

Long-range topotaxis as a result of supercellular topographic anisotropy during chemotaxis

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Highly motile cells, like patrolling leukocytes during immune response and amoeboid sarcoma cells during metastasis, navigate relatively long distances in complex environments where they encounter a wide variety of topographic structures on different length scales. Subcellular topographic substrate anisotropies have been shown to cause a drift in cell migration (topotaxis) *in vitro*, indicating that the topography of the extracellular environment can function as a physical guidance cue. Here, we expand on these previously reported subcellular findings by showing that supercellular asymmetries spanning long distances can also induce topotactic drift. Such long-range topotactic drift was generated by exposing highly motile cells to a subtle spatial gradient in the arrangement of cell-sized pillars. Using an analytical model and mean first-passage time analyses, we show that this type of long-range topotaxis emerges through the many interactions of persistently random migrating cells with the anisotropic confinement imposed by the gradient pillar field. We demonstrate that pillar-induced long-range topotaxis is conserved during chemotaxis, the cellular drift in response to chemical gradients. Topotactic and chemotactic drifts were found to add linearly, a finding that could have implications for *in vivo* studies on directed cell migration, which mostly focus on chemotactic responses alone. Moreover, as long-range topotaxis is an independent physical effect, it offers a way to steer highly motile cells independently from chemotactic guidance, which could have potential applications in cell sorting and tissue engineering.

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I. INTRODUCTION

Directed, single-cell migration is driven by environmental guidance cues, such as chemical, electrical, temperature, or stiffness stimuli [1–6]. Along with these stimuli, the topography of the environment can also induce directed cell migration [6–11]. Particularly, it has been shown that *in vitro* assays including channels [12–16], ratchets [7,17,18], grooves [19–21], pillars [22–24], curves [25], or areas with increased alignment of extracellular matrix fibers [14,26–28] can act as topographical guidance cues for eukaryotic cells. These *in*

vitro assays rely on anisotropy in environmental topography to influence the direction and speed of cell migration, and exploit differences in slope, confinement, or alignment to affect motility. An excellent umbrella term to subsume all such migratory responses to topography is *topotaxis* [11], directed cell movement in response to topographic gradients, a term recently reintroduced by Park *et al.* to describe topographical guidance of melanoma cells using subcellular pillar geometries [22].

So far, studies involving topotaxis have largely focused on the influence of topographic differences smaller than the cell body (subcellular). In such systems, the cell is in uninterrupted contact with one or multiple steps of a topotactic gradient. However, apart from subcellular stimuli, migrating cells encounter topographic differences on other length scales as well. Especially highly motile cells, which migrate large distances (up to several millimeters), navigate a landscape with topographic differences at many different length scales. Amoeba moving through soil [29], or mammalian cells squeezing through tissue, like leukocytes during immune response [30,31] or metastasizing cancer cells [32], are prime examples of highly motile cells traveling long distances. During such long-range navigation, the availability of space in dense cell, matrix, or otherwise crowded environments can be a deciding factor. Although cells can sometimes remodel

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their direct environment [33], it is often more efficient to squeeze through existing space during movement [34,35]. When differences in the availability of space are positionally dependent, even on a scale larger than a cell (supercellular), they could constitute a topotactic gradient. The possibility of topotaxis as a result of supercellular topographic anisotropy or gradients is still an open question.

In nature, cells do not travel through topographies in isolation, but often in the presence of other guidance cues. The immune response is a well-known example process involving cell migration through multicue environments, as neutrophils extravasate and subsequently navigate across various tissue topographies reacting to secreted chemokines after wounding [31,36,37]. Elucidating the interplay between various guidance cues is of recent interest [2,17,38], but a daunting problem due to the complexity of most extracellular environments and the many types of cells and migration mechanisms. Cells may ignore certain stimuli in favor of others, or different cues might add up in affecting cell movement. Focusing on topotaxis, not much is known about the interplay between topotactic guidance and other motion cues, even though it could be argued that the potential relevance of any guidance effect can only be gauged in the context of other stimuli. Chemotaxis, directed cell movement in response to chemical stimuli, is a good candidate benchmarking tool for topotactic guidance, as it is tunable by varying chemotactic molecule concentration [39,40], and is one of the most well-studied guidance cues through its omnipresence in nature [41].

The influence of isotropic topographies on cell migration and chemical stimuli has only been studied separately using *Dictyostelium discoideum* (*D. discoideum*), a well-known model organism for researching amoeboid cell migration [29,40,42,43]. When starved, the amoeba become highly motile and have a strong chemotactic response to cyclic-adenosine monophosphate (cAMP) gradients [40]. While moving in the starved state, the cells repeat a process of pseudopod splitting and elongation [3,44,45], which is common to many other cell types that perform amoeboid migration, like metastasizing cancer cells and leukocytes [46,47]. Amoeboid migration is characterized by a mix of random motion (through pseudopod splitting) and persistence (elongation) on short timescales, but evolves into fully random motility over longer timescales [48]. When exposed to a gradient in cAMP concentration, the persistent-random motion of starved *D. discoideum* becomes biased, resulting in an overall drift, i.e., chemotaxis [39].

