4.4(a). Further in agreement with the smooth-swimming mutant is that the polar angle $\alpha$ is approximately constant for most impact parameters followed by a decrease for the largest impact parameters. However, Fig. 4.4(b) also showed only a small $R$-dependence of $\alpha$ for $\Delta$cheY, whereas in Fig. 4.7(b) $\alpha$ clearly differs by $\sim 0.1$ between different radii for the wild-type. Interestingly, the $\alpha$-values for both radii are below the values for the smooth-swimming mutant. The polar angle $\alpha$ relates to the arc-length via $l_{\text{arc}} = \alpha R$. Thus, taken together,
Fig. 4.8 Effect of obstacle lattice density on reorientation of E. coli wild-type. (a) The average impact parameter slightly increases for denser lattices. (b) The mean cosine of the reorientation angle $\psi$ is mainly constant for radius $R = 16\mu m$ while $\langle \cos \psi \rangle$ for $R = 36\mu m$ increases with decreasing obstacle separation, $d$. A larger value of $\langle \cos \psi \rangle$ corresponds to a higher diffusivity. The value at the smallest $R/d$, $\langle \cos \psi \rangle = 0.35$, suggest a reorientation angle of $\psi_T = 69^\circ$, which is close to previously reported tumbling angles [79]. The error bars correspond to the standard deviation of different repeats.

As shown in Fig 4.8(a), the average impact parameter increases slightly with $R/d$ but the values are comparable to those obtained for the smooth-swimming mutant in Fig. 4.5(a). The mean cosine of reorientation, on the other hand, is significantly smaller for the run-and-tumble wild-type than the smooth-swimming mutant. As can be seen from Fig. 4.8(b), $\langle \cos \psi \rangle$ is approximately constant at $\langle \cos \psi \rangle = 0.45$ for obstacle lattices with the small pillar radius (green curve), while the average for the smooth swimming is $\langle \cos \psi \rangle \sim 0.65$. The analysis for the larger pillar radius, on the other hand, shows a large variation in the impact parameter for dense obstacle lattices (purple curve in Fig. 4.8(a)). In addition, the mean cosine of the reorientation angle is seen to increase for denser lattices, which may be caused by the change in average impact parameter. Since the distribution of impact parameters shifts to larger $b$ for larger $R/d$ (see as well Fig. 4.6(a)), we move to the right on Fig. 4.7(a) and thus to smaller $\psi$. A smaller $\psi$ in turn means an increase in $\langle \cos \psi \rangle$ (assuming $0 < \psi < \pi/2$).

The motility pattern of the wild-type differs from the smooth-swimming mutant by the occurrence of tumbling events. Tumbling may be suppressed close to surfaces but could also be a means of escape by reducing the residence time $\tau_R$. As shown
4.2 Particle scattering at microfluidic pillars

Fig. 4.9 Tumbling in an obstacle lattice. (a) Residence time $\tau_R$ at an obstacle for smooth-swimming mutant $\Delta$cheY (solid line, circle marker) and wild-type (wt) $E. coli$ (dashed line, diamond marker). The residence time is larger for a larger obstacle. Tumbling reduces the time spent at an obstacle for small impact parameter $|b|$ for both obstacle radii. The error bars correspond to the standard error over all included samples (bars not shown if smaller than marker). (b) Effect of obstacle lattice density on tumble frequency $\mu$ of $E. coli$ wild-type. Tumbles were identified using the heuristic tumble recognizer described in section 4.1.3 using the speed criterion. The tumble frequency remains approximately constant for obstacle lattices with pillars of radius $R = 16\mu m$ (green curve) but increases for $R = 36\mu m$ (purple curve). The error bars correspond to the standard deviation of different repeats.

in Fig. 4.9(a), the residence time decreases significantly for small impact parameters compared to the smooth-swimming mutant $\Delta$cheY while the difference vanishes for large $|b|$, in particular for the smaller obstacle radius. For large impact parameters, the orientation of the cell is already close to aligned with the surface and tumbling might not influence the residence time much, whereas the influence is large at small $b$. Alternatively, head-on collisions with small impact parameters could possibly induce tumbling events. The residence time, in addition, depends strongly on the pillar radius as expected for both steric and hydrodynamic interaction effects. A larger curvature increases hydrodynamic attraction as well as the role of noise to escape the obstacle due to the decreased curvature [43] (also see discussion about the diffusive limit of the slide-off condition in section 2.3.2). Tumbling could have a stronger influence on the residence time at larger obstacles because a larger reorientation is required at smaller curvature. This effect could then be the cause for the $R$-dependence in the $\alpha$-values determined for the wild-type in Fig. 4.7(b) compared to the smooth-swimmer in Fig. 4.4(b).
The presence of an obstacle lattice might also alter the tumbling motility itself. Values previously reported in the literature for the tumbling frequency of E. coli in a two-dimensional experimental system vary between $0.42s^{-1}$ \cite{115} to $2.86s^{-1}$ \cite{116}. Here, the value obtained for the most dilute obstacle lattice is $\mu = 0.63s^{-1}$ and therefore within the reported range. Fig. 4.9(b) shows the value of the tumbling rate as a function of the lattice density. In lattices with pillars of radius $R = 36\mu m$ (purple curve), the tumble rate obtained using the heuristic algorithm does not change appreciably with the lattice density. In lattices with pillars of the smaller radius $R = 16\mu m$, on the other hand, the tumbling frequency increases in denser obstacle lattices. This trend is robust to varying the parameters of the heuristic tumble recognizer, $\delta_v$ and $\delta_t$, or applying the turn rate criterion instead. The heuristic tumble recognizer may falsely identify some obstacle interactions as tumbles since both speed can decrease and turn rate increase upon obstacle collision. Because the frequency of obstacle collisions increases with lattice density, this could artificially increase the tumbling frequency. Furthermore, at the same obstacle density, a lattice with smaller pillars has a larger contact area at which cells could ‘tumble’. Although a visual inspection of the identified trajectories did not suggest that this could be a significant fraction of the identified events, further analysis is required, and the increase observed in Fig. 4.9(b) should be considered inconclusive.

4.3 Diffusive transport: comparison between experiment & theory

The analysis of the microscopic pillar interactions in the previous section is used to independently estimate a diffusion coefficient based on the Run-and-Tumble model developed in chapter 2 and chapter 3. For the smooth-swimming E.coli $\Delta$cheY, we can calculate the effective diffusion coefficient as

$$\tilde{D} = \frac{\tilde{v}^2}{2 \left[ D_R + \langle \cos \psi \rangle / \tau \right]}$$

(4.13)

where $\tilde{v}$ is the effective swimming speed (defined below), $D_R$ is the rotational diffusion coefficient and $\tau$ is the reorientation time scale. The rotational diffusion coefficient was estimated using particle tracks from the lattice with the smallest $R/d$. The mean squared angular deviation was fitted according to $\langle \varphi(t)^2 \rangle = 2D_R t$ at long times to obtain $D_R = 0.16rad^2/s$. This value is larger than the value estimated by Berg \cite{68} but within the range of previous estimates \cite{74}. As described in chapter 2, the reorientation
4.3 Diffusive transport: comparison between experiment & theory

Fig. 4.10 Relative diffusion coefficient for two different pillar radii at different obstacle separations. (a) Smooth-swimming mutant ΔcheY. The diffusion coefficient obtained by fitting the MSD with Eq. (4.2) is rescaled by the expected free diffusion coefficient $D_0 = 1250\mu m^2/s$ (see main text). The dashed line is the theoretical prediction based on Eq. (4.13). The diffusive transport is large even for dense obstacle lattices. (b) *E.coli* wild-type. The dashed line is the theoretical prediction based on Eq. (4.17). The expected free diffusion coefficient is $D_0 = 351\mu m^2/s$. Here too the diffusive transport is large even at dense obstacle lattices. The error bars correspond to the standard deviation of different repeats.

time scale $\tau$ is given by $\tau = \tau_c + \tau_R$. The time between collisions is $\tau_c = \lambda/v$, where the mean free path $\lambda$ for a hexagonal lattice of obstacles according to the Santalo formula (see e.g. Eq. 2.5) is given by

$$\lambda = \frac{\sqrt{3}d^2 - 2\pi R^2}{4R}. \quad (4.14)$$

As $\alpha$ is approximately constant for different impact parameter, I will use the theory developed for the sliding boundary condition. The residence time $\tau_R$ can then be calculated as $\tau_R = R\alpha/v$, where the polar angle $\alpha = 0.39\text{rad}$ is the average over all interactions. The effective swimming speed is assumed to be given by the linear interpolation

$$\tilde{v} = v\frac{\tau_c}{\tau} + v_{\text{obs}}\frac{\tau_R}{\tau}, \quad (4.15)$$

where the swimming speed at the obstacle is approximated as (see section 2.3.1)

$$v_{\text{obs}} = \frac{R}{\tau_R} \sqrt{2(1 - \cos \alpha)}. \quad (4.16)$$

Using $v = 20\mu m/s$ [117] and $D_R = 0.16\text{rad}^2/s$ we expect a free diffusion coefficient of $D_0 = 1250\mu m^2/s$, which was used to rescale the diffusion coefficient from the MSD.
The rescaled diffusion coefficient is shown in Fig. 4.10(a) for the two pillar radii tested. The theoretical prediction using Eq. (4.13) is shown as dashed line. It should be noted that much of the variation in diffusion coefficient reflects variations in the swimming speed of the culture. Therefore, the speed $v$ in Eq. (4.15) was averaged separately for each $R$ and $d$ combination. Accounting for this speed variation, the predicted diffusion coefficient is close to the diffusion coefficient determined from fitting the MSD. For both pillar radii studied, the diffusion coefficient retains its magnitude even in dense obstacle lattices. At large obstacle densities, the theory underestimates the average diffusion coefficient, which might be caused by the guiding effect observed in chapter 2.

The theoretical estimate for the wild-type $E. coli$ needs to consider biologically-induced tumbles in addition to the obstacle induced ‘tumbles’ in the RTP model 

$$
\tilde{D} = \frac{\tilde{v}^2}{2 [D_R + (1 - \langle \cos \psi \rangle)/\tau + (1 - \langle \cos \psi_T \rangle)\mu]},
$$

(4.17)

where $\mu$ is the tumbling rate of the bacterium, which was approximated using the tumbling rate at low density $\mu = 0.63 s^{-1}$. Thus, in evaluating $\tilde{D}$ we ignore any potential effect of the lattice on the tumbling rate here. The effective speed $\tilde{v}$ and obstacle reorientation time $\tau$ are calculated as described for the smooth-swimming mutant. The average polar angle is adjusted to $\alpha = 0.32 \text{rad}$ for $R = 16 \mu m$ and $0.25 \text{rad}$ for $R = 36 \mu m$, respectively.

Using $v = 20 \mu m/s$, $D_R = 0.16 \text{rad}^2/s$, $\mu = 0.63 s^{-1}$ and $\langle \cos \psi_T \rangle = 0.35 (\psi_T = 69^\circ [79])$ in Eq. (4.17) we expect a free diffusion coefficient of $D_0 = 351 \mu m^2/s$, which was used to rescale the diffusion coefficient obtained from MSD fits. The relationship between the rescaled diffusion coefficient and the obstacle density is shown in Fig. 4.10(b). Accounting for the speed variation, the predicted diffusion coefficient is close to the diffusion coefficient determined from fitting the MSD for most measurements.

### 4.4 Discussion

The experimental results presented in this chapter provide the first detailed measurements of the scattering interaction between bacteria and a microfluidic lattice. The results I have obtained allow the first evaluation of the relative importance of hydrodynamic and steric effects for bacteria scattering at convex surfaces as single events, as well as the at the level of a population diffusing through a collection of obstacles in a regular lattice.
The microscopic analysis predicts that hydrodynamics plays a significant role in determining scattering events. A first indication of hydrodynamic influence before direct contact is the distribution of impact parameters at low obstacle densities, which is markedly different to the expectation from theory and simulations in chapter 2. The theoretical derivation of the collision angle distribution in section 2.3.1, \( P(\beta) = \sin(\beta)/2 \), assumed a particle travelling in a straight line before collision. This assumption is not strictly satisfied for a particle subject to rotational diffusion, but nevertheless the simulations - which included noise in the orientation - agreed with this prediction at low obstacle density. However, neither theoretical derivation nor simulations took hydrodynamic effects into account. Previous hydrodynamic analysis predicted an alignment prior to surface contact [43], at least for small collision angles. Hydrodynamic interaction and resulting alignment prior to collision could explain the distribution of impact parameters shown in Fig. 4.6(a). Increasing the circle layer \( \epsilon \) indeed shifted the distribution towards smaller impact values in Fig. 4.6(b), and gave an indication of the size range of hydrodynamic interaction. The obtained values predict an astonishingly large range of hydrodynamic interaction for the microfluidic pillars. Spagnolie et al. derived the basin of attraction for trapping of microswimmers at large obstacles [43]. In this case, the approaching cells were trapped only within a radius of \( 1.5 - 2.5 \) times their body length at very large obstacles. In the case of \( E. coli \), we would expect at most \( 2 \times 2 \mu \text{m} \) based on the actual cell size, but it might increase up to \( 2 \times 12 \mu \text{m} \) if the flagella length is included. However, the range of hydrodynamic effects remains a puzzle since the limit derived in [43] was based on much larger obstacles than considered here and is, thus, probably not applicable here.

Another indication of hydrodynamic influence can be gained by examining single particle trajectories. Some trajectories with \( |b|/R \sim 1 \) appear to follow the obstacle surface for some time and effectively bend inwards. For a steric interaction (as in the slide-off condition) we expect a particle to be only briefly in contact with the obstacle upon collision with \( |b|/R \sim 1 \). Hydrodynamic effects, on the other hand, might lead to the observed interactions due to hydrodynamic attraction to the pillar [43]. Simulations of a squirmer particle with pusher dipole found that the scattering angle \( \psi \) is a non-monotonic function of the impact parameter \( b \), i.e. the particle trajectory bends inwards for large impact parameter as shown in Fig 4.11(a). Some individual particle trajectories from the experiments seem to qualitatively agree with this prediction as highlighted in Fig. 4.11(b).

