Quantitative characterization of chemorepulsive alignment-induced interactions in active emulsions

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The constituent elements of active matter in nature often communicate with their counterparts or the environment by chemical signaling which is central to many biological processes. Examples range from bacteria or sperm that bias their motion in response to an external chemical gradient, to collective cell migration in response to a self-generated gradient. Here, in a purely physicochemical system based on self-propelling oil droplets, we report a novel mechanism of dynamical arrest in active emulsions: swimmers are caged between each other’s trails of secreted chemicals. We explore this mechanism quantitatively both on the scale of individual agent-trail collisions as well as on the collective scale where the transition to caging happens as a result of autochemotactic interactions.

INTRODUCTION

Motile micro-organisms have evolved to sense their environment and react to it, e.g. by reorientation. They sense the gradients of an external stimulus or a field in their surroundings and migrate up or down that gradient; a behavioral response called taxis. If the gradient is formed in the concentration field of a chemical species, and the organism responds to it in the form of a change in their propulsion directionality/strategy, they have shown chemotaxis.

In single cellular organisms, chemotaxis guides many processes like colony migration [1] or biofilm formation (cf. Dictyostelium or Physarum). The response of an organism to a chemical gradient that is produced by the microorganisms themselves, is called autochemotaxis. Whether the chemical species is a chemottractant or a chemorepellent the system displays positive or negative autochemotaxis, respectively. Existing studies are usually based on attractive signaling; however, repulsive signaling is also of practical importance, e.g. if a colony wants to explore space efficiently by mutual avoidance.

Autochemotaxis in colonies of living organisms causes complex behavior governed by an interplay between physical effects and biological processes [2, 3]. One way to understand the underlying mechanism for the emergence of these complex behaviors is to untangle the biological effects and the physical mechanisms. To this end, the rapidly growing field of artificial active matter is attempting to design and develop synthetic micro-swimmers that can mimic for example chemotaxis strategies using principles of non-equilibrium physics. Self-phoretic particles are the broadly-studied example of artificial micro-swimmers that locally generate a chemical gradient and drift in this self-made gradient. It has been shown that suspensions of these particles exhibit non-trivial dynamics influenced by autochemotaxis [4–8]. We have previously demonstrated that self-propelling droplets [9] can be used as a model system for repulsive chemical signaling [10, 11]. The droplets leave behind a trace of ‘used fuel’ which acts as a chemorepellent to other droplets, where the the motion of such a droplet is affected by the previous passage of another droplet. The phenomenology of the system has similarities to the collective chemotaxis of trail-following bacteria [12, 13], except that the interactions with the slow chemicals are repulsive rather than attractive.

In this study, we present a quantitative analysis of individual ‘delayed collisions’ and compare it to an analytical model based on time lag, angle of incidence and chemical coupling strength. We show that these parameters determine whether a droplet can go through the trail laid out by another active droplet or bounce back from it. In the second part of the study, we demonstrate how individual binary collisions lead to a novel state of autochemotactic arrest in swimmer ensembles, a kind of ‘history caging’, where droplets are temporarily trapped in an evolving network of repulsive trails.

METHODS AND GENERAL SWIMMING BEHAVIOUR

Unless noted otherwise, all experiments were done in microfluidic cells using a quasi 2D Hele-Shaw geometry, with a typical cell area of 75×50 mm² and height of 50µm and observed using bright field or fluorescent microscopy. The active emulsions we used consisted of microdroplets of the oil CB15 with a diameter \( d_{\text{drop}} = 50 \mu m \) placed, at low number densities, in an aqueous, supramicellar solution of the surfactant TTAB (at 5wt.%, unless otherwise stated, with CMC = 0.13wt.%). If the surfactant concentration in the bulk medium exceeds a threshold value the droplet spontaneously undergoes a dynamical instabil-

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FIG. 1. Visualization of the chemical trail. (a) Schematic of the experimental setup for fluorescent microscopy of the filled micelle trail. (b) A micrograph of the chemical trails made by droplets under fluorescent microscopy. We used a higher surfactant concentration (15 wt.%) to increase the solubilization rate and thereby the trail fluorescence. (c) Zoomed view of the panel (b). AA' is the cross-section at which the intensity profile was quantified. (d) The schematic for the propulsion mechanism of the droplet. The black arrow shows the direction of motion. (e) The flow field generated by Marangoni flow at the droplet interface visualized via streak lines of 0.5 µm tracer particles. (f) The time evolution of light intensity profiles along AA' superimposed with the Gaussian fits. (g) The peak values of intensity profiles versus time. To account for the initial shape of the trail intensity profile, we model it with a linear chemical source that was released and started diffusing 20 seconds (obtained from fitting) before the passage of the droplet (see Methods). Scale bars show 50 µm.