Here, we show that a subtle supercellular gradient of cell-sized obstacles induces long-range topotactic drift for highly motile cell migration, even in the presence of chemotactic cues. For our experiments, we observed starved *D. discoideum* motility inside micropillar fields with a gradient in interpillar spacing. The gradient pillar field was found to induce topotactic drift, although the cells are never in full contact with an entire gradient step (change of spacing). After combining the topotactic stimulus with a chemotactic cAMP gradient, the topotactic drift was conserved and found to linearly add to chemotaxis. Then, the origin of pillar-induced long-range topotaxis was explored using a mean first-passage time (MFPT) analysis. Specifically, we focused on the emergence of long-range topographical guidance from the interaction of

inherently stochastic cell movement in combination with the asymmetric structure of the gradient pillar field. Cell movement through pillar enclosures was coarse-grained as cell passages through single-pillar domains with different pore sizes. The dynamics inside these domains was connected to motion across the entire pillar field by modeling cell motility as a jump process between different zones of equal pillar spacing. The model suggests that gradient pillar fields will always result in topotaxis for quasirandom cell motility if the escape rates across the pillar field are positionally dependent. Our results indicate that even in multicue environments subtle topographic anisotropies may result in effective drift for quasirandom cell movement. As many cell types move with some degree of stochasticity, long-range topotaxis as a result of supercellular topographic anisotropy could significantly contribute to the long-range navigation of cells in natural environments.

II. RESULTS

A. Combining topotactic and chemotactic gradients in a single multicue assay

Long-range topotaxis of highly motile amoeboid cell migration was studied inside an anisotropic micropillar field with a gradient in pillar spacing using the model organism *D. discoideum* in the starved state [Figs. 1(a)–1(e)]. As we will show, long-range persistent-random cell motion in the anisotropic pillar field results in topotactic drift of cells. To examine the relevance of this physical effect, both the pillars and cells were embedded in a microfluidic device to enable an additional chemotactic gradient by controlled diffusion [Fig. 1(a) and Sec. III] of cAMP, a well-studied chemical attractor for *D. discoideum* [3,39]. By adding the second gradient, we created an experimental assay with two independent, simultaneous stimuli for cell motility: an anisotropic topography and a chemical gradient in solution.

To study the topotactic effect in isolation, cell migration was first measured on pillar fields with a spatial gradient in a chemotactically neutral buffer [Fig. 1(f)]. Then, to investigate whether long-range topotaxis is conserved in the presence of chemotactic stimuli, cAMP gradients were aligned (A) and opposed (O) to the topographical guidance cue in either negative [Fig. 1(g)] or positive [Fig. 1(h)] x direction. These two configurations ensure competitive or synergistic effects depending on the orientation of both external stimuli. As controls, cell migration was also studied on two isotropic pillar fields (spaced 15 and 20 μm) and on flat surfaces, each with and without cAMP gradients.

Figure 1(b) shows a top view of the primary pillar gradient (T) used. The pillars are fabricated from the biocompatible transparent polymer polydimethylsiloxane (PDMS) and its surface can be assumed chemically homogeneous (Sec. III B). The height and diameter (d) of the pillars are equal (8 μm) and comparable to the typical diameter of a *D. discoideum* cell, which varies between 8 and 12 μm [50]. Pillar size was based on previous studies [43,51], and the ratio between pillar height and cell size was chosen to keep the motion of cells primarily two-dimensional (2D). As such, the pillars act as obstacles, interrupting persistent cell motion, and the spaces between pillars (pillar domains) act as sequences of