Beyond this qualitative analysis, the quantitative relationship between reorientation \( \psi \) and impact parameter \( b \) offers surprising insights since it does not meet the expectation
Scattering and diffusive transport of *Escherichia coli* in microfluidic lattices

Fig. 4.11 Particle trajectory bending. (a) Hydrodynamic simulations suggest that the scattering angle $\Theta$ (corresponds to $\psi$ in this study) is a non-monotonic function of the impact parameter and active particles can scatter inwards at large impact parameters due to hydrodynamic attraction. Adapted from [43]. (b) A selection of particle trajectories obtained for the smooth-swimming mutant *E.coli* $\Delta$cheY is coloured according to impact parameter. An example trajectory that scatters inwards is highlighted (arrow and purple trajectory). Trapping or orbiting of the pillar is observed very rarely.

of either a purely steric or purely hydrodynamic interaction. As described in section 2.3.2, scattering based on a steric interaction (slide-off condition) leads to $\psi \to 0$ when $\beta \to 0$ or $\beta \to \pi$ (i.e. $|b|/R \to 1$). In general, $\psi$ and $\alpha$ depend on the collision angle $\beta$ as well as the pillar radius $R$, with a larger collision angle and a larger pillar radius increasing the reorientation. Similarly, the work done in [43] showed that approximating the scattering angle for small collision angles based on hydrodynamic theory predicted a dependence on the radius and the collision angle [43]. Indeed, for both strains $\psi$ decreases with increasing $|b|$ and increases with increasing $R$. Contrary to the expectation for the slide-off condition though, both smooth-swimming mutant and wild-type *E.coli* are significantly reorientated even for large $b$ ($\psi > 0$ in Fig. 4.4(a) and Fig. 4.7(a)). As the reorientation $\psi$ needs to be large enough to direct the cell away from the obstacle surface, the angle $\psi$ is a function of $\beta = \cos^{-1}(b/R)$. To disentangle potential hydrodynamic or steric effects at the obstacle from this minimum $\beta$ dependence, we can study the polar angle $\alpha$ as a function of the impact parameter $b$ and the obstacle radius. Astonishingly, $\alpha$ only depends on the impact parameter for very large $|b|$ for both strains studied. Furthermore, only the RTP wild-type strain
shows a dependence of $\alpha$ on $R$. Yet, the residence time $\tau_R$ clearly depends on $R$ and $b$ for both strains. For a constant $\alpha$ we know that

$$\tau_R = \frac{R\alpha}{v},$$

(4.18)

where $v$ is the swimming speed. Let us consider the different cases for the smooth-swimming mutant $\Delta$cheY: i) First, we decrease the impact parameter $b$ while keeping $R$ constant. In this case, we know that $\alpha$ stays approximately constant, while $\tau_R$ increases sharply with decreasing $b$. According to Eq. (4.18), the swimming speed then has to decrease with a decrease in impact parameter. We will see that a similar conclusion can be drawn for all the other cases. ii) We increase $R$ while keeping $b$ constant. Again, $\tau_R$ clearly increases while $\alpha$ stays approximately constant. Thus, $v$ has to decrease or at least remain constant upon an increase in $R$. For the wild-type, case i) follows the same argument as $\Delta$cheY. For case ii), when we keep $b$ constant and increase $R$, we observed a decrease in $\alpha$ while $\tau_R$ increased. Thus, the swimming speed has to remain constant or decrease. Taken together, these results strongly suggest that the swimming speed decreases with a decrease in $b$.

Such a decrease in swimming speed upon decrease in $b$ could be explained by both steric or hydrodynamic effects. For example, for steric interactions the swimming speed follows $v = v_0 \cos \beta = v_0 (b/R)$ and, hence, decreases from the free swimming speed as $b/R$ decreases. In [42], the speed of cells indeed decreased by $\approx 5 \mu\text{ms}^{-1}$ from the free swimming speed for cells tracing large obstacles. However, the results here predict that the speed depends on the orientation towards the surface and may, thus, vary along the trajectory tracing the surface of smaller obstacles. Indeed, the scattering interaction for *E. coli* wild-type at colloids ($R \sim 1.5 \mu\text{m}$) was found to differ depending on the angle of approach in [46]. Head-on collisions showed a decrease in speed and large reorientation (termed tumble-collisions), whereas collisions with a small collision angle showed no change in swimming speed and a small reorientation. This is in stark contrast with theoretical studies predicting an increase above the bulk swimming speed for squirmers at flat walls [118, 119]. However, a reduction of the average swimming speed parallel to the surface has recently been observed for squirmers at surfaces with increasing curvature [120]. Hence, future work should attempt to determine the instantaneous speed along the trajectory of bacteria interacting with obstacles as a function of their impact parameter. This could then be compared with the expectation from steric and hydrodynamic models.

The slide-off condition is appealing for its simplicity in terms of implementation and is used routinely in computational studies of active particles in complex environments.
[55, 107, 108]. However, taken together, the observations made in the microscopic analysis in this work suggest that the (pure) slide-off condition based on steric interaction is not an adequate model for bacteria interacting with microfluidic pillars below the trapping radius. By contrast, the sliding condition may be a reasonable approximation for both wild-type and smooth-swimming mutant, even though individual particle trajectories show a large variation as illustrated in Fig. 4.11(b). Future studies might explore whether this conclusion also applies to synthetic microswimmers.

Given the potential role of hydrodynamic interactions, future work could compare these results to soil bacteria such as Pseudomonas putida to study whether they are adapted differently to swimming in porous media. P. putida has multiple polar flagella and performs a run-reverse motility. During the reverse mode, the bacterium wraps the flagella around its cell body [121]. This different propulsion mechanism might lead to a change in the dipole strength, which could affect the hydrodynamic interaction with obstacles.

The presence of tumbles in the motility pattern alters the observed microscopic obstacle interaction. To summarise, tumbling reduces the residence time for collisions with small impact parameter, increases the reorientation angle $\psi$ and reduces the polar angle $\alpha$. The effect of tumbling is larger for smaller impact angles and larger obstacle radius. Thus, tumbling might be a means to escape surface interaction with large tumbling angles. Similarly, the presence of obstacles may modify the motility pattern by increasing the tumbling frequency as suggested by Fig. 4.9(b). Fluorescent staining of the flagella could improve the analysis of tumbling, and clarify whether the tumbling frequency is modified close to a microfluidic pillar. In particular, it would be interesting to resolve whether small impact parameters increase the likelihood of tumbling events, or whether tumbling only has a larger effect on the residence time for these impact parameters. In addition, the direct observation of flagella would remove the reliance on signatures of tumbling events in trajectories, which may be similar to and, therefore, confused with those observed for some obstacle interactions. Future research into the hydrodynamics of tumbling close to a (convex) surface could also shed more light on possible hydrodynamic effects on tumbling.

The diffusion coefficient at dense obstacle lattices is significantly above the theoretical prediction for both smooth-swimming mutant and wild-type E.coli. Examining individual particle tracks, some bacteria seem to follow a lattice channel for a significant time, as was also observed for the simulations in chapter 2. Therefore, future work should aim to study geometrical guiding effects in the dense obstacle lattices. Overall,

4 E.coli has peritrichous flagella, which are distributed over the cell body [11]
the RTP model is estimating values close to the MSD derived coefficients based on the microscopic values. Future work could improve the model by incorporating the tumbling effects on obstacle interactions as both processes may not be considered independent Poisson processes.

Finally, we remark on how systems with different obstacles sizes and topologies will lead to different microscopic interactions and macroscopic transport than that observed here. In particular, although the results presented here suggest a smaller effect of the pillar radius on the scattering process than expected, this observation might be restricted to pillars in a certain range. The interaction with colloids of the size of the bacteria, such as used in [60, 61], is likely different from the obstacles used in this study. Similarly, larger pillars that approach the critical trapping radius could increase the probability of long residence times at the obstacle. In addition, the topology of the porous medium can affect the transport. In a three-dimensional disordered porous medium made of crosslinked copolymers, the type of random walk changed from run-and-tumble motility to intermittent hopping and trapping [92]. The transport of bacteria in these media is dominated by the properties of the porous medium, similar to transport of passive particles [106]. This work represents the first step towards systematically studying the effect of increasing complexity, for instance, by varying the obstacle size, shape or arrangement.
Chapter 5

Active Brownian Particles in obstacle gradients

5.1 Active particles biased by lattice topography

Heterogeneity is prevalent in the natural settings of microswimmers, e.g. in the distribution of chemicals, light exposure, water saturation, flow rates or density of porous media [122, 123]. As the previous chapters have shown that interactions with a regular obstacle lattice can significantly modify the macroscopic transport of active particles, in this chapter I turn to the question of transport in non-uniform environments. It is well-known that random porous media alter transport, e.g., by trapping particles [54]. Yet, does a non-uniform distribution of obstacles still modify the transport in the absence of traps? Without explicitly considering obstacles, we can gain qualitative predictions from a simple model in 1D: let us consider an RTP with moving speed $v(x)$. Let $R(x,t)$ and $L(x,t)$ denote the probability density of the particle being at position $x$ and moving either to the right or to the left, respectively. Tumbles are independent reorientation events with rate $\mu(x)$. Since in one-dimension half of the tumbles do not result in a change in direction (i.e. $R \rightarrow R$ and $L \rightarrow L$), the probability densities can be modelled as [26, 27]

$$\frac{\partial R}{\partial t} = -\frac{\partial (vR)}{\partial x} - \frac{\mu R}{2} + \frac{\mu L}{2}, \quad (5.1)$$
$$\frac{\partial L}{\partial t} = \frac{\partial (vL)}{\partial x} + \frac{\mu R}{2} - \frac{\mu L}{2}. \quad (5.2)$$

These telegraph equations may be combined to derive an equation for the one-particle probability density $p \equiv R + L$

$$\frac{\partial p}{\partial t} = -\frac{\partial J}{\partial x}, \quad (5.3)$$
where the flux has been defined as \( J \equiv v(R - L) \). Differentiating Eq. (5.3) with respect to \( t \) and eliminating \( R \), \( L \) and their derivatives gives

\[
\frac{\partial^2 p}{\partial t^2} = \frac{\partial}{\partial x} \left[ v \frac{\partial(p v)}{\partial x} + \mu J \right].
\]

(5.4)

The diffusive limit is derived by neglecting ballistic movements, i.e. \( \partial^2 p/\partial t^2 \to 0 \), which yields a drift-diffusion equation for the flux

\[
J(x) = -\frac{v(x)^2}{\mu(x)} \frac{\partial p(x)}{\partial x} - \frac{v(x)}{\mu(x)} \frac{\partial v(x)}{\partial x} p(x),
\]

(5.5)

where the first term on the RHS corresponds to diffusive motion, whereas the term proportional to \( p \) is the drift caused by a spatially-varying swimming speed. Using this simple telegraph equation, Schnitzer [26] showed that the steady-state distribution for non-interacting RTPs depends on the inverse of the swimming speed

\[
p(x) = p(0) \frac{v(0)}{v(x)}.
\]

(5.6)

Tailleur & Cates extended this relation to interacting RTPs with a swimming speed that can be expressed as \( v(x) = v(p(x)) \) [27], which can be further extended to ABPs at long times \( t \gg D_{\text{rot}}^{-1} \), where \( D_{\text{rot}} \) is the rotational diffusion coefficient [124]. This result sparked an intense interest in the field of active matter, in particular in Motility-Induced Phase Separation (MIPS). In systems exhibiting MIPS, particles interact via their swimming speed and slow down at large particle density, e.g. due to biochemical or steric effects [125]. The resulting positive feedback loop leads to particle clustering and eventual phase separation. Relation (5.6) was tested qualitatively in the experimental work in [126, 127], which used genetically modified \( E. coli \) to independently control the swimming speed by light intensity. The bacteria indeed accumulated at regions of low speed defined by imposed light patterns. Recently, the relation has been shown to hold quantitatively as well [128]. It is important to stress that while MIPS requires particle interaction, drift due to speed variations is independent of interactions.

As described in the previous chapters, an obstacle lattice introduces reorientation events due to scattering collisions. A spatially-varying obstacle density then introduces a spatially varying reorientation frequency. However, it has been shown that this variation does not affect the population up to first order approximation [27]. Therefore, particles undergoing classical specular collisions are distributed uniformly in an obstacle lattice with varying density. On the other hand, non-classical scattering rules, such as
the slide-off condition, additionally reduce the effective swimming speed due to the time spent at the surface. From chapter 2, we know that the mean effective run time is $\tau = \tau_c + \tau_R$ and
\[
v_{\text{eff}} = v \frac{\tau_c}{\tau} + v_{\text{obs}} \frac{\tau_R}{\tau},
\]
where $v_{\text{obs}}$ is the speed at an obstacle and $\tau_c$ and $\tau_R$ are the mean collision time and residence time, respectively. As a consequence, a spatially-varying obstacle density introduces a spatially varying effective swimming speed. The reduction in speed is larger the more the particles interact with obstacles. Thus, we may expect from Eqs. (5.5) and (5.6) that particles accumulate at high obstacle densities. This prediction is independent of any trapping, which might occur in random media, but follows solely from the predicted gradient in swimming speed. Indeed, *E.coli* previously accumulated at regions with larger obstacle density in a microfluidic device in [129].

The described trend is expected purely from the variation in swimming speed and, therefore, not caused by a directional change in the motility pattern of the cells in response to a gradient in environmental conditions. Thus, the expected phenomenon is rather a ‘kinesis’ than a ‘taxis’. However, the term ‘topotaxis’ has been used to describe the migration of eukaryotic cells which can be biased by topographical cues in the environment [130]. For example, recent experiments investigated the topotactic migration of the slime mould *Dictyostelium discoideum* in a lattice of microfluidic posts [131]. Different areas of the lattice had different (regular) obstacle densities, and it was found that the cells migrated to areas of lower obstacle densities. To explain the observed drift, a recent computational study investigated the average population drift of ABP in an obstacle density gradient [107]. In agreement with the experimental study [131], the authors found in simulations a drift towards regions of lower obstacle density, where the drift velocity increased for larger gradients in obstacle density. This drift was explained with a larger effective persistence length at lower obstacle densities. However, the previous research described above predicts a drift towards areas of smaller speed and smaller effective persistence length [26, 27, 124]. As a result, it remains unclear which effect a varying obstacle density may have on active particles, and which factors might influence the direction of drift.