ity which breaks the symmetry and propels the droplet at a speed $V \approx 25 \mu$m/s. The underlying propulsion mechanism [9, 14] can be summarized as follows: The oil droplets gradually solubilize, with oil molecules migrating into surfactant micelles. Since the interfacial surfactant density is coupled to the respective local densities of oil-filled and empty micelles, the trail of oil-filled micelles in the wake of a moving droplet locally increases the interfacial tension (Fig. 1 d), thus creating a self-sustaining Marangoni stress at the interface that pushes the droplet forward.

To visualize the chemical trail we added the hydrophobic fluorescent dye NileRed to the oil phase [15]. As the dye co-migrates with the oil into the micelles, we can obtain and quantify the distribution of oil field micelles from fluorescent microscopy (Fig. 1 a,b). Fig. 1 c, d and e respectively, present the zoomed-in view of a fluorescent image, the schematic of the propulsion mechanism, and the streak lines of tracer particles that visualize the flow generated by the droplet (in droplet reference frame). We note that, for illustration purposes, the micrographs in Fig. 1 b,c and Fig. 5 a were recorded at a higher surfactant concentration of 15 wt.%, to increase the solubilization rate and thereby the trail fluorescence. However, a lower concentration yields very ballistic droplet motion and long cruising ranges and is therefore preferable in quantitative experiments. To model the trail diffusion, we approximate the droplet as a moving point source emitting chemorepellent at a constant rate. Accordingly, the chemorepellent profile along a line perpendicular to the trail (e.g. AA’ in Fig. 1 f inset), represented by the fluorescence intensity, should be Gaussian with a peak height scaling with $t^{-1/2}$. We have plotted Gaussian fits of the time dependent recorded intensity along AA’, as well as the respective amplitudes from the fits in Fig. 1 e,f, with excellent agreement, validating both the Gaussian distribution and $t^{-1/2}$ decay, and yielding a diffusion constant for filled micelles $D_{fm} = 52.5 \mu$m²/s. This is consistent with our observations from previous studies [10] and common literature values for micellar diffusion (for details of the fitting please see Supp. Mat.). Based on this highly regular behaviour, we can in the following extract a chemical gradient map for the entire experiment by tracking the motion of all droplets in the system and treating them as chemorepellent point sources, even without recording micellar fluorescence. Using the experimentally-obtained quantities we can estimate the diffusive time scale of the trail decay: $\tau_{diff} = a_{drop}^2 / D_{fm} \approx 24$ s, which is quite long due to the large size of the filled micelles ($\approx 5$nm), and remarkably longer than the droplet’s advective time scale $a_{drop}/V \approx 2$ s. Such long-living chemical gradients can change the chemical potential landscape and influence the propulsion dynamics of other droplets even without direct hydrodynamic interaction. Beyond the advective timescale, we can therefore model droplet-trail interactions by a Chemically Active Polar Particle model approach [16], as follows.
CHEMICALLY ACTIVE POLAR PARTICLE MODEL

The fore-aft flow at the interface is determined by the gradient in the local concentration of the empty micelles. We assume that, in the steady swimming state, the fore-aft asymmetry of the droplet is stably maintained and thus the swimming droplet can be modelled as a chemically-active polar particle [16, 17]. Thus, when a droplet encounters a trail, it experiences an effective torque governed by a coupling constant Ω, and an effective force with coupling α. We note that the general theory of chemically-active polar particles also allows for an effective force projected along the axis of the particle, with a third coupling constant, but we will neglect this possibility here as the data can be explained within the more minimal model with two coupling constants.