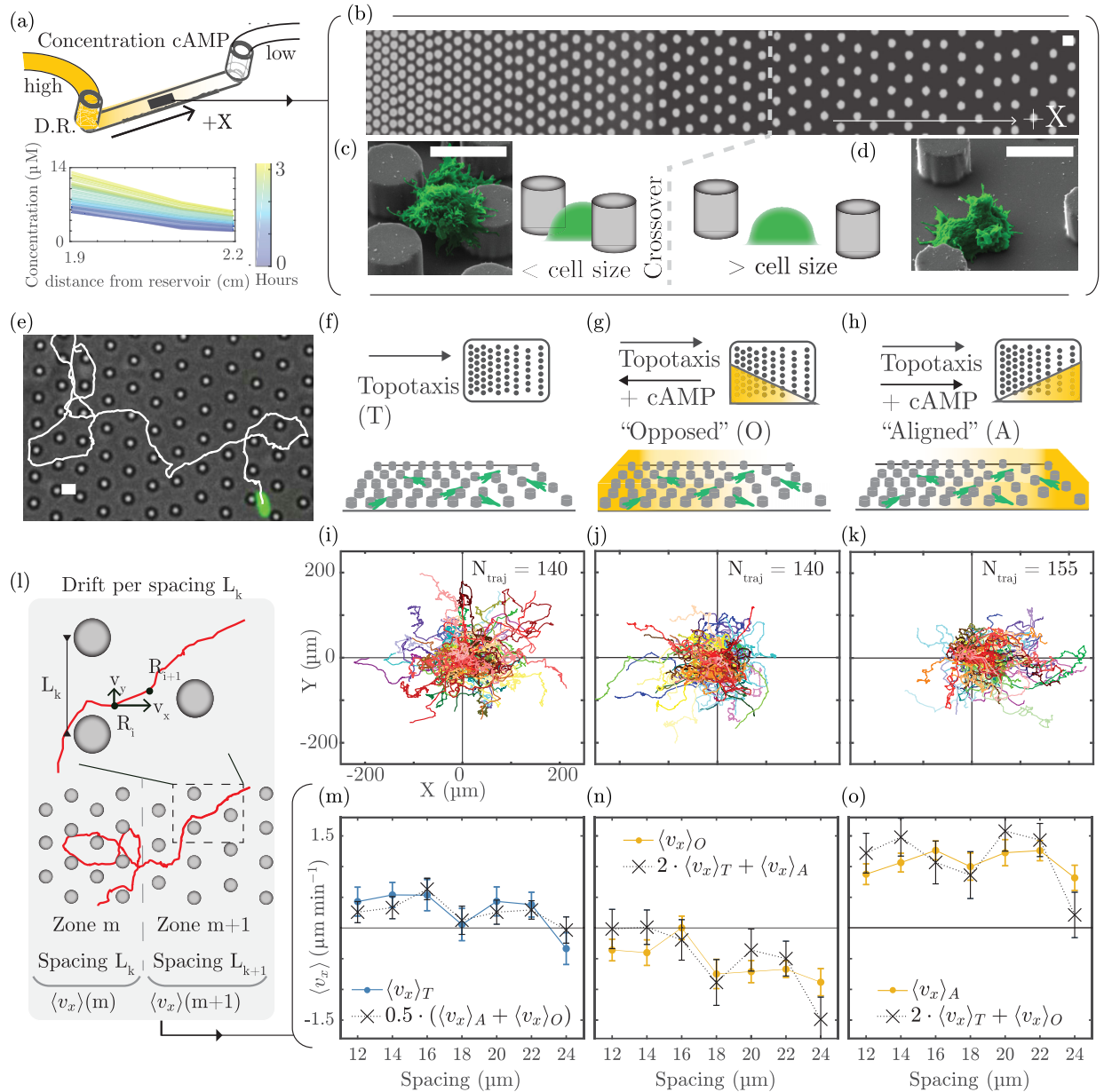


FIG. 1. Supercellular spatial gradient in arrangement of cell-sized obstacles generates physically induced drift, which is conserved in a chemotactic gradient. (a) For the multicue assay, fields of PDMS micropillars (black) were placed in microfluidic devices, where cAMP gradients (yellow) could be applied through controlled diffusion using a reservoir (D.R.). The gradient was stable in and around the pillars over the course of a time-lapse measurement (inset graph and see Appendix E). (b) The micropillar array was varied in lattice spacing (L_k), functioning as a topotactic gradient for highly motile cells. The entire field is shown in x (size: 0.9 mm), but only a slice is shown in y (actual size: 2.2 mm). (c), (d) Every four pillar rows (in $\pm x$), the space between pillars was varied from much smaller to much larger than the cell size, illustrated by two SEM micrographs and a schematic. The crossover from spacing smaller to larger than the cell size is indicated with a dotted line. (e) Example trajectory (white) of a starved *D. discoideum* cell (green, free GFP) inside the micropillar array (bright field image) during 72 min of a *T* measurement (also see Movie S1 in Supplemental Material [49]). (f)–(h) In the multicue assay, three principal configurations were studied, only topotaxis (*T*), and with an extra chemotactic gradient (cAMP, yellow), either opposed (*O*) or aligned (*A*). (i)–(k) Center plot of recorded cell trajectories for three experimental conditions. Cells migrate toward the more spacious side of the array, and when a chemotactic gradient is applied, cell migration is opposed or enhanced depending on aligning or opposing both cues. (l) To calculate migratory drift per spacing (L_k), all cell displacements in the x direction are averaged per pillar zone (m) of equal pillar spacing (L_k) and per configuration. (m) Drift measured in the direction of the topotactic gradient (blue line) as a function of the interpillar spacing. Error bars are 95% confidence intervals (CIs). Topotactic drift is similar to the “reconstructed” topotactic drift (black line), found by a linear addition of the drifts of the complementing aligned and opposed experiments. The overlap between measured and “reconstructed drift” indicates topotaxis is conserved during chemotaxis. (n) Drift measured in the opposed experiment (gold). The data closely follow the “reconstructed drift” (black) generated by using the data from the other topotaxis-only and aligned experiments. (o) Drift (gold) and “reconstructed drift” (black) for the aligned experiment. All scale bars are 10 μm .

enclosures with varying pore size. Cells fully mounting pillars are rarely observed, and if encountered, are excluded from the analyzed data. The pillar fields [Fig. 1(b)] were arranged in a triangular lattice to ensure that cells encounter pillars during migration in the direction of the topotactic and chemotactic gradients ($\pm x$ direction), thereby excluding the possibility of cells simply migrating along alleys in between lines of pillars in the gradient direction.