In order to investigate potential causes for the seemingly contradicting expectations, in the following I will test the effect of a gradient in obstacle density on the motion of ABP in complex environments using the agent-based model developed in chapter 2. Then, I will discuss preliminary results of a microfluidic experiment using the green alga *Chlamydomonas reinhardtii* as model active particle.
Fig. 5.1 Gradient in obstacle lattice. Effect of changing the gradient parameter in Eq. (5.9) with \( R = 40 \) and \( d/R = 2 \). (a) \( r = 0.05 \), (b) \( r = 0.20 \).

5.2 Gradient in obstacle density

First, we can test the theoretical predictions using simulations of the model established in chapter 2 (see section 2.1) with a gradient in the obstacle density. In order to facilitate comparison with the previous computational study, we choose the same obstacle gradient described in [107] (up to a constant shift). The centre of the obstacle in row \( i \) and column \( j \) has coordinates

\begin{align*}
x^{(i,j)} &= \frac{d}{1 - e^{-r}} (e^{jr} - 1) \\
y^{(i,j)} &= \pm d i e^{jr}
\end{align*}

where \( d \) quantifies the distance between obstacle centres in the origin and \( r \) quantifies the gradient in obstacle distances. Obstacles within the same column are separated by \( y^{(i,j)} - y^{(i-1,j)} = de^{jr} \) and rows are similarly separated by \( x^{(i,j)} - x^{(i,j-1)} = de^{jr} \). Thus, spacing depends exponentially on the column number \( j \) and increases with \( x \). The relative change between adjacent obstacle rows, however, is independent of the column number \( j \) as can be seen from

\[ \delta x = \frac{[x^{(i,j+1)} - x^{(i,j)}] - [x^{(i,j)} - x^{(i,j-1)}]}{x^{(i,j)} - x^{(i,j-1)}} = -1 + e^r. \]

For \( r \to 0 \), \( \delta x = 0 \) and we approach a regular square lattice with distance \( d \) between the obstacle centres. The effect of varying \( r \) is illustrated in Fig. 5.1. A smaller \( r \) has a more gradual change in obstacle density with a larger average obstacle density in the simulation domain.
There is an important decision to make regarding the simulation domain. Should the domain be of similar size for the different lattice gradients, or should the largest distance between obstacles be similar for all lattice gradients? I decided to keep the simulation domain constant, such that the overall distance covered between the dense and dilute regions is comparable. Tests fixing the maximum distance between obstacles indicate that the choice of simulation domain may influence the strength of the observed drift but the trends remained the same.

There are different options for dealing with the boundaries of the simulation domain. The region of interest with a gradient in obstacle density could be flanked by regions with constant distance between obstacles, which is equivalent to the distance used at the edge of the gradient region. This approach was chosen in [107] and corresponds to open boundaries for the region of interest. Alternatively, the obstacle lattices shown in Fig 5.1 could be mirrored at the origin along the x-direction. Periodic boundary conditions could then be applied at the boundaries along the x-direction. I chose to implement ‘reflecting’ boundaries, where the particles follow the same slide-off condition at the simulation domain as at the obstacle, which allows for the study of a closed system.

The active particles are initialised uniformly in the simulation domain with a uniform distribution of initial orientations. If an initial position overlaps with an obstacle, the position is resampled. However, only the $y$ component is resampled to keep the distribution uniform along the obstacle gradient.

### 5.3 Drift and steady-state depend on slope of gradient

An example trajectory of a particle moving in an obstacle density gradient is shown in Fig. 5.2. The obstacle density decreases with increasing $\tilde{x} = x/x_{\text{max}}$, where $x_{\text{max}}$ is the domain size. The average population position is shown in Fig. 5.3(a), where time has been rescaled by the persistence time $\tau_p = 1/D_{\text{rot}}$. As the particles are distributed uniformly at the start of the simulation, the initial average position is $\tilde{x} \sim 0.5$. Over time, the uniform distribution relaxes to its steady-state distribution. As can be seen from Fig. 5.3, the position of the population drifts towards $\tilde{x} < 0.5$, that is towards denser obstacle regions. This shift in the average position of the population is larger for shallower gradients (i.e. smaller $r$). We can fit the average position over time with the expression

$$\tilde{x}(t) = \tilde{x}_0 e^{-\lambda t} + \tilde{x}_{SS}, \quad (5.11)$$
Fig. 5.2 Particle moving in an obstacle gradient. The obstacle lattice is determined by Eq. (5.9) with \( r = 0.1 \) and \( d/R = 2 \). For all simulations, the domain size is \([x, y] = [20l_p, 4l_p]\) where \( l_p = v/D_{\text{rot}} \) is the persistence length of the particle. The track is colour-coded according to time rescaled by the persistence time \( \tau_p = D_{\text{rot}}^{-1} \).

Parameters: \( v = 20\mu m/s, D_{\text{rot}} = 0.1\text{rad}^2/s \).

where \( \lambda \) is a relaxation rate, \( x_0 \) is the initial position and \( x_{SS} \) is the steady-state position of the average population coordinate. The relaxation rate obtained from the fit is influenced by the initial value \( x_0 \), but for all simulations it takes approximately two orders of magnitude of persistence time to reach the steady-state distribution. At steady-state, diffusive flux and advective flux are balanced, i.e. \( J(x) = 0 \) in Eq. (5.5).

Thus, a smaller \( x_{SS} \) corresponds to a stronger drift towards dense obstacle regions as the steady-state distribution is more strongly skewed. The final average position \( \bar{x}_{SS} \) is not influenced by the initial distribution, and is therefore used to compare lattices with differently sized obstacles. Fig. 5.3(b) shows the \( \bar{x}_{SS} \) determined from fitting for different obstacle radii, where \( R \) has been rescaled by the persistence length \( l_p = v/D_{\text{rot}} \). For all obstacle radii studied, the shift in the population position is larger for a smaller gradient \( r \). However, the average population position at steady-state is a non-monotonic function of the obstacle radius. A larger obstacle radius reduces \( v_{\text{obs}} \) in Eq. (5.7). At the same time, \( \tau_R \) increases with increasing \( R \) (see Eq. (2.27)) and the particle can spend less time exploring the domain. Due to this trade-off, medium sized obstacles elicit stronger drifts.

It seems counter-intuitive that the drift is stronger for smaller values of \( r \), i.e. a smaller gradient in obstacle spacing. For example, clustering results observed in MIPS rely on a strong decrease in swimming speed, e.g. in the form of an exponential function. However, the lattice geometry described by Eq. (5.9) does not create a monotonic
5.3 Drift and steady-state depend on slope of gradient

Fig. 5.3 ABPs accumulate at higher obstacle densities. (a) Average population position over time in a lattice with $R/l_p = 0.05$ for different gradient parameters $r$. Initially, the particles are distributed uniformly in the domain and the average position is $x/x_{\text{max}} \sim 0.5$, where $x_{\text{max}}$ is the size of the domain. The gradient in obstacle density introduces a drift towards denser obstacle regions over time, where $\tau_p = D_{\text{rot}}^{-1}$ is the persistence time. The drift and steady-state depend on the slope of the obstacle gradient $r$ in Eq. (5.9). The dashed line is a fit of an exponential decay according to Eq. (5.11). (b) The average population position at steady state, $\tilde{x}_{SS}$, is skewed more strongly for smaller gradient slopes $r$. Medium sizes of obstacles generate a stronger drift. For all simulations, $N_P = 10^5$ particles were used in the simulations.

change in the effective swimming speed profile. It rather creates an alternation between decrease in effective speed in the proximity to obstacles and a subsequent increase up to the free speed level between obstacle rows. Consider an obstacle lattice with a large gradient, as illustrated in Fig. 5.1(b). At the dense end on the left side of the domain, the particles slow down and accumulate. However, when a particle randomly moves towards the right side of the domain, it quickly moves into regions with obstacles that are spaced far apart - more so the similar particle would do in the lattice with smaller $r$ in Fig. 5.1(a). However, in the dilute region on the left end of the domain in 5.1(b), the collision frequency with obstacles is small and, hence, the speed is close to free swimming speed as only a contact with an obstacle will reduce the speed. Thus, a more gradual change in obstacle density (i.e. smaller $r$) creates a more sustained change in swimming speed and, thereby, a stronger drift towards lower swimming speed.

The observed accumulation at low densities may indeed be closer to the phenomenon of rectification (see chapter 1). In the classic example described in chapter 1, a sequence of V-shaped obstacles rectified bacterial motion and concentrated cells on one side of the array of obstacles. In general, rectification occurs for passive Brownian particles
in the presence of an asymmetric substrate under a driving force [132]. For active Brownian particles, on the other hand, self- propulsion on an asymmetric substrate is sufficient and there is no need for a driving force [132], which has been shown to hold in general for self-propelled particles in periodic asymmetric potentials [133]. The gradient lattice considered here is not periodic and the obstacles only decrease the swimming speed rather than favouring a certain direction of motion as in the case of V-shaped obstacles. Nevertheless, the asymmetric environment leads to an asymmetric swimming speed similar to a potential in the domain and might produce a ‘ratchet-like’ effect as in previously described rectification studies. Placing the ‘ratchets’ closer to each other reduces the time spent in free space before encountering another ‘ratchet’, i.e. a smaller gradient may increase the channelling towards higher obstacle densities.

5.4 Microalgae in microfluidic lattices

In the following, I will present preliminary tests of the theory using the microalgae *Chlamydomonas reinhardtii*. These algae scatter off convex surfaces mainly due to steric interaction of their flagella with boundaries [134] and differ significantly from the slide-off boundary condition considered in the simulations just discussed. Nevertheless, the non-classical surface interaction reduces the effective speed of algae at surfaces. An advantage of using algae is the reduced accumulation at flat boundaries (such as those enclosing a microfluidic channel) due to their hydrodynamic flow field, which has been described as a puller-type [3, 16]. Furthermore, rectification towards certain areas of the microfluidic channel could locally create high densities of confined microswimmers. In those conditions, pusher-type bacteria may interact via their flow fields [135], which could complicate the analysis by large scale density fluctuations [136]. For puller-type algae, on the other hand, this effect is reduced.

5.4.1 Growth conditions

The wild-type alga *Chlamydomonas reinhardtii* CC125 was used for the experiments. Stock cultures were grown on agar slants of TAP medium (see Appendix B) at room temperature. Liquid cultures were prepared by inoculating 15mL of Tris-minimal medium (see Appendix B) with an inoculation loop of stock culture. The culture was then grown under a 14 : 10h day/night illumination cycle at 100rpm and 25°C. After 5 – 7 days, the culture reached exponential growth phase and a cell density of 3-5 × 10^6 cells/mL. The cell count was determined using a Coulter counter (Z2 Dual Threshold
Cell Counter, Beckman, US). Subsequently, the culture was diluted to $5 \times 10^5$ cells/mL and grown for another 3 – 4 days. The culture was harvested $\sim 1 h$ after the onset of the day cycle, and the experiment was performed at $3 \times 10^6$ cells/mL. The motility pattern of this microswimmer has been described by Run-and-Tumble dynamics \cite{24} with a persistence length of approximately $180 \mu m$ \cite{56}. While the simulations presented earlier were based on parameters motivated by smooth-swimming \textit{E. coli}, the resulting persistence length is comparable to those values given for \textit{C. reinhardtii}.

5.4.2 Microfluidics

The microfluidic channels were provided by Marco Polin at University of Warwick, and were $30 \mu m$ thick with pillars of $30 \mu m$ radius. Therefore, the $R/l_p$ in these experiments is $\approx 0.17$. The PDMS channel was prepared as described in chapter 4. The channel contained one inlet and two outlet holes, each of $1.5 mm$ diameter, to facilitate loading. After bonding glass slide and PDMS channel, the algal culture was loaded into the microfluidic channel at $100 \mu L/min$ without further preparation. Finally, the inlet and outlet tubes were closed off by clamps, which is a crucial step as drift due to flow impedes the measurement of any bias in the distribution due to the obstacle lattice.

5.4.3 Imaging and data analysis

The microalgae were imaged 30min after loading at 4x magnification and 15fps under brightfield illumination for $30 s$. A red filter was used to avoid bias due to phototaxis and flagella sticking to surfaces \cite{137}.

First, the median image was subtracted from the image stack to remove small impurities as well as the pillar outline for the analysis; the resulting image is shown in Fig. 5.4(a). To obtain the concentration of algal cells as a function of the obstacle density, we may average the cell density over constant obstacle densities. During the plasma bonding and due to the imaging set-up, the obstacle lattice is not guaranteed to be aligned with the borders of the image. Yet, averaging across constant obstacle density requires a known angle of the lattice, which is best achieved if the lattice is aligned with the image border. Therefore, pillars in the median image were identified by fitting circles using matlab’s \textit{imfindcircles} function. The centres in the same obstacle row were fitted by a line to obtain the slope of the obstacle positions in the image as shown in Fig. 5.4(a). The slopes obtained for all rows individually were averaged and the image stack was rotated according to the appropriate average angle. Finally, the
Fig. 5.4 Data analysis. (a) Aligning the channel. The pillars of 30 µm radius were identified as circles. A line was fitted through the centres of the pillars in the same row to get the slope of the row. The average slope identified from the rows was used to rotated the image to align the pillar axis with the image borders. (b) An example image from the rotated stack, which was adjusted by subtracting the median image. Pillars identified in the median image have been added for illustration as black circles. The scale bar corresponds to 100 µm. The arrow indicates the direction of increasing y in the data analysis.

image stack was cropped to the maximum possible size. Fig. 5.4(b) shows an example image from the image stack.

The spatial distribution of algae was approximated by the image intensity. Under brightfield imaging, the algae appear as dark spots. However, due to diffraction, the intensity can vary for cells at slightly different heights and within one spot. Therefore, I applied an intensity threshold to create a binary image, dilate the binary gradient mask and fill in interior gaps. The intensity value was then averaged along constant obstacle density, i.e. along the x-direction of the (rotated) image.