We define droplets to have a position \( \mathbf{r} \) and orientation \( \mathbf{n} \) and to move in the \((x, y)\) plane. The Langevin dynamics for \( \mathbf{r} \) and \( \mathbf{n} \) are as follows [16]:

\[
\begin{align*}
\dot{\mathbf{r}} &= V_0 \mathbf{n} - \alpha \nabla c + \sqrt{2D_t} \xi_t, \\
\dot{\mathbf{n}} &= \Omega \mathbf{n} \times (\mathbf{n} \times \nabla c) + \sqrt{2D_r} \mathbf{n} \times \xi_r,
\end{align*}
\]

where \( \xi_t \) and \( \xi_r \) represent Gaussian distributed translational and rotational noise with unit strength, and \( D_t \) and \( D_r \) are the corresponding translational and rotational diffusion coefficients of the droplet. Analysis of the mean squared displacement of the droplets in dilute conditions reveals ballistic trajectories over periods larger than 100 s (see Fig. 5(b)), suggesting that Brownian translational diffusion is negligible, and that the rotational diffusion coefficient is also small, with an upper bound of \( D_r = 0.01 \text{ rad}^2/\text{s} \) corresponding to persistence lengths larger than \( V_0/D_r \approx 2.5 \text{ mm} \).

We model the micelle trail as a chemical field \( c \) with Gaussian profile perpendicular to the direction of motion, with a width that depends on the time elapsed since the preceding droplet moved away from the point of interest. Without loss of generality we assume the preceding droplet to propel along the \( x \)-axis, so that the chemical field is given by

\[
c(y) = \frac{c_0}{\sqrt{4\pi D_{\text{fm}} \Delta t}} \exp\left(-\frac{y^2}{4D_{\text{fm}} \Delta t}\right),
\]

where \( D_{\text{fm}} \) is the diffusion coefficient of the (filled) micelles. Because the diffusion of the micelles is slow compared to the propulsion velocity of the droplets \( (D_{\text{fm}}/(\Delta V_0^2) \ll 1) \), we can neglect the \( x \)-dependence of the concentration profile, as well as its time-dependence, so that we can use (3) to describe the interaction of the incoming droplet with the trail.

We thus solve the following deterministic equations in 2D for the incoming droplet’s position \((x, y)\) and orientation \( \theta \), which is defined as the angle between \( \mathbf{n} \) and the \( x \)-axis (see Fig. 2d):

\[
\begin{align*}
\dot{x} &= V_0 \cos \theta, \\
\dot{y} &= V_0 \sin \theta + \frac{\alpha c_0 y}{\sqrt{2\pi(2D_{\text{fm}} \Delta t)^{3/2}}} \exp\left(-\frac{y^2}{4D_{\text{fm}} \Delta t}\right), \\
\dot{\theta} &= -\frac{\Omega c_0 y}{\sqrt{2\pi(2D_{\text{fm}} \Delta t)^{3/2}}} \exp\left(-\frac{y^2}{4D_{\text{fm}} \Delta t}\right) \cos \theta.
\end{align*}
\]

EXPERIMENTAL QUANTITATIVE ANALYSIS OF DROPLET-TRAIL INTERACTIONS IN 2D

We experimentally studied individual droplet-trail interactions by placing droplets at low number densities in aqueous surfactant solutions in shallow Hele-Shaw microfluidic reservoirs. We recorded and analysed the droplet trajectories via video microscopy and mined the data for trail interactions (see Supp. Mat., section S1 S1.3 for criteria).

Fig. 2 shows examples of crossing and reflecting interactions and the extracted data for the following droplet’s signed trail distance \( d \), speed \( V \) and orientation \( \theta \) (as shown in panels e and f for the interactions in b and c).

Since our model assumes the preceding droplet to move in a pristine medium with isotropic chemical field, we selected interactions where its motion that can locally be well approximated by a straight line (red trajectories in Fig. 2a,b), and where we assume the chemical gradient in the trail to evolve according to Eqn. 3.

The droplet approaches the trail at an incident angle \( \theta_{\text{inc}} \) with respect to the first trajectory (the blue trajectory in Fig. 2a). An interaction starts and ends when the distance \( |d| \) between the droplet and the trail falls below a threshold value \( d_{\text{max}} = 220 \mu\text{m} \). We identify the points of intersection (green points in a and b), or, for reflection, closest approach on each trajectory, and define the time lag \( \Delta t \) as the interval between each droplet passing these points, and the time origin \( t_0 \) as the respective point in time for the following droplet. We note that for non-specular reflections with \( \alpha_0 \neq 0 \) (Eqn. 2) the time of maximum rotation rate, \( t_{\text{turn}} = |d\theta/dt|_{\text{max}} \), should be slightly delayed with respect to the time of closest approach.