The topotactic gradient acts on a length scale much larger than the cell body, i.e., at long-range, as center-to-center pillar spacing L_k was increased by $1\text{ }\mu\text{m}$ after every fourth pillar column in the $+x$ direction (size of field, x, y : 0.9 by 2.2 mm). Here, k denotes the discrete increase in pillar spacing. By not increasing the distance between every subsequent column of pillars, cells are more likely to reorient at least once within each zone of equal pillar spacing m during exploration, as trajectories have persistence lengths measuring between 17 and $40\text{ }\mu\text{m}$ (Appendix A and Fig. 4). Across the entire field, interpillar spacing increases from 3 to $17\text{ }\mu\text{m}$, resulting in pillar center-to-center distances of $L_k = 11, 12, \dots, 25\text{ }\mu\text{m}$, and pillar zones [Fig. 1(l)] of widths $L_m \approx 38, \dots, 87\text{ }\mu\text{m}$. Cells moved smoothly through each spacing chosen. Locally, the available space between pillars spans three different length scales when compared to the cell diameter: (1) a squeezed environment with continuous pillar contact [$3\text{--}9\text{ }\mu\text{m}$; Fig. 1(c)], (2) an environment where the cell periodically loses contact with pillars [$13\text{--}17\text{ }\mu\text{m}$; Fig. 1(d)], and (3) a crossover region with pillar domains of about cell size ($11\text{--}12\text{ }\mu\text{m}$).

B. Supercellular topotactic gradient induces biased cell migration

To quantify cell migration in the pillar fields and study the gradient effects, we recorded cell center-of-mass trajectories and analyzed (averaged) statistical observables, such as drift [52]. Figure 1(f) (Movie S1 in Supplemental Material [49]) shows an example trajectory projected onto the pillar field. Cell migration in the anisotropic pillar field produces heterogeneous, persistent-random-like trajectories, similar to those measured on flat PDMS surfaces (Movie S2 in Supplemental Material [49]). Contrary to movement on flat surfaces, there is a drift in cell motion to the more spacious side of the anisotropic pillar field, measuring approximately $\langle v_x \rangle_T < 0.5\text{ }\mu\text{m min}^{-1}$ over all spacings [Fig. 1(m)]. Surprisingly, this *topotactic drift* is conserved in the presence of a chemotactic cue. When the topotactic cue is aligned with chemotaxis (A), cells are drawn more toward the source of cAMP [Fig. 1(j)], than when the two cues are configured oppositely (O) [Fig. 1(k)]. In the aligned case A, the drift is approximately $\langle v_x \rangle_A > 1\text{ }\mu\text{m min}^{-1}$ for each spacing [Fig. 1(o)], whereas for the opposing gradients O, the drift measures $\langle v_x \rangle_O < -1\text{ }\mu\text{m min}^{-1}$ [Fig. 1(n)]. Clearly, the topotactic drift generated by the anisotropy inside the pillar field increases or decreases the chemotactic response of *D. discoideum*, as $|\langle v_x \rangle_A| > |\langle v_x \rangle_O|$ across the pillar gradient.

The averaged drift for all pillar field experiments, including controls, is shown in Fig. 2(c), normalized to the average instantaneous velocity $\langle v_0 \rangle$. The averaged instantaneous velocity is a measure of overall cell motility and normalization to this observable is advantageous for comparing relative drift

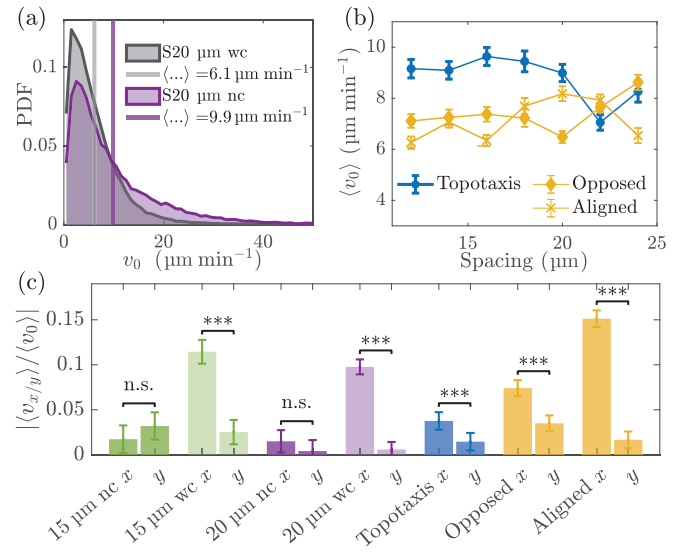


FIG. 2. The topotactic drift response for all pillar field experiments. (a) PDF of the instantaneous velocity v_0 of cell migration on the $20\text{ }\mu\text{m}$ spaced fields with cAMP (wc, gray) and without (nc, purple). (b) Average instantaneous velocities per pillar spacing L_k measured for the three principal experimental configurations. (c) Averaged, normalized drift in the x, y directions for all isotropic (15 and $20\text{ }\mu\text{m}$) and anisotropic (T, O, A) experimental conditions, when applying no cAMP (nc) or with cAMP (wc). The drifts of $15, 20\text{ }\mu\text{m}, T$, and A are all in the $+x$ direction; the O drift is in $-x$ direction. The drift in x of the other experiments and in all y directions is randomly oriented.

responses across different experiment types. *D. discoideum* motility can vary across experiments due to overall population heterogeneity (elaborated in Sec. II D), but primarily due to a systematic drop in speed of starved cells in the presence of cAMP gradients [53]. Figure 2(a) shows the systematic drop in velocity, from 9.9 to $6.1\text{ }\mu\text{m min}^{-1}$, by plotting the v_0 probability density functions (PDFs) of the $20\text{ }\mu\text{m}$ fields with (wc) and without (nc) a chemotactic gradient. Apart from control experiments, the systematic drop in speed was also measured for the topotactic (T) and multicue experiments (O, A) [Fig. 2(b)]. The shape of the v_0 PDF resembles a generalized Gamma distribution and is similar to persistent-random amoeboid motility on flat surfaces [54,55].