5.4.4 *Chlamydomonas reinhardtii* accumulates at high obstacle densities

The obtained density profile shows large fluctuations, see the green curve in Fig. 5.5. These coincide with the obstacle rows and are caused by variations in free space available for cells to accumulate. Between obstacle rows, many cells can accumulate, while at the position of obstacle rows the available free space is reduced. Due to the gradient in obstacle density, free space within an obstacle row also varies between different rows. To account for this variation in free space, I adjusted the cell intensity
Fig. 5.5 Algae accumulate at large obstacle densities. First, the image was rotated to have uniform obstacle density along the x-direction. Then, the image intensity was averaged along the x-direction (green curve). Next, the profile was adjusted by the free space as given in Eq. (5.12). Finally, the density was smoothed using a moving average with heuristically determined window size.

profile by the free space available at each point along $y$

$$\tilde{A} = 1 - \frac{A_O(y)}{A_T(y)}, \quad (5.12)$$

where $A_O(y)$ is the obstacle area, $A_T(y)$ is the total area. The areas were quantified by the number of pixels in a row of the image. I then divided the image intensity by $\tilde{A}$ to obtain the adjusted density shown as the orange curve in Fig. 5.5. The procedure reduced the large fluctuations significantly but it is still possible to identify larger intensity values close to obstacles. These ‘overshoots’ that are often close to obstacle positions are caused by algae accumulating at the boundaries. Finally, the profile was smoothed using a a moving average filter (purple curve in Fig. 5.5). The final profile shows a clear and steady increase in cell density towards regions of higher obstacle density. Thus, a density gradient in a microfluidic obstacle lattice can introduce a drift in the motility of algal cells and, thereby, bias the density profile towards higher obstacle densities.
5.5 Discussion

Both the computational and experimental results presented here contrast with the recent study by Schakenraad et al. [107] but agree with the expectation of a drift towards regions of lower speed derived from theoretical results in [26, 27, 124]. The simulations considered here and in [107] are comparable but with notable differences in the boundary conditions of the simulation domain and the initialisation of particles. In the previous study [107], the particles left the gradient domain via effectively open boundaries into regions with regular obstacle lattices. Thus, a comparison between steady-states is not possible. Furthermore, an open boundary could cause the observation of a transient drift. As Schnitzer noted in his seminal paper [26] for a system with constant speed but spatial variations in tumbling rate, particles can initially move towards regions of low reorientation frequency in a process termed ‘pseudochemotaxis’. At long times in a closed domain, however, particles have visited all regions many times and distribute uniformly in the domain because the spatial variations in tumbling frequency do not affect the steady-state distribution. An open boundary, on the other hand, could lead to an escape of particles based on this initial drift. In a gradient of obstacle density, the swimming speed varies in addition to the collision frequency, but the speed variation is locally restricted to areas with obstacles, in particular at dilute obstacle densities. Hence, the speed variation might not take effect initially and the drift towards lower obstacle densities observed in [107] may be caused by the open boundary conditions of the domain. Such a set-up might then be better described as a first passage problem. The time scale of (experimental) observation is, therefore, a crucial parameter in order to determine whether an observed drift might be caused by a similar ‘pseudotaxis’. For example, the time to reach steady-state in the simulations presented here is an order of magnitude larger than the time scale studied in [107].

Why then did we not observe an initial drift towards lower obstacle densities in the simulations presented in Fig. 5.3(a)? Besides the domain boundary, the initial position of particles used here differs from [107]. While we used a uniform initial distribution of particles, Schakenraad et al. [107] initialised the bacteria in the centre of the gradient domain. Consequently, we are averaging over space and sampling the domain boundaries from the beginning, whereas the problem set-up in [107] may be better suited for studying the single particle propagator without encountering the domain boundaries initially. When we instead initialised all particles in the centre of our simulation domain, we indeed observed an initial preliminary drift towards lower
obstacle densities for some lattice gradients studied. Further simulations could explore the role of domain boundary and initial conditions in more detail.

Not only the direction of the drift but also the predictions for the drift dependence on the strength of the density gradient obtained here contrast with the results presented in [107]. While the previous study [107] found that larger gradients (i.e. increasing $r$ in Eq. (5.9)) cause a larger drift velocity, we observed stronger drifts for smaller gradients. Similar to the drift direction, this discrepancy may be caused by the differences in simulation set-up. By the same token, the results presented here predict that the $r$-dependence of the drift is non-monotonic as there is no drift in a uniform obstacle lattice (where $r = 0$).

The discrepancy of the results of this study with the experimental observation of the slime mould *D. discoideum* moving in a microfluidic lattice towards lower obstacle densities in [131] could be caused by a variety of different factors. In [131], the distance between pillars was at most slightly larger than the diameter of the cell, which could therefore be in contact with several pillars. Similarly, Gorelashvili et al. [138] reported that cells accumulate at regions with free space of size similar to the cell. By contrast, the ABPs in the simulations are never in contact with more than one obstacle as they are by definition point particles. The microalgae studied experimentally here are much closer to this assumption. This raises several questions for future studies: If the system in [131] is indeed in steady-state, what causes the drift towards lower densities? The relative size of the pillar spacing, or an actual taxis? As most studies are motivated by applications such as immune cells moving in the extracellular matrix: is the steady-state distribution in a closed domain even relevant for those applications, or is the open boundary indeed a more realistic assumption?

Preliminary experiments with microalgae seem to support the theoretical prediction of accumulation at dense obstacle regions. In addition, the unadjusted density profile in Fig. 5.5 illustrates the ratchet-like effect of the gradient lattice, which was put forward to explain the simulation result of smaller obstacle gradients leading to stronger drifts. In particular, I propose to consider the problem at hand as a special case of rectification, which is caused by the asymmetry of the obstacle density gradient. In this case, the obstacles could be regarded as ‘traps’ for the particles as seen as overshoots in the adjusted density profile in Fig. 5.5. The problem could be examined from this angle theoretically, e.g. by considering a potential in the swimming speed or a Markov chain with asymmetric jumping probabilities between traps. Further experimental work should complement the theoretical analysis by investigating in more detail the influence of the gradient parameter $r$ and the obstacle size. Specifically, it would be interesting
to explore whether the obstacle radius can be optimized to induce the strongest drift possible as suggested by the results in Fig. 5.3(b). Theoretical analysis is also required to predict a population density at steady-state, which could be used to compare to experimental results. However, this should include a modified scattering rule in the model to account for the specifics of algal scattering since the speed at the obstacle, $v_{\text{obs}}$ in Eq. (5.7), depends on the characteristics of the surface interaction. Finally, based on the parameters used the simulation results presented here could be useful to design channels appropriate for smooth-swimming *E. coli*.

Many microorganisms inhabit porous environments; bacteria or algae that dwell in soil are prime examples. The above discussed tendency of microswimmers to accumulate at high obstacle densities could be employed to increase bioremediation in regions of low permeability \[129\]. Without background flow, they should accumulate in dense regions. That being said, there are a multitude of biasing drifts on the transport of microorganisms, which might interfere with topographic drifts. For example, bacteria may follow a chemical field of pollutant in bioremediation applications, whereas swimming algae are biased by the gravitational field of the earth. It would be interesting to study the effect of combining different mechanisms to bias cell movement, which may be useful in applications in bioengineering.

This chapter introduced spatial variations in the swimming speed imposed by the environment, which the microswimmer cannot control. The next chapter, on the other hand, will look at the speed as a parameter in the motility response that can be changed independently by bacteria. Much of the discussion in this chapter is also relevant in the next chapter.
Chapter 6

Motility response augmenting chemotaxis by chemokinesis

6.1 Swimming speed as a response parameter

The run-and-tumble model was originally introduced for enteric bacteria such as *E. coli* and *Salmonella typhimurium* [11, 81], which commonly live in nutrient-rich environments, such as the gut. Marine and soil bacteria, however, often experience heterogeneous and nutrient-scarce environments, and have been found to display different motility patterns. For example, several species living in these harsher environments respond to higher concentrations of attractant by increasing their swimming speed [103, 139, 140]. This response, known as ‘chemokinesis’, modifies the swimming speed in response to the local chemical concentration without affecting the tumbling rate, as illustrated in Fig 6.1(b) - as opposed to chemotaxis, which varies the tumbling frequency in response to a chemical gradient (as discussed in chapter 3). A positive chemokinetic response leads to a higher swimming speed at higher attractant concentrations, whereas a negative response lowers the speed at those concentrations. A wide range of the strength of positive chemokinetic response has been reported, even for a single species. The responses have been found to vary for the legume-symbiotic soil bacteria *Sinorhizobium meliloti* and *Azospirillum basilense* from 7.5 to 35% [141–143] and 40 to 77% [144], respectively; 7.5 to 73% for the soil and freshwater purple bacterium *Rhodobacter sphaeroides* [145, 146]; 26 to 53% for the enterobacterium *E.coli* [147]; 48% [140] or 6 to 64% [148] for the marine pathogen *Vibrio coralliilyticus*. The marine bacterium *V.alginolyticus* showed an increase of up to 80% upon stimulation with glucose in [149]. However, to the best of my knowledge, the pure chemokinetic speed increase as a function of attractant concentration has not been systematically measured for any of these species.
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Fig. 6.1 Chemotaxis vs. chemokinesis: (a) Chemotaxis is the biased movement of bacteria in response to a chemical gradient of attractant by reducing the tumbling rate $\mu$ and, thereby, increasing the length of runs in a favourable direction. (b) Positive chemokinesis leads to an increase in swimming speed $v$ in response to an increase in the local attractant concentration.

The biological significance of the chemokinetic response of some marine and soil bacteria has yet to be fully elucidated. Based on the environment that chemokinetic bacteria have been found in, (positive) chemokinesis might be beneficial in heterogeneous environments with scarce sources of nutrients (attractants). For example, alga-sized microbeads coated with various amino acids were used to study the response of marine bacteria to point-like sources of attractants [139]. All marine bacteria studied were observed to accumulate in bands around the point-like sources while displaying a chemokinetic response. Furthermore, chemokinesis could allow marine bacteria to track algae, helping to foster symbioses with these microorganisms, as well as permitting to respond quickly to short bursts of nutrients, such as those generated from lysing algae [150]. Another example of a chemokinetic marine bacterium is the coral pathogen *Vibrio coralliilyticus*. Microfluidic experiments on this pathogenic bacterium in combination with mathematical modelling have suggested that the maximum accumulation in response to chemical cues produced by heat-stressed coral hosts is larger and is reached faster than in the absence of chemokinesis [140, 148]. As heat-stressed corals are more susceptible to pathogens, chemokinesis could be a crucial evolutionary advantage in oceans heating up due to climate change. In fact, the chemokinetic response was shown to be even stronger at elevated temperatures; increasing from 6% at 20°C to 64% at 30°C [148].

Recent interest in chemokinesis has also been sparked by synthetic microswimmers, such as Janus particles. Janus particles are synthetic colloids in a bath of fuel (e.g. H$_2$O$_2$) that propel due to an asymmetric chemical reaction on their surface [151]. These particles show a positive chemokinetic response since their swimming speed increases with increasing fuel concentration [14], and therefore accumulate in areas of lower fuel concentration.
To date, theoretical work on the combination of positive chemokinesis and chemotaxis has focussed on single-cell level using agent-based models [140, 149, 152]. The chemokinetic response of the marine pathogen *V. coralliilyticus* has been modelled as a step increase in swimming speed when effector concentration increases beyond a threshold attractant value [140]. This agent-based model was used to analyse the chemotactic response to a transient attractant gradient in a microfluidic device after an initial release of attractant. The chemotactic index (i.e. the enhancement in cell concentration over a control region) suggests that chemokinesis enables a stronger and faster response. This model has been further adapted to include speed dependent changes in the probability of flicking and reorientation frequency of *V. alginolyticus* [149, 153]. In this particular case, the speed induced changes in motility pattern are responsible for a significant part of the chemotaxis improvement, as shown by the agent-based model in [149].

In this chapter, we develop a continuum model to study the spatio-temporal dynamics of bacterial populations with chemokinetic and chemotactic responses. We incorporate chemokinesis into the standard Keller-Segel model for chemotaxis by deriving the model from microscopic run and tumble dynamics. The Keller-Segel model is a classical tool to study chemotaxis in bacteria but has not been applied to chemokinetic bacteria so far. The model is based on partial differential equations, unlike the agent-based models used in the previous chapters. The PDE approach will allow us to focus on the population-level response in a continuous manner, and compare it to observations made in previous studies on chemokinesis using agent-based models, which so far implemented sudden changes such as a step response in speed. The extended Keller-Segel model is then used to obtain analytical conditions for chemokinetic drift, and solved numerically for three different examples of attractant distributions that are inspired by existing experimental systems to study bacterial chemotaxis.

### 6.2 A modified Keller-Segel model

We derive a model for chemotaxis in combination with positive chemokinesis by considering a one-dimensional system in which a cell can move either to the right or left with speed $v$. We follow the same approach as discussed in section 5.1 of the previous chapter but adopt asymmetric tumbling frequencies: a right (left) moving particle changes direction with rate $\mu_R$ ($\mu_L$). The one-particle probability density for bacteria again evolves according to $\partial b / \partial t = -\partial J / \partial x$, and the bacterial flux $J$ can be derived as a drift-diffusion equation (e.g., see chapter 5)
Motility response augmenting chemotaxis by chemokinesis

\[ J(x) = -D_b(x) \frac{\partial b}{\partial x} + V(x)b \]  \hspace{1cm} (6.1)

with

\[ D_b(x) = \frac{v^2}{2\mu}, \quad V(x) = V_k + V_\chi, \]  \hspace{1cm} (6.2)

where \( \mu_R + \mu_L = 2\mu \), and where we have defined the chemokinetic

\[ V_k = -\frac{v}{\mu} \frac{\partial v}{\partial x} \]  \hspace{1cm} (6.3)

and chemotactic

\[ V_\chi = \frac{v}{2\mu}(\mu_L - \mu_R), \]  \hspace{1cm} (6.4)

drift speeds, respectively. We will now connect the bacterial flux (6.1) to the commonly used Keller-Segel model of chemotaxis, adapted to the description of chemokinetic populations in dynamic environments.