We observe that the probability of crossing versus reflection depends both on \( \theta_{\text{inc}} \) – for a shallow angle of incidence, the required turning rate for reflection is lower – and on \( \Delta t \), which determines the gradient strength (Eqn. 3). We illustrate this in Fig. 2a,b: we observe, for similar \( \theta_{\text{inc}} \), a transition from reflection to crossing with increasing \( \Delta t \). As shown in panels (d) and (f), there is also evidence of non-specular reflection, as well as the following droplet slowing down and speeding up around \( t_0 \), suggesting a negative \( \alpha_0 \).

To derive a phase diagram for crossing and reflection in \((\Delta t, \theta_{\text{inc}})\) space, we will now proceed to a quantitative estimate of the model’s parameters by numerical fits to our recorded data.
FIG. 2. Autochemotactic interaction between droplets. (a) The fluorescent micrographs of crossing and reflecting interactions. The red trajectory corresponds to the first passing droplet (secreting the trail) and the blue trajectory corresponds to the second droplet. (c) and (d) data from bright field microscopy, typical trajectories of crossing and reflecting interactions. (e) and (f) Plots of distance, swimming speed and rotation rate (angular velocity) for the interactions in (c) and (e), respectively. All scale bars are 50 µm.

FIG. 3. Three example fits of the chemically-active polar particle theory to experimental trajectories, where each column corresponds to a different trajectory. Two fit parameters ($\Omega_0$ and $\alpha_0$) were adjusted. The blue lines correspond to the resulting best fit for the given trajectory, whereas the red lines are the theoretically predicted trajectories using the median values of all fits analyzed, $\Omega_0 = 7 \cdot 10^3 \mu m^2/s$ and $\alpha_0 = 3 \cdot 10^4 \mu m^3/s$.

FITS OF MODEL AND EXPERIMENT DROPLET-TRAIL INTERACTIONS

We fit the theoretical trajectories obtained from numerical solution of (3–6) to the experimental trajectories in order to obtain estimates for the two unknown coupling constants $\Omega_0$ and $\alpha_0$, which were used as fitting parameters. For the diffusion coefficient of the micelles, we used the measured value $D_{mf} = 52.5 \mu m^2/s$. The droplet velocity $v$ was estimated as the average velocity in the given trajectory, and the experimentally-measured time lag $\Delta t$ was used as an input. Initial conditions for the time evolution of $(x(t), y(t), \theta(t))$ were obtained from the data as described in the previous section: $y(0) \approx 200 \mu m$ corresponds to the initial value of the signed distance $d_{max}$, $\theta(0)$ to the incidence angle, and $x(0) = 0$ without loss of generality.

For our analysis of the fits, we focused on sharp reflection events with $\theta_{inc} > 60^\circ$, for which our minimal model of the trail as a static Gaussian with constant width is best justified (for low incidence angles, interactions occur over longer times and a wider range of $x$-values, which may affect the validity of our approximation and introduce differences between parallel and antiparallel reflection events). The 54 reflection events were fitted and ordered from best to worst fit according to the fit error. By analyzing the median values of $\Omega_0$ and $\alpha_0$ calculated from the $n$ best fits as a function of $n$, we found that the median values stabilize at $\Omega_0 \approx 7 \cdot 10^3 \mu m^2/s$ and $\alpha_0 \approx 3 \cdot 10^4 \mu m^3/s$ between $n \approx 20$ and $n \approx 40$. Smaller $n$ values are susceptible to the noise due to small number statistics, whereas for larger $n$ we would include bad fits that skew the distribution, presumably corresponding to non-ideal trajectories (e.g. interactions with curved trails or measurement artifacts due to global drift in the chamber). Details can be found in Fig. S4 of the
Supplement.

Examples of the fits are shown in Fig. 3 for three experimental trajectories. Here, blue lines correspond to the actual fit to the given trajectory, whereas red lines correspond to the theoretical prediction using the values $\Omega_0 = 7 \times 10^3 \, \mu m^2/s$ and $\alpha_0 = 3 \times 10^2 \, \mu m^3/s$. The characteristic features of the evolution of the position, velocity, and angular velocity with time are well recapitulated by the model, both for each particular fit and when using the median values. Importantly, $\Omega > 0$ implies that the droplet reorients to point away from the trail, whereas $\alpha > 0$ implies that the droplet is also directly repelled by the trail. While we found that the trajectory shapes are most sensitive to changes in $\Omega$, which is the key parameter governing the interaction, the presence of a positive $\alpha$ is essential in order to correctly capture the time evolution of the droplet velocity (second row in Fig. 3), which decreases before the turning point and increases after it.