The overall drifts on isotropic fields (15 and $20\text{ }\mu\text{m}$) without a chemotactic gradient (nc) are negligible, whereas the drift response of all experiments with chemotactic gradients stands out ($15, 20\text{ }\mu\text{m}, O$, and A) [Fig. 2(c)]. This is expected, as the chemotactic response of starved *D. discoideum* to the chemokine cAMP is exceptionally well tuned to even the most shallow gradients [56]. The aligned A and opposed O configurations measure averaged drifts above and below the control experiments on isotropic pillar fields with cAMP [Fig. 2(c)], demonstrating the influence of the topotactic gradient. The topotactic gradient shows a smaller averaged drift than all experiments involving a chemotactic gradient, and measures half the difference between the O and A configurations (suggesting linear summation of the cues; see Sec. II C). Given the strong chemotactic response of *D. discoideum* [40,45], it is surprising that a relatively weak spatial asymmetry, a change

in spacing of 1 μm after multiple cell lengths, has a significant effect during multicue experiments.

C. Long-range topotaxis and quasidirected chemotaxis add linearly

The addition of topotactic and chemotactic cues can be further investigated by comparing each of the principal experimental configurations to the combined response of the two complementary experiments [Figs. 1(f)–1(h)]. For example, the topotactic drift $\langle v_x(k) \rangle_T$ can be compared to a “reconstructed” topotactic drift found by linearly adding the measured drifts of the other two multicue experiments, $\frac{1}{2} [\langle v_x(k) \rangle_A + \langle v_x(k) \rangle_O]$, for each pillar spacing. Figure 1(m) (black line) shows this “reconstructed” topotactic drift, and indeed, it overlaps with the drift measured in the actual topotaxis experiment. This finding thereby provides evidence of linear summation of the chemotactic and pillar gradient-generated topotactic cues. This procedure can also be performed for the multicue experiments *O* and *A*; the “reconstructed drifts” are found by $\langle v_x(k) \rangle_A = 2 \langle v_x(k) \rangle_T - \langle v_x(k) \rangle_O$ and $\langle v_x(k) \rangle_O = 2 \langle v_x(k) \rangle_T - \langle v_x(k) \rangle_A$, respectively. These results are shown in Figs. 1(n) and 1(o) (black lines), and equally, the reconstructed drifts coincide for most spacings. As a side observation, the drifts at or around the 17–19 μm spacing suddenly lower and then rise for all topotaxis experiments [Figs. 1(m)–1(o)], which is further elucidated in the discussion.

As the previous paragraph explained, the averaged drifts provide evidence of summation of the topotactic and chemotactic cues studied [Figs. 1(m)–1(o) and 2(c)]. However, this method of direct drift calculation is prone to noise, as some cells hardly transition to a different pillar spacing during movement, or parts of motion trajectories do not complete a full transition cycle to a different pillar domain. Although both examples do not add information on cell navigation up and down the pillar gradient, they still weigh in on averaged drifts. As our aim is to investigate the influence of just the gradients, we also analyzed cell movement as just a sequence of transitions between pillar columns in the gradient direction (Fig. 3). By just focusing on completed transitions between pillar columns, this simple empirical method allows the calculation of (frequentist) transition probabilities that only take full migratory cycles between pillar domains into account. We found that this approach reduced the noise in analyzing the overall directional preference compared to averaged drift calculations $\langle v_x \rangle(k)$.

Figures 3(a)–3(c) (Movie S3 in Supplemental Material [49]) show an example of how transition probabilities to the spacious (P_+) and less spacious side (P_-), and their difference, $\Delta P(k)_E = P_{+,E}(k) - P_{-,E}(k)$, are computed for a single-cell trajectory. The transition probabilities and their differences are displayed in a histogram and binned per spacing L_k , to highlight the net directional preference of migration with respect to the pillar gradient [Fig. 3(c)]. The same scheme was used to determine the net difference for each principal configuration [$\Delta P_T(P_+, P_-)$, $\Delta P_O(P_+, P_-)$, and $\Delta P_A(P_+, P_-)$] using the transitions of all cells per configuration. All net differences can be expressed in terms of P_+ and P_- and were mapped to $[-1, 1]$ for ease of comparison (see Sec. III E).