### 6.2.1 Chemotactic sensitivity

For chemotactic populations, the drift-diffusion flux (6.1) is coupled to a chemoattractant density field \( c \) via the chemotactic drift speed, given by Eq. (6.4). In the standard Keller-Segel model it is phenomenologically asserted that this chemotactic drift speed is proportional to the change in the chemical attractant in space,

\[ V_\chi = \chi \nabla f_\chi, \]  \hspace{1cm} (6.5)

where \( \chi \) is the chemotactic sensitivity parameter and \( f_\chi \) is a function of \( c \) that ensures that the chemotactic drift is biased towards higher attractant concentrations [28]. This definition of the chemotactic drift speed assumes that the chemical attractant profile changes in space but not in time. However, as mentioned, known chemokinetic bacteria inhabit dynamic environments, such as in the ocean or soil. As pointed out by Hein et al [154], the effective gradient perceived by a bacterium changes in a temporally varying attractant profile depending on the direction of its run. Consider a source of attractant at one point of space, and the associated negative gradients of concentration as one gets away from it. If these gradients are steady in time, for example as in a microfluidic setting, a bacterium exploiting the attractant landscape will correctly detect the gradient and swim towards the source, as in standard chemotaxis. However, if the source corresponds to a single point-like release of attractant, and the concentration at the source position also decays due to diffusion, a bacterium travelling toward the
source perceives a smaller increase (or even a decrease) in concentration compared to the steady case. On the other hand, a bacterium moving away from the source perceives a decrease, which is reduced in magnitude compared to the steady case.

In the following we derive new relations for the chemotactic drift speed $V_\chi$ and sensitivity parameter $\chi$ for bacteria undergoing chemotaxis and chemokinesis in dynamic environments, connecting the macroscopic parameter $\chi$ with the microscopic swimming speed $v$ and tumbling rate $\mu$. We follow the approach of de Gennes [88, 89, 155], who considered run-and-tumble bacteria with a simplified E. coli-like chemotactic response. The modifications of the established Keller-Segel model presented here include: i) a swimming speed that is a function of position; ii) a temporally varying contribution to the gradient perceived by the bacteria. As in [88, 89], we consider a one-dimensional model of run-and-tumble bacteria propagating in a gradient of attractant, to which bacteria are exhibiting a ‘small response’, that is the tumbling response remains close to the adapted value (as described in chapter 3) [156]. Chemotactic memory is modelled through a kernel integral, reflecting the fact that bacteria ‘remember’ their chemical environment over a characteristic delay time of a few seconds (see chapter 3). We neglect directional persistence and rotational diffusion, which can be addressed in the same framework [155], but are here ignored for simplicity. The modified de Gennes model we employ could be applicable to chemokinetic species like rhizobia, which display run and tumble dynamics [103]. The model neglects features of the motility pattern of chemokinetic marine bacteria, such as a run-reverse-flick mechanism or the influence of a change in swimming speed on reorientation frequency, as observed for V. alginolyticus [149], but instead focus on the pure effect of speed change.

As the tumbling frequency follows an exponential distribution, tumbling events can be treated as independent events of a Poisson process with rate $\mu(t) = \mu_0 e^{\Delta(t)}$, where $\mu_0$ is the tumbling rate in the absence of a gradient and where $\Delta(t)$ is the chemotactic bias given by the memory integral

$$\Delta(t) = \int_{-\infty}^{t} dt' K(t-t') f_\chi(x(t')).$$  \hfill (6.6)

In chapter 3, $f_\chi(x(t)) = c(x, t)$ for simplicity, but here we choose a more realistic concentration function

$$f_\chi = \frac{c(x, t)}{c(x, t) + k_\chi},$$  \hfill (6.7)

where $k_\chi$ is the chemotactic sensitivity parameter [157]. The memory kernel $K(t)$ was first measured for E. coli [158] and more recently also for V. alginolyticus [159]. Based on these experiments we assume that the kernel obeys $\int_{0}^{\infty} K(t)dt = 0$, i.e. the tumbling
rate perfectly adapts. As mentioned, we shall assume a small response ($\Delta(t) \ll 1$), so that the bacterial tumble rate can be linearised to

$$\mu(t) \approx \mu_0(1 - \Delta(t)),$$

(6.8)

The assumption made so far are similar to those made in chapter 3 discussing chemotaxis in obstacle lattices. However, to solve the system we will now follow an approach based on average run time intervals as in [88]. Considering a run that starts at $t = 0$, the probability density of a tumble event in the interval $[t, t + dt]$ is $\mu(t) \exp(-\int_0^t dt' \mu(t'))$. The mean run duration is given by

$$\tau = \left\langle \int_0^\infty \mu(t) \exp \left( - \int_0^t dt' \mu(t') \right) t dt \right\rangle_{\text{paths}},$$

(6.9)

where angled brackets denote averaging over all possible bacterial swimming paths, as the tumble rate is path dependent (the ‘paths’ subscript will be omitted henceforth). Integrating (6.9) by parts and recalling (6.8) we can write

$$\tau = \left\langle \int_0^\infty e^{-\mu_0 t} \exp \left( \int_0^t dt' \Delta(t') \right) dt \right\rangle.$$

(6.10)

Next, since $\Delta(t) \ll 1$, we can linearise the exponential with the integral to obtain

$$\tau = \frac{1}{\mu_0} + \mu_0 \int_0^\infty dt e^{-\mu_0 t} \left\langle \int_0^t dt' \Delta(t') \right\rangle,$$

(6.11)

where we have brought the angled brackets inside the time integral to surround only path dependent quantities. Substituting the expression for the bias (6.6) and performing a change of variables in the memory integral by defining $u = t - t'$, we then obtain

$$\tau = \frac{1}{\mu_0} + \mu_0 \int_0^\infty dt e^{-\mu_0 t} \times \left\langle \int_0^t dt' \int_0^\infty du \ K(u) f_{\chi}(x(u) - t') \right\rangle.$$

(6.12)

As the time interval of interest is small compared to the gradient variations, we can Taylor expand the concentration function about a reference position and time:

$$f_{\chi}(x(t - u)) \approx \text{const} + x(t - u) \nabla f_{\chi} |_t + (t - u) \partial_t f_{\chi} |_{x(t)}.$$

(6.13)

We note that the constant term does not influence the integral in (6.12), since the response function $K(u)$ integrates to zero. Further analysis is simplified by considering
the special response function

\[ K(u) = A\delta(u - \theta), \]  

(6.14)

where \( \theta \) is a single delay time [88]. We note that this function does not represent a physical response and is considered solely for the purpose of facilitating the calculation. Then, substituting equations (6.14) and (6.13) into (6.12) and integrating over the delta function we obtain

\[ \tau \approx \frac{1}{\mu_0} + A\mu_0 \int_0^\infty e^{-\mu_0 t}[I_1(t) + I_2(t)]dt, \]  

(6.15)

where we have defined

\[ I_1(t) = \left\langle \int_0^t \nabla f_x x(t' - \theta) dt' \right\rangle \]

\[ = \nabla f_x \int_0^t \langle x(t' - \theta) \rangle dt'. \]  

(6.16)

The average over paths can be taken inside the time integral since tumbles for \( t > 0 \) are treated as having no effect on cell motion, so that paths are independent of time on the interval considered. Proceeding similarly we define

\[ I_2(t) = \int_0^t (t' - \theta) \left\langle \partial_t f_x |_{x(t')} \right\rangle dt'. \]  

(6.17)

To evaluate (6.16), we recall that the bacteria we are considering do not undergo rotational diffusion and do not possess directional persistence. Thus, following [88], we see that for times preceding a run (when a bacterium is tumbling) the position of a bacterium is, when averaging over paths, not correlated to the velocity. On the other hand, during a run, the position correlates with velocity. In this way, in (6.16) we have

\[ \langle x(t' - \theta) \rangle = \begin{cases} 0, & t' < \theta \\ \pm v(x)(t' - \theta), & t' > \theta \end{cases} \]  

(6.18)

So that integral (6.16) becomes:

\[ I_1(t) = \pm \nabla f_x v(x) \int_0^t (t' - \theta) dt' = \pm \nabla f_x v(x) \frac{(t' - \theta)^2}{2}. \]  

(6.19)

We proceed analogously to evaluate integral (6.17). In this case, the integral over paths for times preceding a run does not average to zero, but sums up the contributions from
the temporal variation of the gradient during tumbles. During a run, on the other hand, the temporal variation is evaluated for when bacteria are travelling up (down) the gradient, providing positive (negative) weights to the path integration, which provides

\[
\langle \partial_t f_X \mid x(v) \rangle = \begin{cases} 
F(t', \theta), & t' < \theta \\
\pm \partial_t f_X, & t' > \theta 
\end{cases}
\]  (6.20)

where \( F(t', \theta) \) is a function of time and delay. In this way, we can carry out integral (6.17)

\[
I_2(t) = \int_0^\theta F(t', \theta)(t' - \theta) \, dt' \pm \int_0^\theta \partial_t f_X \mid x (t' - \theta) \, dt'
\]  (6.21)

which can be integrated to obtain

\[
\tau_{L,R} \approx \frac{1}{\mu_0} + A \mu_0 \int_0^\infty dt e^{-\mu_0 t} \times \pm [v(x) \nabla f_X + \partial_t f_X] \frac{(t - \theta)^2}{2},
\]  (6.22)

Extending to the general response function \( K(\theta) \) with a distribution of delays [88], the run time is

\[
\tau_{L,R} \approx \frac{1}{\mu_0} \pm \frac{v(x)}{\mu_0^2} \left[ \nabla f_X + \frac{1}{v(x)} \partial_t f_X \right] A e^{-\mu_0 \theta}.
\]  (6.23)

Since \( \mu_{L,R} = 1/\tau_{L,R} \) and (see Eq. (6.4))

\[
V_X = v(x) \frac{\mu_L - \mu_R}{\mu_R + \mu_L},
\]  (6.25)
we then arrive at an expression for the chemotactic drift

\[ V_\chi = \frac{v(x)^2}{\mu_0} \beta \left[ \nabla f_\chi + \frac{1}{v(x)} \partial_t f_\chi \right], \]  

(6.26)

where we have defined the constant \( \beta = \int_0^\infty d\theta K(\theta)e^{-\mu_0 \theta} \). The standard definition of the chemotactic sensitivity is obtained from the empirical drift Eq. (6.5), where as previously \( \chi \) is the chemotactic sensitivity. We extend here the definition to include temporal gradients, and define

\[ \chi(x) = \frac{v(x)^2}{\mu_0} \beta, \]  

(6.27)

so that the chemotactic drift is given by

\[ V_\chi = \chi(x) \left( \nabla f_\chi + \frac{1}{v(x)} \partial_t f_\chi \right), \]  

(6.28)

Thus, the presence of a temporally increasing (decreasing) gradient increases (decreases) the chemotactic drift speed. Further, we note that the perturbation to the chemotactic drift speed is smaller the larger the value of the swimming speed. In the absence of chemokinetic alterations to the swimming speed (i.e. \( v(x) = v_0 \)), the sensitivity is simply

\[ \chi_0 = \frac{v_0^2}{\mu_0} \beta, \]  

(6.29)

where subscripts denote a constant swimming speed. Dividing (6.27) by (6.29) provides the chemokinetic relationship

\[ \chi(x) = \chi_0 \left( \frac{v(x)}{v_0} \right)^2. \]  

(6.30)

### 6.2.2 Chemokinetic function

To implement \( v(x) \) (and thus \( \chi(x) \)), we consider how to quantify the chemokinetic coupling between speed and local concentration of attractant. We need to make assumptions about the relationship between swimming speed and attractant concentration, as experimental studies have not been carried out to provide this. Firstly, we assume that cells swim at a base level speed, \( v_0 \). Secondly, we reasonably posit that chemokinesis monotonically increases the swimming speed up to a maximum speed denoted as \( v_0 + v_c \).
Motility response augmenting chemotaxis by chemokinesis

Fig. 6.2 Chemokinetic response function. The swimming speed increases from a reference speed, $v_0$, by at most $v_c$, depending on the local concentration of the attractant according to a Hill type function as given by Eq. (6.32). The parameter $n$ determines the strength of the gradient at the half-saturation concentration, $k_c$.

The dynamics between these limits are given by an unknown function characterising the chemokinetic response. For example, a Michaelis-Menten type equation

$$v(x) = v_0 + v_c \frac{c(x)}{c(x) + k_c}$$

(6.31)

could satisfy the above assumptions. The speed monotonically increases from $v_0$ to the maximum $v_0 + v_c$ ($v_c > 0$) with increasing attractant concentration, where the half-maximum speed is reached at the attractant concentration $k_c$. Although Eq. (6.31) satisfies all the assumptions we have made, this particular chemokinetic function is limited by the absence of an inflection point. The chemokinetic drift velocity scales with the gradient of swimming speed, $\partial_x v = \partial_x v \cdot \partial_x c$, which is maximised at an inflection point of the chemokinetic function in presence of a linear concentration field ($\partial_x c = \text{const}$). Thus, we choose a Hill-type equation

$$v(x) = v_0 + v_c \frac{c(x)^n}{c(x)^n + k_c^n}$$

(6.32)

to approximate the chemokinetic response. The Hill parameter $n$ allows us to introduce an inflection point in the response and change the gradient $\partial_x v$. As can be seen from Fig 6.2, the speed increases monotonically with increasing attractant concentrations $c$ for all $n$ and $v_c > 0$. For any $n$, the half-maximum speed is $v = v_0 + v_c/2$ at $c = k_c$ as all functions covered by Eq. (6.32) have the same half-saturation constant $k_c$. Note that upon setting $n = 1$, one recovers Eq. (6.31); for $n \to \infty$, Eq. (6.32) approaches
6.2 A modified Keller-Segel model

Finally, in situations where bacterial growth cannot be ignored (e.g. migration across agar plates considered in section 6.3.2), bacteria are also assumed to undergo logistic growth, which comprises growth and death terms \([89]\). We note, however, that the chemoeffectors eliciting chemotaxis and chemokinesis need not in general be metabolisable nutrients that induce growth. Using Eqs. (6.1), (6.2) and (6.28), the full model equations for the chemical attractant field and the chemotactic and chemokinetic bacterial population field, therefore, are

\[
\begin{align*}
\frac{\partial c}{\partial t} &= D_c \frac{\partial^2 c}{\partial x^2} - k_g g(c) \frac{b}{Y}, \\
\frac{\partial b}{\partial t} &= -\frac{\partial J}{\partial x} + k_g g(c) b \left(1 - \frac{b}{k_b}\right), \\
J(x) &= -\frac{v^2}{\mu} \frac{\partial b}{\partial x} - \frac{v}{\mu} \frac{\partial v}{\partial x} b + \chi(x) \left(\nabla f + v^{-1} \partial_t f\chi\right) b,
\end{align*}
\]

where \(k_g\) is the maximum growth rate, \(Y\) is the bacterial yield, and \(k_b\) is the carrying capacity. Furthermore, \(g(c)\) is chosen as a Monod-type growth function \(g(c) = c/(c+k_s)\) with the half-saturation constant \(k_s\). Note that \(\chi(x)\) is given by Eq. (6.30), \(f_\chi\) by Eq. (6.7) and \(v(x)\) by Eq. (6.32).