**PHASE DIAGRAM USING RESULTS OF THE FIT**

We determined $\theta_{inc}$ and $\Delta t$ for all selected interactions and plotted them in the phase diagram in Fig. 4 a, with reflections marked by white and crossings by black data points (164 reflection and 90 crossing events). We note that we do not distinguish between parallel and antiparallel interactions – for $\theta_{inc} > 90^\circ$, we convert to $180^\circ - \theta_{inc}$. The background colour map interpolates the maximum rotation rate during the particle-trail interaction $|d\theta/dt|_{\text{max}}$ as obtained by fitting to the white (reflection events) and green (crossing events) points. The dashed line is the boundary between the crossing and reflecting events predicted by the theoretical model (including the uncertainty caused by rotational diffusion).

**COLLECTIVE DYNAMICS GOVERNED BY AUTOCHEMOTACTIC INTERACTIONS: HISTORY CAGING**

In a more crowded system, moving droplets trace out a network of diffuse particles, leading to frequent reorientations, and causing a dynamically evolving chemical potential landscape. This causes droplets to get transiently trapped in the interstitial spaces of the trail network.

To study the collective dynamics in 2D, we placed suspensions of swimmers at number densities between $n \approx 0.025$ and $8.58 \, \text{nm}^{-2}$ in a quasi-2D Hele-Shaw cell and recorded their trajectories for long times ($\approx 5 \, \text{min}$). We first illustrate the trapping behaviour by snapshots from a fluorescently dyed sample in Fig. 5 a. We follow one swimmer by marking its trajectory in cyan. The first image was chosen shortly after the addition of the droplets, hence, the experiment starts with almost no secreted trails. Initially, all droplets move persistently, and their first reorientation is a result of the first encounter with a trail. As they travel the secreted trails gradually form a network of trails. A potential landscape based on $Vc$, evolves, with local minima (dark regions) between the trails. The swimmers get dynamically arrested by multiple reflections at the walls of these transient cages – escape is possible only when $Vc$ has decreased sufficiently at the boundaries or when the chemical buildup caused by the droplet itself forces it out of the cage.

Fig. 5 b shows, for increasing number densities, the mean squared displacement profiles obtained by ensem-
ble averaging over the trajectories,

$$\text{MSD}(t) = \frac{1}{N} \sum_{i=0}^{N} (r_i(t) - r_i(t_0))^2,$$

where $t_0$ is the starting moment of the experiment and $N$ the number of droplets. For any number density, droplets initially undergo ballistic propulsion ($\text{MSD} \sim t^2$). For a single droplet (no interactions), we do not see a transition to diffusive scaling. For intermediate number densities, we observe a change in the slope of the MSD profiles, which is associated with the reorientations caused by the autochemotactic interactions. At large number densities (150 droplets and more), after a short ballistic period, the MSD reaches a plateau. This plateau is reminiscent of the caging signature of in the MSD for colloidal glasses [18], however, here the caging is caused by trail-droplet instead of direct droplet-droplet collisions and is therefore observed at much lower volume fractions ($\phi_{\text{droplets}} \approx 10^{-2}$, cf. [19]). For more crowded systems, the crossover to caging happens earlier, the lifetime of the cage is longer, and the cage size is smaller. The plateau is followed by a crossover to a third, subballistic regime caused by consecutive caging events. We have illustrated this for an example trajectory in Fig. 5 c, for a droplet that undergoes three caging events in a very crowded system (300 droplets). The droplet trajectory is mapped by circles colour coded by time in the experiment. To highlight the cage formation by spatiotemporal density fluctuations, we plotted in the image background, using the same colour code, all droplet positions recorded within a window of $d < 220 \mu m$ and $0 < \Delta t < 50s$ around the current droplet position. Entering an area with increased density, the droplet reorients frequently, staying in place until it is ejected by the chemical buildup into a less populated space, where it proceeds ballistically until it encounters the next high-density location (‘cage escape’). Since ballistic runs are uncorrelated, the long-time dynamics presumably correspond to a Gaussian random walk, however, it is not feasible to reliably quantify the respective exponent in the MSD due to the limited lifetime of the droplets and the finite size of the experimental cell.

**EXTENDING THE SYSTEM TO 3D**

One intriguing feature to study is how far dimensionality matters in caging effects. While, in two dimensions, fully confining cages exist even for point-like particles and one-dimensionally parameterized trajectories, in three dimensions the cage would always have holes the droplet can escape through. Caging in 3D is therefore only possible owing to the finite volume of the diffusing trail.