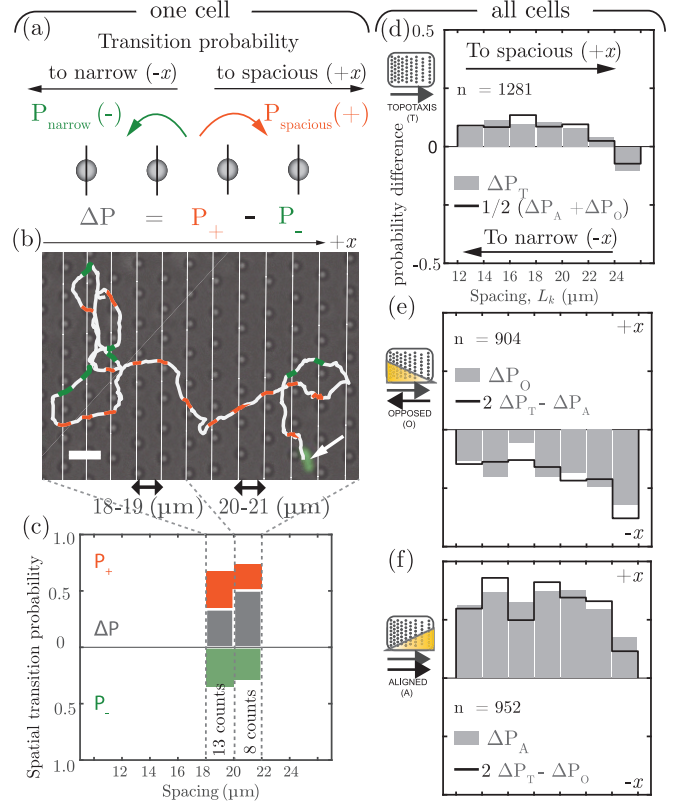


FIG. 3. Pillar gradient-induced topotaxis is conserved during chemotaxis and the impact of both cues adds up linearly for cell motion. (a), (b) Pillar fields are computationally divided into pillar columns in the direction of the gradients to track the dynamics of the migrating cell (arrow) as a function of the spacing L_k between pillars. Each time a cell transitions to an enclosure (pillar domain), with respect to the direction of the gradient, a transition is recorded to either the more spacious (orange) or narrower (green) part of the pillar field. Scale bar is 15 μm . (c) A spatial transition probability histogram can be constructed using the recorded transitions, by calculating the probability of the cell to transition to narrower (P_{orange}) or to more spacious (P_{green}) at each pillar spacing and their sum (ΔP , gray). (d) Histogram of all transitions measured for the topotaxis only experiment, showing that cells migrate to areas with bigger interpillar spacing (gray). The “reconstructed” ΔP (black) shows that the difference between the opposed and aligned experiment is exactly the topotactic cue. (e) Histogram with the topotactic and chemotactic effects opposed. The net spatial transition probability ΔP (gray) and “reconstructed” ΔP (black) show close resemblance, thereby indicating linear addition of both cues. (f) Histogram with the topotactic and chemotactic effects aligned, and the sum of the opposed and topotaxis configurations, equally indicating linear addition.

The difference of all transitions in the topotaxis field [Fig. 3(d)] shows that cells are topographically guided toward the spacious side of the pillar array. The difference ranges between $\Delta P_T = 0.05$ –0.15 and “points” toward the more spacious side of the pillar field. Only when pillars are spaced furthest apart (23–25 μm), cells prefer migration to the opposite direction ($\Delta P_T = -0.07$). When the chemical and topographical gradients are aligned [Fig. 3(f)], cells transition more readily $\Delta P_A = 0.17$ –0.38 toward the source of cAMP

TABLE I. Overview of the cell motility measurements. The error on instantaneous velocity $\langle v_{in} \rangle$ is the 95% CIs, using the number of data points as the degrees of freedom. For this table, the effective diffusion constant D_{eff} was determined by a linear fit for experiments with no drift, and a BRW model (see Appendix A) for those with drift. The error on D_{eff} was determined by doing the same fits on the upper and lower 95% CI of the averaged MSDs. The number of trajectories was chosen as degrees of freedom for the D_{eff} 95% CIs.

Exp.	Meas.	Traj.	Data	$\langle v_{in} \rangle \mu\text{m s}^{-1}$	$D_{eff} \mu\text{m}^2 \text{s}^{-1}$
S15 μm n.c.	2	51	15 807	0.152 ± 0.001	0.74 ± 0.47
S20 μm n.c.	2	63	20 219	0.164 ± 0.002	1.06 ± 0.56
S15 μm w.c.	2	78	24 542	0.153 ± 0.002	0.94 ± 0.28
S20 μm w.c.	2	99	48 633	0.102 ± 0.001	0.37 ± 0.02
Topotaxis	5	134	41 503	0.148 ± 0.001	0.54 ± 0.43
Opposed	3	135	41 838	0.125 ± 0.001	0.34 ± 0.11
Aligned	4	155	37 536	0.122 ± 0.001	0.32 ± 0.04
Flat n.c.	5	159	33 976	0.199 ± 0.001	1.76 ± 0.51
Flat w.c.	4	186	48 756	0.174 ± 0.002	1.72 ± 0.28

compared to when the gradients oppose [Fig. 3(e)] $\Delta P_O = 0.06$ – 0.27 . Again, this indicates that the topotactic gradient influences the chemotactic response of the cells. The chemotactic response was found by averaging over the data gathered on isotropic pillar fields in presence of a cAMP gradient ($\Delta P_{cAMP} \approx 0.25$). All experimentally determined transition probabilities are displayed in Table II.

To study the combinatorial response, the “linear addition” of cell transition probabilities was computed, following the same scheme as employed for averaged drifts. The topotactic cue can be distinctly reconstructed from the aligned and opposed experiments $\Delta P_T = \frac{1}{2} (\Delta P_A + \Delta P_O)$, and similarly, twice the difference between the topotaxis, and either aligned or opposed chemotactic gradient experiments, should generate the respective other: $\Delta P_A = 2 \Delta P_T - \Delta P_O$ and $\Delta P_O = 2 \Delta P_T - \Delta P_A$. The corresponding histograms are shown in Figs. 3(d)–3(f) (black lines) and provide further confirmation of linear addition. The measured (gray histograms) and reconstructed probability differences (black lines) coincide for all three experiments, evidence that the same topographical guidance is indeed measured in the experiments with and without a chemical gradient. Moreover, the conserved topotactic effect adds up to chemotaxis with approximately similar strength.