Let us summarise the effects of chemokinesis in this model. A spatially varying speed affects all three terms of the bacterial flux \(J\) in Eq. (6.33c) as: (i) there are regions with a higher diffusivity since \(D_b \sim v^2\) (first term); (ii) it introduces a drift where \(\partial v/\partial x \neq 0\) (second term); and (iii) there are regions with a larger chemotactic drift as \(\chi \sim v^2\) (third term).

6.2.3 Non-dimensional model

The system of partial differential equations (6.33) is non-dimensionalised using the characteristic time and length scales \(t_0 = k_g^{-1}\) and \(x_0 = \sqrt{t_0 D_b^0}\), where the bacterial diffusivity is \(D_b^0 = v_0^2 \mu^{-1}\) recalling \(v_0\) is the base line speed. We rescale the attractant and bacterial densities by their respective initial densities, \(c_0\) and \(b_0\). The system of PDEs in dimensionless form hence reads
Motility response augmenting chemotaxis by chemokinesis

\[
\frac{\partial C}{\partial t} = N\nabla^2 C - HB \frac{C}{C + K_S} \tag{6.34a}
\]

\[
\frac{\partial B}{\partial t} = -\nabla J + B \frac{C}{C + K_S} (1 - B) \tag{6.34b}
\]

\[
J = \mathcal{V}(X)^2 \nabla B + V_k B + V_X B \tag{6.34c}
\]

\[
V_k = -\mathcal{V}(X) \frac{\eta \omega^n C^n - 1}{(C^n + \omega^n)^2} \nabla C \tag{6.34d}
\]

\[
V_X = \mathcal{V}(X)^2 \frac{\delta_0 K_X}{(C + K_X)^2} \left( \nabla C + \frac{\zeta}{\mathcal{V}(X) \frac{\partial C}{\partial t}} \right) \tag{6.34e}
\]

with non-dimensional parameters \(B = b/b_0, C = c/c_0, N = D_c/D_0^b, H = b_0/(Y c_0), K_S = k_s/c_0, \eta = v_c/v_0, \omega = k_c/c_0, \delta_0 = \chi_0/D_0^b, \zeta = v_0/(\mu x_0)\) and \(K_X = k_X/c_0\). The non-dimensional speed function in Eq. (6.34) is

\[
\mathcal{V}(X) = 1 + \eta \frac{C^n}{C^n + \omega^n}, \tag{6.35}
\]

where \(\eta\) is the maximum increase in swimming speed, \(\omega\) is the non-dimensional attractant concentration at which the half maximum speed increase is reached, and \(n\) is the Hill parameter. Note that \(\omega\) corresponds to the inflection point of Eq. (6.35). The chemokinetic response is positive for \(\eta > 0\). To model migration in agar plate, the model is finally extended to the 2D axisymmetric case by introducing polar coordinates as

\[
\nabla B = \frac{\partial B}{\partial R} \tag{6.36a}
\]

\[
\nabla^2 B = \frac{\partial^2 B}{\partial R^2} + \frac{1}{R} \frac{\partial B}{\partial R}, \tag{6.36b}
\]

and the equivalent equations for the chemical field, \(C\).

6.2.4 Numerical implementation

A finite difference scheme was chosen to compute the numerical solution of the system of PDEs (6.34). The domain \(\Omega\) is represented by a vector of \(M\) equally spaced grid points, \(X_1, ..., X_M\). A fourth order scheme in space and a first order scheme in time are used to approximate the derivatives. Homogeneous Neumann boundary conditions
### Table 6.1 Non-dimensional parameters used in simulations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Figure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H = \frac{b_0}{Y c_0}$</td>
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<td>6.3, 6.4</td>
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<td></td>
<td>3.5</td>
<td>6.5, 6.7, 6.8</td>
<td>[89]</td>
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<td>6.5, 6.7, 6.8</td>
<td>[89]</td>
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<td></td>
<td>105.0</td>
<td>6.5</td>
<td>[89]</td>
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<tr>
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<td>6.5</td>
<td>-</td>
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<td>[140]</td>
</tr>
<tr>
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</tr>
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<td>$K_S = \frac{k_s}{c_0}$</td>
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<td>$K_\chi = \frac{k_\chi}{c_0}$</td>
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<td>[89]</td>
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<tr>
<td>$S$</td>
<td>0.5</td>
<td>6.7, 6.8</td>
<td>-</td>
</tr>
</tbody>
</table>
are imposed for both substrate and bacterial concentration, i.e. \( \frac{\partial C}{\partial X} \big|_{X=1, M} = 0 \) and \( \frac{\partial B}{\partial X} \big|_{X=1, M} = 0 \).

The parameters used in the non-dimensional model are summarised in Table 6.1. The parameters are mostly based on literature values for \( E. coli \). Due to the lack of experimental data required for a full estimate of the functional form of the chemokinetic response of, e.g., a single species, the values for the chemokinetic response (\( \eta, \omega, n \)) are motivated by several studies and chosen to best illustrate the response. For example, \( \eta = 0.5 \) corresponds to a 50% maximum increase in the swimming speed, which is on the order of magnitude that has been reported for several species [140, 146, 147]. The largest increase reported, to the best of my knowledge, is 80% for \( V. alginolyticus \) [149]. Thus, \( \eta = 2 \) is chosen as an extreme value to illustrate the effect of chemokinesis more clearly. The half-saturation constant \( \omega \), which is required for the chemokinetic function given by Eq. (6.35), has not been reported directly. From (visual) inspection of published results, half-saturation constants may be about 0.1mM of glucose and 0.1mM of acetate for \( E. coli \) [147] and \( R. sphaeroides \) [146], respectively. In [140], the half maximum speed seems to be reached at about 30% relative mucus concentration, while the maximum is reached at about 60%. As pointed out in [154], absolute concentration above a threshold can be detected before gradients can be accurately measured. Therefore, we assumed that the half-saturation constant of chemokinetic response (\( \omega \)) should be below or on the same order as the chemotactic half-saturation constant (\( K_\chi \)). As there has not been a functional fit to chemokinesis measurements, an estimate for the Hill factor \( n \) is difficult. From visual inspection of results in [140] and [147] we may assume a factor between 1 and 3. Simulations with larger Hill factors were performed to compare to results based on the assumption of a step-change in swimming speed (i.e. \( n \to \infty \)) as done in agent-based models [140, 149]. The parameters given in table 6.1 were chosen to illustrate the effect of chemokinesis best.

In the following section, we will present numerical solutions for three different types of attractant gradient. First, we consider a steady linear attractant gradient with distribution \( C(X, 0) = 0.01X \), while the bacteria are initially uniformly distributed \( B(X, 0) = 1.0 \). Next, to study the response to a self-generated gradient in soft agar plates we assume a Gaussian bacterial inoculum, \( B(R, 0) = \exp(-R^2/\sigma^2) \), with a corresponding attractant distribution, \( C(R, 0) = 1 - \exp(-R^2/\sigma^2) \) [89]. Finally, we examine the response to a transient point source with initial distribution \( C(R, 0) = S(4\pi NT)^{-1} \exp(-R^2/4TN) \), where \( S = 0.5 \) and \( T = 0.02 \), while the bacteria are initially uniformly distributed \( B(R, 0) = 0.2 \).
6.3 Chemokinesis alters the chemotactic response quantitatively and qualitatively

6.3.1 Steady linear attractant profile

Condition for dominant chemokinetic drift

Before solving the extended Keller-Segel model numerically, we can use Eq. (6.34) to analytically derive a condition on the relative importance of chemokinetic and chemotactic contributions to the drift of the bacterial population. The drift due to a spatially varying swimming speed causes cells to accumulate in regions where they have low speeds. For $\eta > 0$, by construction of the velocity function (6.35), the speed is low at low attractant concentrations. The chemotactic drift, on the other hand, is directed towards higher attractant concentrations by virtue of Eq. (6.7). Hence, the bacterial density is governed by two competing drifts, as can be seen from the opposing signs in Eq. (6.34d) and (6.34e). If the chemokinetic drift is larger than the chemotactic drift for a large part of the spatial domain, this could lead to accumulation at low attractant concentrations, instead of the biologically-desirable accumulation at high concentrations. Assuming a stationary and linear attractant profile (i.e. $\partial C/\partial T = 0$ and $\partial C/\partial X = \text{const}$), we have from Eq. (6.34d) and (6.34e) that the chemokinetic drift is larger than the chemotactic drift if

$$\frac{n\eta\omega^n}{(C^n + \omega^n)^2} V^{-1} C^{-1} > \frac{\delta_0 K_X}{(C + K_X)^2}, \quad \forall X \in \Omega,$$

(6.37)

where $\Omega$ is the spatial domain. In the case of a linear attractant profile, we know from Eq. (6.35) (and Fig. 6.2) that the gradient $\partial V/\partial C$ is maximum close to the half-saturation constant $\omega$. Thus, we evaluate condition (6.37) with $C = \omega$, which yields the condition

$$n > \frac{4\delta_0 \omega K_X}{(\omega + K_X)^2} \left(\frac{1}{\eta} + \frac{1}{2}\right), \quad \forall X \in \Omega : C(X) = \omega.$$

(6.38)

If the Hill parameter $n$ exceeds this threshold, the chemokinetic drift is predicted to be larger than the chemotactic drift at the attractant concentration $C^* = \omega$ for $\eta > 0$. Note that, for a step function, condition (6.38) is always met at the threshold concentration $C^* = \omega$ since $n \to \infty$. Conversely, if $\eta < 0$ (i.e. modelling a negative chemokinetic response), condition (6.38) will never be met as the chemotactic and chemokinetic drift have the same direction (chemokinesis in this case is stabilising).
Motility response augmenting chemotaxis by chemokinesis

Fig. 6.3 Chemokinesis in steady linear attractant profile. Bacterial response (bottom row) to a fixed linear attractant profile (top row) using pure chemotaxis (blue curve) versus chemotaxis with chemokinesis (orange curve) for three time points. Note the changing range of the y-axis in the bottom row. The initial bacterial profile is indicated by the grey line. The position at which $C = \omega$ is highlighted by a dotted red line. Parameters $H = 0$, $N = 0$, $K_\chi = 0.53$, $\delta_0 = 50$, $\eta = 2$, $\omega = 0.2$, $n = 5$, $T = 1, 9, 17$ (as shown).

Numerical solution

The full model Eq. (6.34) includes the effect of growth and consumption as well as chemotaxis and chemokinesis. Thus, any change in the spatial distribution of the bacterial population due to chemotaxis and chemokinesis will feed back onto the attractant distribution due to consumption by the bacteria. In order to identify the influence of chemokinesis without such additional complications, we first solve the model for a steady attractant gradient, i.e. $\partial C/\partial T = 0$. We furthermore assume a linear profile such that $\partial C/\partial X = \text{const}$ and ignore consumption, and thus population growth. This situation might be achieved experimentally in microfluidic devices [160], where the gradient may be fixed and bacterial growth can be neglected on experimental timescales short compared to growth timescales $\sim k_g^{-1}$.

Fig. 6.3 compares the response of a purely chemotactic population to the response of a chemotactic-chemokinetic population. Globally, chemokinesis leads to a stronger and faster accumulation at high attractant concentrations than in the purely chemotactic case. At the critical concentration, $C = \omega$, however, the chemokinetic drift holds back a
6.3 Chemokinesis alters the chemotactic response quantitatively and qualitatively

Fig. 6.4 Effect of Hill parameter on chemokinesis in steady linear attractant profile. Chemotactic-chemokinetic bacterial response to a fixed linear attractant profile at $T = 0.5$ (same constant attractant profile as in Fig. 6.3). The Hill parameter $n$ in Eq. (6.35) and, thus, the speed gradient is varied. The position at which $C = \omega$ is highlighted by a dotted red line, while the slower subpopulation is highlighted by the green arrow in the case $n = 40$. Parameters: $H = 0$, $N = 0$, $K_\chi = 0.53$, $\delta_0 = 50$, $\eta = 2$, $\omega = 0.2$, $n = 1, 5, 10, 40$. For the chosen parameters, $n = 40$ is just above the threshold given by (6.38).

subset of the population because it is directed towards lower attractant concentrations as described above. This can be seen in the form of an accumulation of cells at low attractant concentrations. As the chemotactic sensitivity parameter, $\delta_0$, is large in this simulation, the population subset overcomes the drift and accumulates at high attractant concentrations at long times. However, if condition (6.38) is fulfilled, the chemokinetic drift is larger than the chemotactic drift. Thus, there is a subpopulation driven to small attractant concentrations by chemokinesis. The effect of varying the Hill parameter $n$ in Eq. (6.35) is illustrated in Fig. 6.4. For the parameters chosen in Figure 6.3, condition (6.38) is met for $n > 39.78$. Testing for this effect experimentally would require observing the transient bacterial concentration profiles in addition to the commonly reported steady-state profiles.