To investigate caging in 3D, we studied droplet ensembles in unconfined, force free bulk media. We eliminated the effect of gravity, matching the density of the swimming droplets with the aqueous surfactant solution by adding heavy water, D$_2$O. We observed dyed droplets using a scanning light sheet fluorescence microscope over a volume of $3 \times 3 \times 3 \text{ mm}^3$.

To validate the diffusive spreading of the trail in 3D, we first captured the time-dependent fluorescence in the trail of a droplet sedimenting under gravity along the light sheet normal, taken at a fixed sheet position in $z$ (Fig. 6 a). We extracted the fluorescence intensity profile $I(x, t)$ of a cross section of the trail along the line AA’ (Fig. 6 c), as shown in the false-colour sample image in Fig. 6 b. The peak values $I_{\text{peak}}$ scale with $t^{-1/2}$ (inset), similar to the 2D behaviour analyzed in Fig. 1.

Next, to study the collective dynamics in 3D, we recorded the 3D trajectories of droplets in active emulsions over long times. Fig. 6 d illustrates the simultaneous tracking for an experiment at intermediate number density, $n = 8 \text{ mm}^{-3}$, showing a 3D reconstruction of all droplet positions at an arbitrary time in the experiment, superimposed with trajectories for the droplets we were able to track for sufficiently long times ($\approx 5 \text{ min}$). We have plotted typical trajectories for systems with different number densities in Fig. 6 e-g. In the dilute case ($n = 2 \text{ mm}^{-3}$), the trajectory is quite straight, while in the system with intermediate number density, the droplet experiences a few reorientation events. The trajectory for the dense system shows alternating quasi-ballistic and caged sections similar to the dynamics in 2D (Fig. 5 c).

In Fig. 6 h, we plotted the mean squared displacement of a set of 3D trajectories of sufficient length extracted from the data sets used for Fig. 6 e-g. The signatures of caging can be observed in the form of plateaus in the MSD profiles (in particular for the $n = 22 \text{ mm}^{-3}$ case). These observations suggest that even in 3D, the droplet can get trapped in an evolving chemical potential landscape created by the trails of other swimmers.

**CONCLUSION AND OUTLOOK**

In conclusion, we used dilute active emulsions as artificial systems to explore the collective dynamics of active particles governed by negative autochemotactic interactions. Based on fluorescent imaging, we visualized and measured the diffusion coefficient of the filled micelle trails left in the wake of the droplets; a quantity necessary for theoretical modeling of the interactions. We quantified these autochemotactic interactions and showed that whether a droplet crosses or is reflected by a trail can be predicted by the time lag between droplet passages and the angle of incident.

We further explored the collective dynamics in active emulsions and observed a novel dynamical arrest mechanism: transient autochemotactic caging. We extended our system to 3D and showed that caging also happens in unconfined suspensions of active droplets. Interestingly, caging already happens at significantly lower volume fractions ($\phi \approx 10^{-4}$), compared to the 2D case. Apart from dimensional effects, one contributing factor
FIG. 5. 2D caging. (a) Snapshots from fluorescent microscopy of an active emulsion in a Hele-Shaw cell (droplet size 50 µm). The experiment was conducted at high droplet solubilization rate to increase trail visibility. One sample trajectory is plotted to show the evolution from ballistic propulsion to a caging event. The scale bar is 1 mm. (b) Mean squared displacement profiles for emulsions with different number densities. The ★ denotes the cross-over to the caging regime. The ★ denotes the cross-over to the third regime (cage-escape). (c) A typical trajectory $s(t)$ undergoing several caging and cage-escape events (thick symbols). Thin symbols: other droplets approaching within a spatiotemporal window of $d < 220$ µm and $0 < t_0 - t < 50$ s around the current trajectory point $s(t_0)$. The colormap represents the time.

might be that the droplet motion in 3D is not rectified by the cell boundaries and that reflection is therefore already effected by weaker gradients. This question, however, cannot be resolved yet without further quantitative modelling of the coupling parameters.

While it is in principle feasible to treat individual droplet-trail interactions including hydrodynamic feedback [20], the chemically-active polar particle model does a very good job in capturing essential features of the collision behaviour: Due to the slow trail diffusion, our experiments show that reflection is still frequent after the flows caused by the leading droplet have decayed, and statistically the majority of observed interactions would happen when there is no advective contribution from the leading droplet. This makes the collective behaviour far more accessible to numerical and analytical modelling by using a similar simplified paradigm.