Summarizing the results thus far, anisotropic pillar fields with a supercellular gradient in interpillar spacing generate long-range topotactic drift for highly motile cell migration. Moreover, the topotactic drift is conserved during chemotaxis and adds up linearly to the competing chemotactic cue.

TABLE II. Averaged transition probabilities for all topotactic and chemotactic gradient configurations. Standard errors are ± 0.005 – 0.015 for all values.

	T	$15W$	$20W$	O	A
P_+	0.5435	0.6308	0.6150	0.3993	0.6586
P_-	0.4565	0.3692	0.3850	0.6007	0.3414
ΔP	0.0869	0.2615	0.2301	0.2014	0.3171

Both the calculation of drift as a function of pillar spacing and the net difference between transition probabilities support these findings. How well the probability differences align with their reconstructed counterparts is remarkable, and reveals the power of a more coarse-grained approach, as excess data are trimmed by only taking into account movement across multiple enclosures in the x direction. Building on the success of this empirical approach, the second part of this paper (Secs. II E–II H) will introduce an analytical model of pillar gradient-induced topotaxis. The cornerstone of this model will be to coarse-grain cell movement throughout the pillar field as series of escapes from pillar-delimited domains, and subsequently as a jump process between multiple domains (pillar zones) on the entire field. Before averaging out any details, however, the heterogeneity between and within cell trajectories is investigated further. Doing so will not only garner appreciation for the high degree of complexity due to cell-to-cell variability, intertrajectory variations, and the pillar field, but will also provide insight on choosing a suitable time and spatial scale for a coarse-grained model of cell movement later on.

D. Pillar obstacles enhance stochasticity of inherently heterogeneous cell trajectories

The measured cell trajectories were found heterogeneous on two different levels: Within each trajectory, there are alternating types of migration, and in addition, we measured cell-to-cell motion variability. Both these levels of heterogeneity are apparent from two example trajectories shown in Figs. 4(a) and 4(b). Here, each recorded position of the cell is shown using a dot, which allows a more clear visualization of temporal variations in motion as opposed to using a line [as seen in Fig. 1(e)]. These example trajectories indicate cell-to-cell variability, as both examples were recorded during the same measurement on an isotropic pillar field, but also clearly highlight the existence of different states of motion within each trajectory itself.

Within each trajectory, cells intermittently alternated between directed runs and diffusivelike (i.e., quasirandom) states. This behavior is shown in red and blue in Figs. 4(a) and 4(b), respectively, by using a local mean squared displacement (l-MSD) analysis [57]. In short, this analysis assigns each trajectory point j to be part of a directed or quasirandom motion state based on the value of the local α exponent extracted from the MSD in a sliding window ($M = 12$) around that point (Sec. III D). The α_j exponent is found by a fit to the MSD for anomalous diffusion, $\langle r(t)^2 \rangle_j = A_j \cdot t^{\alpha_j}$, where t is the lag time. The two-state motion of starved *D. discoideum* arises from periodic reorientation, in order to respond, or search for, changing chemotactic signals, and was measured on pillar and flat substrates (Appendix A).

Although random motion states are measured on flat substrates, the introduction of pillars further randomizes motion, as cells are obstructed by, and spend time navigating around, vertical pillar surfaces [Figs. 4(a) and 4(b)]. The average duration of directed t_{dir} and quasirandom t_{ran} motion states is shown in Fig. 4(d) and demonstrates pillar-induced randomness through a comparison of flat and pillar substrate experiments. On flat PDMS, cells moved quasirandom for,