As the attractant concentration is fixed, we can determine a steady-state for the bacterial population, i.e. by setting $\partial B/\partial T = \partial J/\partial X = 0$. Due to the homogeneous Neumann boundary conditions of the problem, we have $J = 0$ in Eq. (6.34), which yields the differential equation.
\[
\frac{\partial B}{\partial X} + B \left[ \frac{1}{2D} \frac{\partial D}{\partial X} - \delta_0 \frac{d}{dX} \left( \frac{C}{C + K\chi} \right) \right] = 0,
\]

where we used the non-dimensional diffusivity \(D(X) = \mathcal{V}(X)^2\). This equation can be integrated to give the steady-state

\[
\frac{B}{B^*} = \frac{\mathcal{V}^*}{\mathcal{V}} \exp \left\{ \delta_0 \left( \frac{C}{C + K\chi} - \frac{C^*}{C^* + K\chi} \right) \right\},
\]

where \(B^*, C^*, V^*\) are reference values at a chosen reference point \(X^*\). It is thus clear that, in addition to the influence on the dynamics, chemokinesis affects the steady-state solution via the term \(V^*/\mathcal{V}\), where \(\mathcal{V}\) varies in space due to chemokinesis. In the case of chemokinesis but no chemotaxis (i.e. \(\delta_0 = 0\)), the steady-state is determined by the inverse of the speed distribution, i.e. the bacteria accumulate at low speed, as expected and shown previously [26, 127, 161]. For non-zero \(\delta_0\), if the speed is uniform in space, \(V^*/\mathcal{V} = 1\) and Eq. (6.40) reduces to the chemotactic steady-state solution. The exponential term in Eq. (6.40) represents the chemotactic contribution to the steady state, which does not depend on the swimming speed. Thus, the increase in chemotactic sensitivity \(\chi\) (see Eq. (6.30)) must be balanced by the increase in diffusivity at steady state in a fixed chemical gradient. However, chemokinesis still affects the steady state via the term \(V^*/\mathcal{V}\). This chemokinetic effect in the steady-state may only be detectable in experiments with small \(\delta_0 = \chi_0/D_b\), since the chemotactic exponential term will dominate over \(V^*/\mathcal{V}\) for large \(\delta_0\).

### 6.3.2 Self-generated gradient: agar plate

We now consider an evolving attractant field generated by a bacterial population able to consume and grow. The attractant is initially uniformly distributed in a 2D axisymmetric setting. This set-up is reminiscent of the classical agar plate experiments, in which bacteria are inoculated in the centre of a nutrient agar plate, see e.g. [89, 162]. While growing and consuming nutrient, the population creates a gradient of attractant, which it then follows outwards in a chemotactic wave. The attractant profile is a travelling wave itself, and we assume here that the profile relative to the bacterial travelling wave is stationary, i.e. \(\zeta = 0\) in the chemotactic drift (6.34e).

In Fig. 6.5, we compare two chemotactic populations to a chemotactic-chemokinetic population. The chemotactic populations travel at a constant speed, either \(\mathcal{V} = 1\) (blue curve) or \(\mathcal{V} = 1 + \eta\) (purple curve). Both populations develop a sharp travelling wave, with a larger wave speed for the population with speed \(\mathcal{V} = 1 + \eta\). The chemotactic-
6.3 Chemokinesis alters the chemotactic response quantitatively and qualitatively

Fig. 6.5 Self-generated gradient. Bacterial populations (bottom row) create an attractant gradient (top row) via consumption, which they respond to with chemotaxis at base speed $V = 1$ (blue curve) or chemotaxis-chemokinesis (orange curve). Chemokinesis leads to a faster but also broader, less pronounced bacterial wave. The chemotactic population travelling at constant speed $V = 1 + \eta$ (purple curve) has the fastest travelling pulse. Parameters $H = 3.5, K_S = 1, N = 0.5, K_\chi = 0.53, \delta_0 = 105, \eta = 0.5, \omega = 0.5, n = 5, T = 1, 8.8, 16.4$

Chemokinetic bacterial population, on the other hand, develops a broader wave profile. The peak of the wave front is smaller and is followed by a plateau. This effect is more pronounced at late times, as can be seen in the third lower panel of Fig. 6.5. The reduced pulse also travels slower than the pulse of the chemotactic population at elevated speed $V = 1 + \eta$ because the front speed scales with the number of bacteria in the pulse [163]. This observation of band broadening might explain why in agar plate experiments testing for chemotaxis, chemokinetic species such as Sinorhizobium meliloti lack the sharp bands [142, 164], which are known to be a hallmark of chemotaxis for other species, e.g. E. coli [89, 162].

Chemokinesis confers an additional biological advantage in the form of increased population growth as can be seen in Fig. 6.6, which shows the integrated number of cells over time. At any point in time, the chemotactic population is smaller than the chemotactic-chemokinetic population. However, the chemotactic population at elevated speed, $V = 1 + \eta$, (purple curve) shows a stronger population growth than the chemotactic-chemokinetic population due to the faster travelling pulse, which is caused
Motility response augmenting chemotaxis by chemokinesis

Fig. 6.6 Population growth. The size of the populations in Fig. 6.5 is the bacterial density integrated over the simulation domain. Faster travelling waves of the chemokinetic-chemotactic population (orange curve) and the chemotactic population at constant speed $V = 1 + \eta$ (purple curve) also induce a faster population growth due to consumption of nutrients, compared to the chemotactic population travelling at $V = 1$ (blue curve). Parameters as in Fig. 6.5

by the increased swimming speed. This increase in swimming speed is associated with metabolic cost [165]. Thus, permanently swimming faster independent of the attractant concentration could be a beneficial strategy, if the metabolically available energy is not constrained by nutrient supply. When nutrient concentrations are low, on the other hand, increasing swimming speed provides no benefit to bacteria and metabolism is a limiting factor. Chemokinesis could provide an advantageous speed enhancement when it is both metabolically affordable and beneficial [139, 150]. While the situation considered in this section assumed an abundant supply of chemoeffectors (and in this case nutrients), the next section will consider the response to a transient burst of chemoeffectors.

### 6.3.3 Transient source

A localised burst of chemoeffectector may, e.g., occur in the sea if algae/phytoplankton lyse and release their content, as has been recently studied in the laboratory [166], or when marine particles exude plumes of chemoeffectector [167]. In soil, plant roots exude sugars and other potential nutrients, which locally create a high concentration of
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Fig. 6.7 Diffusing attractant from a transient source. Bacterial populations (bottom row) are attracted to source of diffusing attractant (top row). The chemokinetic-chemotactic population (orange curve) shows a faster and stronger accumulation than the purely chemotactic population (blue curve). Parameters $H = 3.5$, $K_S = 1$, $N = 0.5$, $K_X = 0.53$, $\delta_0 = 50$, $\eta = 2$, $\omega = 0.2$, $n = 1$, $S = 0.5$, $T = 0.01, 0.05, 0.64$; no bacterial growth.

chemical attractants. In the following, we consider a single strong pulse of chemoeffector originating from a finite-size axisymmetric source that dissipates via diffusion, modelling a potential dynamic environment around roots or marine particles. The attractant profile that develops is $C(R, T) = S(4\pi NT)^{-1}\exp(-R^2/4NT)$, with $S$ representing the amount of chemoeffector contained in the pulse in non-dimensional units. The attractant profile is illustrated in Fig. 6.7 (top row), while the bacteria are initially uniformly distributed in the domain at concentration 0.2. To model the response to such a transient attractant profile, we need to include the chemotactic drift velocity, Eq. (6.34e) with $\zeta > 0$, modified to account for the effective gradient perceived by bacteria as they traverse the temporally varying pulse (see section 6.2.1).

As can be seen from Fig. 6.7, bacteria with chemotaxis and chemokinesis display a faster and stronger response than those with chemotaxis alone. This strong accumulation occurs even though diffusivity close to the source is higher for these bacteria due to chemokinesis. Temporal variations in the concentration ($\sim \partial_t f_\chi$) need to be considered when modelling the chemotactic response for these transient pulses. Indeed, the run duration for a bacterium travelling up/down the gradient is $\tau^{L,R} \propto \pm (v\nabla f_\chi + \partial_t f_\chi)$
Motility response augmenting chemotaxis by chemokinesis (see Eq. (6.24)). Thus, for chemokinetic bacteria with higher \( v \), the temporal perturbation to the chemotactic bias of tumbles is reduced, increasing the accuracy of the chemotactic response. This effect contributes to the stronger accumulation of chemotactic-chemokinetic bacteria. To illustrate this further, in Fig. 6.8 we plot the number of bacteria accumulated at the attractant source, \( B_S \), which is the maximum of the bacterial profiles shown in Fig. 6.7, as a function of time for chemotactic and chemotactic-chemokinetic populations. The plot shows accumulation with \( \zeta > 0 \) (6.34e) or without \( \zeta = 0 \) accounting for the temporal perturbation to the chemotactic response. In the case of a purely chemotactic population, the predicted amount of bacteria accumulated at the source is lower for a model that ignores the temporal perturbation than for one that includes it. Chemokinesis, on the other hand, reduces the relative effect of temporal perturbation so much that there is very little difference between model predictions with \( \zeta = 0 \) and \( \zeta > 0 \).

These results suggest that a chemokinetic population might be able to overtake purely chemotactic competitors in response to a sudden nutrient release. While the difference in \( B_S \) observed in Fig. 6.8 of at most \( \sim 12\% \) may seem small, a corresponding boost to the growth rate can be sufficient to outcompete a purely chemotactic strain within a few generations. In such transient nutrient landscapes, we have further shown that chemokinesis can reduce the adverse effect that a temporal change in attractant profile can have on the chemotactic response.

### 6.4 Discussion

Chemokinesis is a known response for many environmentally relevant species of bacteria, yet its consequences for bacterial population dynamics have been little explored. Using a modified Keller-Segel continuum model we have shown how chemokinesis significantly affects both the dynamics and steady-states of bacterial populations capable of chemotactic and chemokinetic behaviour. This model incorporates the effects of a swimming speed depending on chemoattractant concentration, including an increase in the chemotactic sensitivity, and a recently suggested modification to the chemotactic response in dynamic environments [154], which was derived adapting the microscopic model first suggested by de Gennes [88].

We have solved the model numerically to explore the effect of chemokinesis on migration and accumulation in experimentally realistic gradients. In a fixed attractant gradient, simulations show that chemokinesis can lead to two subpopulations travelling at different speeds, with the slower one being held back by the chemokinetic drift in a
6.4 Discussion

Fig. 6.8 Accumulation at a transient source. The bacterial accumulation at the source is reduced due to the reduced drift $V_\chi$ if the transient nature of the attractant profile is taken into account (i.e. $\zeta \neq 0$). Chemokinesis (CK) mitigates for this effect. Parameters $H = 3.5$, $K_S = 1$, $N = 0.5$, $K_\chi = 0.53$, $\delta_0 = 50$, $\zeta = 8.164 \cdot 10^{-3}$, $\eta = 2$ ($\eta = 0$ if no CK), $\omega = 0.2$, $n = 1$, $S = 0.5$; no bacterial growth. If $V_\chi$ includes the effect of transient source, $\zeta = 8.164 \cdot 10^{-3}$, otherwise $\zeta = 0$.

manner dependent on the chemokinetic response function. In the case of agar plate migration, where bacteria inoculated onto the plate generate their own gradient by consuming nutrients, we find that populations with chemokinesis migrate out from the inoculation point in waves that are faster, but broader than purely chemotactic migrating populations. While the increase in front speed could be explained by a population at a uniformly increased swimming speed, the broadening is only observed in the chemokinetic population. It is a new feature not predicted by previous studies using agent based simulations [149]. It is possible that this broadening might explain why the classic chemotactic Adler bands observed for $E. coli$ [162] are not observed for chemokinetic soil bacteria such as $Sinorhizobium meliloti$ [142, 164]. Furthermore, chemokinesis increases the population growth significantly in comparison to purely chemotactic migration.

The simulations also considered the case of a transient source of nutrients, e.g., a lysed algal cell. In this case, the results show how bacteria with chemokinesis and chemotaxis accumulate faster and more strongly around the source, while concentrations of nutrients are high, with respect to purely chemotactic bacteria. This chemokinetic advantage is both due to the enhanced migration discussed in the previous examples, but also to the fact that chemokinesis mitigates the perturbation to the chemotactic
response due to the transient nature of the attractant profile. We note that, while the model includes the effect of transient chemical fields on tumbles, it does not include a recently modelled effect of chemokinesis on the precision of chemosensing [168]. It will be interesting to include this additional effect, which could lead to further enhancements in chemokinetic accumulations, in future elaborations of this model.

The role of metabolism is an important consideration for chemokinesis. For example, positive chemokinesis might be caused purely by an increase in nutrient availability. A resulting increase in the energy level of the cell may lead to more energy being available for flagella rotation, which allows the cell to swim faster. As the chemotactic sensitivity scales with the swimming speed as $\chi \propto v^2$, a faster swimming population will always show a stronger chemotactic drift. However, swimming is associated with a considerable metabolic cost [165, 169, 170]. Indeed, the energetic cost of swimming increases quadratically with the swimming speed [171]. Therefore, it might be beneficial to swim faster (and, thereby, improve chemotaxis) only if favourable nutrient conditions are available.

If chemokinesis was merely caused by increased energy levels, it should only be observed for chemotactants that are degradable. Most experimental studies so far have tested common metabolisable chemoattractants such as glucose, serine or pyruvate. However, there is some experimental evidence that at least some chemokinetic species show a chemokinetic response that is independent of metabolism. For example, *E. coli* showed a slightly decreased but still significant chemokinetic response to a non-metabolisable analogue of glucose, 2-deoxy-D-glucose (2DG); compared to the response to the metabolisable glucose [147]. Furthermore, *V. corallilyticus* responded to the chemical cue dimethylsulfoniopropionate (DMSP), which is enriched in coral mucus, via chemotaxis and chemokinesis with the strength being comparable to its response to mucus samples, even though the bacterium is unable to degrade DMSP [140]. In addition, *V. alginolyticus* still increased the swimming speed by approximately 40% in response to the (non-metabolisable) 2DG, while glucose elicited an increase of about 80% [149]. Thus, the metabolic state of this marine bacterium might influence the chemokinetic response but it can also be observed independently. The wild-type *R. sphaeroides* indeed showed a strong chemokinetic response to the non-metabolisable malonate in [146]. The role of metabolism was further tested with a mutant that was unable to grow on acetate. Both the wild-type and mutant showed a chemokinetic response of about 60%, further pointing towards an metabolism-independent pathway for chemokinesis in *R. sphaeroides*. The same study found effectors that elicited
either chemokinesis or chemotaxis, or both, suggesting independent pathways for both responses.