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FIG. 6. Extending the system to 3D. (a) The schematic of trail visualization in 3D. The green sheet represents the laser light which scans the trail at different heights obtaining images like (b). (c) The temporal evolution of intensity profiles obtained by laser sheet measurements. The inset shows the peak values versus time. The dashed line is a fit to the data to show the scaling with $t^{-1/2}$. (d) The 3D distribution of the droplets obtained by reconstruction of the scanned images superimposed with the trajectories. (e–g) Typical 3D trajectories of a droplet in emulsions with different number densities (2, 8, and 22 mm$^{-3}$, respectively). The background shows the 3D distribution of the droplets in the emulsion at an arbitrary time during the experiment. The ending points of the trajectories are denoted by the star symbol. (h) Mean squared displacement profiles for emulsions with different number densities.
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S1. MATERIALS AND METHODS

S1.1. Materials and characterisation

Our oil phase consists of CB-15, an isotropic isomer of the common nematic liquid crystal oil 5CB. Monodisperse droplets (size approx. 50 µm, exact values to be added) are mass produced in microfluidic flow junctions. For the purpose of chemical quantification, we dissolved small amounts of the fluorescent dye Nile Red in the oil phase. Nile Red does not fluoresce in water, such that we can assume the fluorescent intensity to stem from micelles filled with oil and co-migrating Nile Red. This assumption is supported by the diffusive spread of fluorescence in the trail mapped in Fig. S1. The swimming medium is 5wt% aqueous solution of TTAB surfactant. For force free bulk measurements, the density of 5CB ($ρ_{5CB} = 1.05 \text{ g cm}^{-3}$) was matched by appropriate heavy water substitution ($ρ_{D2O} = 1.2 \text{ g cm}^{-3}$). Microfluidic Hele-Shaw cells were made from the photoresist polymer SU8 by photolithography.

S1.2. Micellar diffusion quantification by fluorescence

We recorded fluorescent emission by videomicroscopy ($λ_{ex} = 545 \text{ nm, } λ_{em} = 572$) on an Olympus IX-73 at 4x magnification and 4 fps, using a 4Mpx Grasshopper camera. After rotation and rectification of the extracted image series, we extract profiles along a $y = \text{const.}$ image slice (line A-B in Fig. S1 a). We fit the profiles to the time and space dependent decay from a fluorescence quantity $c(x, t = t_0) = M_0δ(0)$. $t_0$ is earlier than the actual moment of droplet passage, since the droplet is not a point source:

$$I_δ(x, t) = \frac{M_0}{\sqrt{4πD_{tm}(t-t_0)}} \exp \left( \frac{-x^2}{4D_{tm}(t-t_0)} \right), \quad (S1)$$

$$M_0 = \int_{-∞}^{∞} I(x, 0) \, dx \quad (S2)$$

We calculate the micellar diffusion coefficient via $σ^2 = 2D_{tm}(t - t_0)$. We note that a more precise model can be based on a decay from two step functions at a distance $d = 50 \mu m$, with $c(x, t = 0) = c_0 [H(x + d/2) - H(x - d/2)]$.

$$I_H(x, t) = \frac{I_0}{2} \left[ \text{erf} \left( \frac{x + d/2}{\sqrt{4D_{tm}t}} \right) - \text{erf} \left( \frac{x - d/2}{\sqrt{4D_{tm}t}} \right) \right] \quad (S3)$$

However, for our times of interest beyond the advective time scale the difference between the models is negligible (Fig. S1).

We note that, since the integrated fluorescence does not decay over time in Fig. S1 b, we do not have to account for possible bleaching effects in our analysis.