To summarise, the energy level of a cell may influence the extent of the chemokinetic response but there are several indications of metabolism-independent chemokinesis for different bacterial species. In a dynamic environment such as considered in section 6.3.3, chemokinesis can then help to reduce temporal bias and improve the chemotactic response. As pointed out by Hein et al, the threshold for detecting absolute concentration is smaller than for gradient detection [154]. Thus, chemokinesis can take place at lower background concentrations to improve chemotaxis only when needed.

The predictions of the model include interesting qualitative effects, which have not previously been observed in agent-based models: the slower subpopulation in the fixed attractant profile, and a broadening of the travelling wave in a self-generated gradient. To test these predictions experimentally, chemotaxis and chemokinesis should be addressed independently. For example, a recently developed *E.coli* system with a swimming speed that is controlled via light [127] could be used to engineer populations with a swimming speed that can be controlled independently of chemotaxis.

Future theoretical investigations would benefit significantly from the experimental measurement of the chemokinetic response function relating swimming speed and local attractant concentration. In this work we assumed a smooth change from a reference speed to an increased speed, where the degree of change in swimming speed changed with a single parameter. As we have shown, a very steep change in swimming speed (e.g. in form of a step change as assumed previously in agent-based models [148, 149]), could actually inhibit chemotaxis rather than promote it. Experimental work so far has been restricted to measurements at very few different attractant concentrations, which makes it difficult to deduce a functional relationship between speed and attractant concentration. Thus, further work is required to determine the function $v(c)$ for chemokinetic bacterial species. Such measurements would also allow elucidation of the rate of adaptation, i.e. how quickly the swimming speed adapts to its new value both for an increase and decrease in attractant concentration, and which we have here assumed instantaneous. The increase in the population-averaged swimming speed in response to a uniform addition of effector occurred on the time-scale of $100 – 200s$ for *V. alginolyticus* [149]. In [146], on the other hand, the chemokinetic response of *R. sphaeroides* was measured within 10s upon uniform addition of chemoeffectors, at which point the swimming speed had already adapted to its increased level. Furthermore, a desensitisation to a sustained higher level of attractant, as observed for chemotaxis, does not seem to occur as the swimming speed remained at elevated levels for hours in
R. sphaeroides and A. brasilense [144, 146]. The discrepancy in the order of magnitude in the response times might be caused by the experimental set-ups, e.g. in [149], the effector first needs to diffuse from two sides in a wide microfluidic channel before a uniform population response can be measured. To conclude, further experiments could shed some light on the chemokinetic response function and adaptation time, which would benefit the further development of the model and its assumptions to understand how bacteria make use of chemokinesis in dynamic environments.
The transport of active particles in complex environments is strongly influenced by their microscopic surface interactions. As a result, the non-equilibrium nature of microswimmers can lead to surprising bulk behaviour, even in the absence of many-body effects. In this thesis, using an agent-based model of ABP, I first showed that scattering rules which align the direction of motion with an obstacle surface allow active particles to traverse obstacle lattices more efficiently (chapter 2). In particular, a reflecting boundary condition, which modelled classical specular reflection, was compared to two scattering rules that preserve the major component of the particle’s direction of motion. The sliding boundary condition assumed that the particle quickly aligns its orientation with the surface of the obstacle and then traverses a fixed polar angle $\alpha$ along the circular obstacle. The stochastic slide-off boundary condition, on the other hand, assumed that the orientation of the particle only changed due to rotational diffusion while the particle travelled along the obstacle until the orientation pointed away from the surface. Interpreting obstacle-induced reorientations as tumbles, an effective RTP model reproduced the main observations made in simulations for the different scattering rules. This approach may be applied to further scattering rules or experimental measurements given that the parameters can be derived from the microscopic scattering rules directly.

When compared to simulations, the RTP model only failed to reproduce the guiding effect observed at high obstacle densities, which was captured by a simple deterministic model. Based on the sliding boundary condition, flights in lattice channels were defined by a sequence of leaving angles. This sequence predicted stable flights for certain values of the polar angle $\alpha$, which corresponded to overshoots in the simulations compared to the RTP model. While the importance of wall accumulation for the formation of
biofilms is often stressed in the literature, RTP and deterministic model showed that transport in complex environments can also benefit from surface alignment.

The biological process of tumbling is used by bacteria such as *E. coli* to bias their random walk up a chemical gradient. Since the ability to bias the motion in a certain direction depends on the ability to move in any direction, chemotactic transport decreases together with diffusive transport in porous media. However, the magnitude of decrease can vary for the two transport processes as shown in chapter 3. A large reorientation at obstacles, such as in the reflecting boundary condition, reduced the chemotactic drift more strongly than expected purely from the diffusive transport because the chemotactic response relies on an internal memory. Boundary conditions that retain the major component of velocity, on the other hand, enabled the cell to respond to chemical gradients even at very large obstacle densities. Yet the specific features of the scattering rules used were important here as well since the magnitude of the chemotactic drift changed significantly if tumbling was suppressed at the obstacle.

Having established the importance of gaining insight into the microscopic scattering rules, the results presented in chapter 4 provided the first experimental contribution toward understanding bacterial scattering at convex surfaces and its contribution to diffusive transport in ordered porous media. The microscopic analysis developed in this work allowed the analysis of scattering events as a function of collision angle without the need for optical tweezers, as used to study wall entrapment in [31]. Optical tweezers could offer more control over the orientation and distance of the cell from an obstacle but also require a more complex experimental set-up. The approach taken here instead determined the impact parameter of trajectories, which is the *y*-component of the collision point where the rotated particle track meets the obstacle and, thus, relates to the collision angle. Tracks with similar impact parameters were then grouped together to analyse the scattering interaction as a function of the impact parameter. This approach yielded a distribution of impact parameters which was peaked towards large impact parameters, i.e. small collision angles. Indeed, this distribution illustrated the potential role of hydrodynamic effects as it suggested a strong alignment with the surface of the pillar prior to impact, which has not been previously observed in the case of wall entrapment. After impact, trajectories of smooth-swimming *E. coli* showed that the residence time decreased with decreasing collision angle, while the polar angle traversed on a pillar only showed a small dependence on either collision angle or pillar radius. Thus, the results strongly indicated a speed reduction at the obstacle as a function of the angle of approach, which could be caused by either steric or hydrodynamic effects. However, given the non-zero reorientation for the
smallest collision angles and the approximately constant polar angle, neither steric nor hydrodynamic models seemed sufficient to explain the observed scattering behaviour on their own, so there is a clear need for further theoretical studies. For example, mesoscale hydrodynamic studies that take into account the finite size of the bacterium as well as flagellar arrangements such as in [38] could provide valuable insights into the scattering process. In addition, three-dimensional imaging techniques (e.g. holographic imaging [31]) could systematically analyse the position and orientation of the bacterium during the scattering process.

The comparison between wild-type and smooth-swimming *E. coli* offered the first insight into the process of tumbling close to a convex surface. Tumbling reduced the time spent at an obstacle for large collision angles but the effect seemed negligible for small collision angles. Tumbling events may either be less common or simply have less influence at small collision angles. However, it was not possible to study the tumbling process at an obstacle directly as the flagella had not been stained. Thus, further experimental investigations are needed to elucidate whether obstacles have any influence on the frequency of tumbling. The results of those studies would allow to refine the theoretical models developed in chapter 3, which showed that a suppression of tumbling close to an obstacle impacts both diffusive and chemotactic transport. Overall, the experimental results presented here in combination with the theoretical analysis in chapter 2 and chapter 3 highlight the importance of a debate around the microscopic scattering rules used in (computational) studies of active particles in complex environments. While volume-exclusion is a convenient assumption for modelling active particles in a Langevin framework, it may not be realistic for bacteria as shown in chapter 4. Yet chapter 2 and 3 showed that different assumptions about the scattering rules can lead to different predictions for the macroscopic transport.

A non-uniform environment can introduce heterogeneity in the motility parameters and, thereby, in the distribution of active particles. As a first example of such an environment, chapter 5 considered a gradient in obstacle density. In simulations, the mean position of a collection of (non-interacting) ABP drifted to high obstacle density because of the reduction in swimming speed at an obstacle caused by the non-classical surface interaction. Preliminary experiments using microalgae as model active particles in microfluidic channels showed promising agreement with the theoretical prediction. Further experiments should aim to gather the dynamic evolution of the population density in lattices with different obstacle sizes and density gradients. As the results here contrast with a study using ameboid cells [131], a gradient in obstacle lattice might be an interesting example to probe differences between different types of active
particles and the underlying dynamics causing them. In addition, the results of chapter 5 underlined the importance of choosing appropriate domain boundary conditions when studying transport of active particles.

Bacteria may also control their swimming speed independently by chemokinesis. Chapter 6 modified the well-known Keller-Segel model to include this attractant-dependent change in swimming speed. While pure chemokinesis leads to an accumulation at low attractant concentrations, chemokinesis in combination with chemotaxis can enhance the chemotactic response and transport. Depending on the steepness of the chemokinetic response function, chemokinesis may in addition modify the chemotactic response qualitatively. In particular, I showed that population waves travelling in self-generated gradients may broaden because of speed variations, while subpopulations propagating at different speeds can develop in steady linear attractant gradients. Furthermore, chemokinesis could serve as an efficient adaptation to dynamic environments by reducing the bias in the gradient estimation introduced by a temporally varying attractant field.

As with any research, caution needs to be exercised when attempting to generalise the conclusions drawn from the present study. For instance, results may change with the architecture of the porous medium as bacteria migrated in a sequence of trapping and hopping in jammed packings of hydrogel particles [92]. Notwithstanding this caveat, the reductionist approach chosen in this thesis lends itself to the investigation of when and how the transport phenomena change upon modifying the environment. Agent-based simulations and microfluidics offer a versatile toolbox to gradually increase the complexity of the studied environment. For instance, the complexity of the porous medium can be increased easily by random placements of obstacles, or obstacles of different shape and size in both simulations and experiments. Aside from the porous medium itself, the effect of chemotaxis on the time spent traversing a porous medium may depend on the type of attractant gradient [172, 173]. In microfluidic experiments, chemical gradients could be established by dissolving attractant in PDMS or using source and sink channels. The gradients established by those methods will differ, and could simulate different environmental distributions of attractant, e.g. chemicals dissolved in groundwater vs. bound to soil particles.

Biophysical research benefits from the crosstalk of experiments and theory. Experiments can give tangible evidence and motivate further theoretical studies. Both analytical as well computational studies could help to answer questions raised by the experiments in chapter 4. On the other hand, theoretical studies can be used to pinpoint differences in biological systems. While some active particles (e.g. the
microalgae in chapter 5) experience a drift in a gradient of obstacle density that can be explained by a density-dependent swimming speed, other active particles may respond to a density gradient with a genuine taxis.
REFERENCES


Appendix A

Distribution of reorientation angles $\psi$

Fig. A.1 Distribution of reorientation angle $\psi$ depends on impact parameter $b$ for *E.coli* $\Delta$cheY. Histogram for trajectories with impact parameter (a) $0.0 \leq b < 0.051$ or (b) $0.867 \geq b < 0.918$. The distribution of the reorientation angle $\psi$ shifts to smaller values with increasing impact parameter, thereby leading to the decrease in mean value shown in the main text in chapter 4. The samples for $R = 16\mu m$ were combined.
Fig. A.2 Distribution of reorientation angle $\psi$ depends on impact parameter $b$ for *E.coli* ΔcheY. Histograms for trajectories with different impact parameters (colour-coded) were smoothed using a moving average filter to improve presentation. The distribution of the reorientation angle $\psi$ shifts to smaller values with increasing impact parameter $b$, thereby leading to the decrease in mean value shown in the main text in chapter 4. The samples for $R = 16 \mu m$ were combined.
Stock solutions were prepared and kept separate. The Phosphate Buffer contained 10.8g of K$_2$HPO$_4$ and 5.6g of KH$_2$PO$_4$ per 100mL distilled water. Solution A was a salt solution made up of 15g NH$_4$Cl, 4.0g MgSO$_4$ · 7H$_2$O and 2.0g of CaCl$_2$ · 2H$_2$O per 500mL water. The 1M TRIS stock was prepared by first dissolving 121.14g of TRIS base in 600mL water. The pH was then adjusted to 7.2 by titration with concentrated HCl. Water was used to reach 1L, and the pH was checked and - if necessary - again adjusted. For 1L of TRISmin media, 20mL of 1M TRIS base (titrated to pH 7.2), 25mL of Solution A, 1mL each of Hutner’s trace elements (see Table B.1) and 1mL of Phosphate Buffer were combined. Water was added to reach 1L and the solution was autoclaved.

For the TAP medium, 2.42g/L of Tris base were weighted in and dissolved in 250mL diWater. Then, 25mL of Solution A, 1mL each of Hutner’s trace elements (see Table B.1), 1mL of Phosphate Buffer and 1mL of glacial acetic acid (17.4mM acetate) were added. Water was used to reach 1L, and the pH was checked and - if necessary - adjusted to 7.2. To prepare solid medium, 1.5g of agar per 100mL of medium were used.
Table B.1 Hutner’s Trace Elements.

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<thead>
<tr>
<th>No.</th>
<th>Components</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EDTA – Na₂</td>
<td>25mM</td>
</tr>
<tr>
<td>2</td>
<td>(NH₄)₆Mo₇O₂₄</td>
<td>28.5µM</td>
</tr>
<tr>
<td>3</td>
<td>CuCl₂ · 2 H₂O</td>
<td>2mM</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td>2mM</td>
</tr>
<tr>
<td>4</td>
<td>ZnSO₄ · 7 H₂O</td>
<td>2.5mM</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td>2.75mM</td>
</tr>
<tr>
<td>5</td>
<td>MnCl₂ · 4 H₂O</td>
<td>6mM</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td>6mM</td>
</tr>
<tr>
<td>6</td>
<td>FeCl₃ · 6 H₂O</td>
<td>20mM</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td>22mM</td>
</tr>
<tr>
<td></td>
<td>Na₂CO₃</td>
<td>22mM</td>
</tr>
<tr>
<td>7</td>
<td>CoCl₂ · 6 H₂O</td>
<td>7mM</td>
</tr>
</tbody>
</table>

The solutions are kept separate. 1mL of each added for 1L of media.