S1.3. Binary collisions: Image processing and data analysis

After region of interest selection, background correction and binarisation, we extracted droplet coordinates via a contour search algorithm [21] combined with a blob size filter for each video frame. To rule out interactions by hydrodynamic entrainment, or direct droplet to droplet interactions, we filtered the resulting coordinate set to exclude droplets whose distance within the same frame are below a set threshold, as well as droplets too close to the cell boundaries. Droplet trajectories were extracted from the filtered set via a Crocker-Grier type algorithm, providing droplet coordinates $x, y$ and speed vectors $v$ for each recorded timestep. We identified and analysed interactions as follows: for each pair of trajectories, we identified matching sections where the trajectory distance fell below a set threshold. Interactions that include a trajectory endpoint were excluded as we cannot guarantee these to be complete. Segment pairs $s_1, s_2$ were sorted by time. We assume the droplet creating $s_1$ to move freely, such that $s_1$ can be safely approximated by a straight line. We note that our swimmers’ dynamics are persistent Brownian rather than strictly ballistic, however, the trajectory persistence length, as seen in Fig. S2, clearly exceeds the typical interaction length, so that our assumption of straight segments is reasonable. We identified the orientation $θ_1$ of $s_1$ via a linear regression fit. If the standard deviation of this fit exceeded a certain value, the segment was considered to be too crooked, i.e. not relating to free motion, and the interaction was discarded. Fig. S2 shows trajectories from one experimental

FIG. S1. Fluorescent emission in the trail of a Nile Red doped droplet. (a) Data vs. $I_δ(x, t)$ ((S1)) and $I_H(x, t)$ ((S3)) fit models (b) The integrated fluorescence does not decay over time.
run with the numerically identified interactions marked in colour. We further discarded, by visual inspection, any interactions that were disturbed by multiple trail collisions. For segment $s_2$, we extracted the following quantities:

1. the time $\Delta t = t_2 - t_1$ elapsed between the two points of closest trajectory approach, which we chose as the time delay of the interaction.

2. for each coordinate in $s_2$, the distance to the closest point in $s_1$, i.e. distance $d(t)$ of swimmer to trail over time. To mark crossing events, by convention, $d$ is signed via $\text{sgn}(d) = \text{sgn}(n_2 \times \hat{e}_1)$, with $n_2$ denoting the $s_2$ trajectory normal and $\times$ the 2D cross product.

3. from a projection of $v_2$ on $\hat{e}_1$, droplet speeds $v_\parallel$ parallel and $v_\perp$ perpendicular to the trail, as well as the angle $\theta$ between $v_2$ and $\hat{e}_1$. To avoid discontinuities in $\theta$ due to $2\pi$ periodicities, $\theta$ was calculated with respect to $-\hat{e}_1$ if $\langle v_\parallel \rangle > 0$.

**S1.4. Laser sheet fluorescent microscopy**

The setup (Fig. S3) consists of an illumination unit (producing the thin laser sheet) and a detection unit (the camera and the objective) that are synchronized and translate vertically (in z-direction) capturing images at a frame size of 1MP in the x-y plane at 4.15 $\mu$m/px resolution. Images are recorded at 150 frames per second, while the z-stages are driven by a sawtooth signal with an amplitude of 3 mm and a frequency of 0.7 Hz, yielding a voxel size of 4.2x4.2x27 $\mu$m$^3$. The laser sheet’s beam waist is $\approx$40 $\mu$m thick. The scanned volume in the square cuvette we use is 3x3x3 mm$^3$. Our samples consist of Nile Red doped droplets with a diameter of 50 $\mu$m in density matched mixtures of TTAB/H$_2$O/D$_2$O.

3D droplet positions are reconstructed from binarized stacks of z-slices for each half period of the sawtooth signal. We extract fluorescent droplet contours for each slice [21] and grouped contours associated with the same droplet within consecutive slices using a mean shift clustering algorithm [22, 23]. Time and z position for each slice are calculated using timestamps provided by the camera and translation stage software interfaces, resulting in full $xyz$ datasets.
FIG. S4. Analysis of the fits to the 54 experimental reflection events with large incidence angle $\theta_{\text{inc}} > 60^\circ$, using two fitting parameters $\Omega_{0}$ and $\alpha_{0}$. (a) The fits are ordered from best to worst according to the error of the fit. (b,c) Median values of (b) $\Omega_{0}$ and (c) $\alpha_{0}$ as a function of the the number $n$ of trajectories used in the calculation of the median value. We observe a plateau at $\Omega_{0} \approx 7 \cdot 10^{3}$ $\mu$m$^2$/s and $\alpha_{0} \approx 3 \cdot 10^{4}$ $\mu$m$^3$/s (grey band), when a sufficient number of trajectories is considered ($n \gtrsim 20$) but the worst fits are left out ($n \lesssim 40$). (d) As an example, we show the histogram for the values of $\Omega_{0}$ and $\alpha_{0}$ (two-dimensional, as well as projected along each dimension) when using the $n = 21$ best fits, which corresponds to using only the fits with relative error $< 4 \cdot 10^{-3}$.