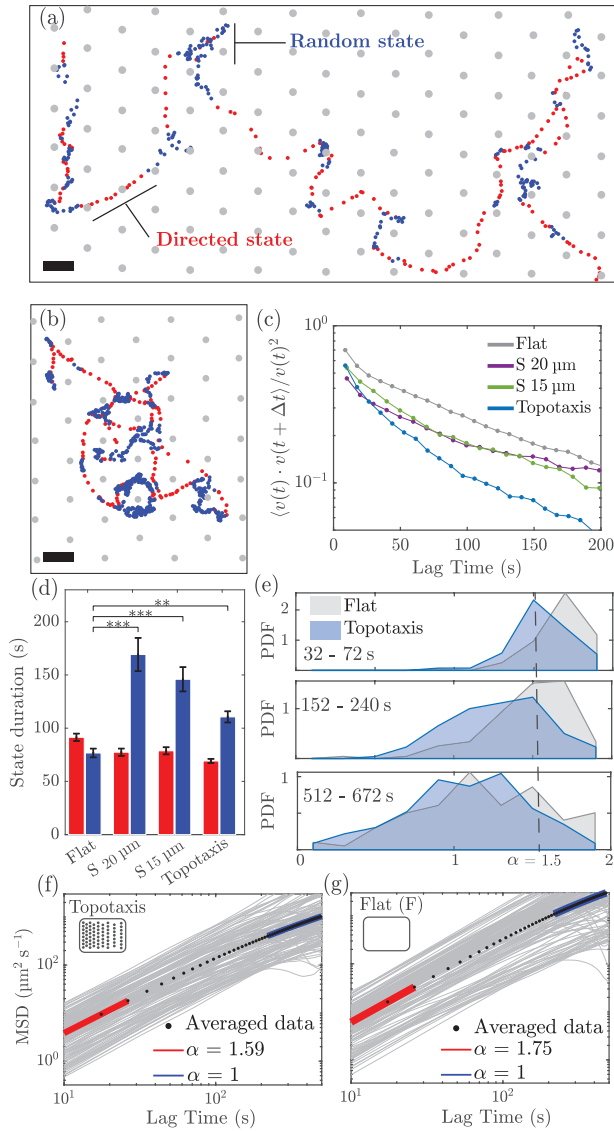


FIG. 4. Cell trajectories are heterogeneous; the pillar lattice increases randomness. (a), (b) Example cell trajectories in a pillar lattice of regular spacing (15 μm). Registered cell positions are visualized by dots ($n = 434$ and $n = 721$, respectively). The tracks show a highly complex and varied structure; the two cells were chosen to represent the typical span in surface explored. L-MSD analysis further highlights trajectory heterogeneity. Trajectory parts that are directed (ballistic, $\alpha > 1.5$) are shown in red, quasirandom in blue ($\alpha \leq 1.5$). The position of pillar centers is shown in gray (not entire pillar shown). (c) Velocity autocorrelation function of the trajectories for the flat surface and pillar arrays. The pillars (15 and 20 μm spacings) disrupt correlations in $\bar{v}$ and a gradient in spacing (topotaxis lattice) introduces additional randomness. (d) Durations of the directed (red) and quasirandom states (blue) for trajectories on different pillar lattice arrays. The pillar lattices induce longer quasirandom states. (e) Probability distributions of MSD α —fits on individual trajectories at different lag times for cells moving on flat and topotaxis surfaces. Cell tracks approach the random regime ($\alpha < 1.5$) sooner when migrating through a pillar field. (f), (g) MSDs of all cells (gray) for the topotaxis and flat datasets and their average (black). Average MSDs are fit in the ballistic regime (red) and diffusive regimes, yielding $D = 0.54 \pm 0.43 \mu\text{m}^2 \text{s}^{-1}$ and $D = 1.76 \pm 0.51 \mu\text{m}^2 \text{s}^{-1}$, respectively.

on average, $\langle t_{\text{ran}} \rangle = 77$ s; this is significantly increased on the topotaxis and isotropic pillar fields: $\langle t_{\text{ran}} \rangle = \{110, 147, 175 \text{ s}\}$. The average duration of the directed states on a flat surface is longer as well, but the difference between experiments is much lower (91 versus 70 s). Hence, the pillar field primarily lengthens the duration of quasirandom, but not ballistic motion on short timescales.

A comparison of the average velocity autocorrelation functions (VACF) and the time-averaged MSD (TAMSD) further supports the hypothesis of pillar-induced randomness. The VACF [Fig. 4(c)] of cell motion decays faster on isotropic fields than on flat substrates. The VACF decays fastest for the topotaxis pillar field, indicating that a mix of pillar spacings seems to add to the randomizing effect. The TAMSD offers insight into the onset of randomness on a longer timescale, as short-time persistence develops into random motion over long periods of time, and is shown in Figs. 4(f) and 4(g) for topotaxis and flat substrate experiments for each individual trajectory (gray) and their average (black). Figure 4(e) shows the PDF of α exponents of individual trajectories at three different lag times. Clearly, at short lag times (30–70 s) the trajectories are still in the ballistic regime for both topotaxis and flat substrates, as the α exponents peak at $\alpha \geq 1.5$. At an intermediate timescale of 150–250 s, most of the α exponents are below $\alpha = 1.5$ for the topotaxis assay. Conversely, the α exponents of flat surface trajectories are still mostly superdiffusive at this timescale, providing more evidence for pillar-induced stochasticity.

All three analyses, TAMSD, l-MSD, and the VACF, suggest that, on average, the crossover between the ballistic and diffusive regimes starts around 100–150 s. At this lag time, the VACF moves below 0.1 and the average directed run ends according to l-MSD analysis. The typical persistence length can be found by multiplying the average directed run time (≈ 100 s) with the instantaneous velocity, and suggests diffusivity starts at a length scale around 10 μm . This is about the spacing of the smallest possible pillar delimited domain (11 μm). This gives a first estimate of the appropriate timescale and length scale of a coarse-grained description of cell movement across the pillar field. The next sections (Secs. IIE and IIF) will discuss cell movement across the pillar gradient as a series of escapes from single-pillar domains, before finally proposing an empirical model to predict long-range topotactic drift (Sec. IIG).

E. Passage time analysis of cell migration through pillar domains

The remainder of this article is dedicated to studying the passage dynamics of single cells from pillar domains and developing a model to approximate cell migration along the gradient, and then use this theory to discuss the origin and strength of the topotactic effect. Our approach is inspired by the empirical transition probability method used in Sec. IIC that projected cell motion on the x axis and analyzed migration along the gradient as a sequence of transitions across pillar columns. We start by investigating the transition dynamics of cell movement in and out of quadrilateral domains delimited by pillars [Figs. 5(a)–5(c)]. The average time to escape from an arbitrary point inside a predefined domain to the boundary

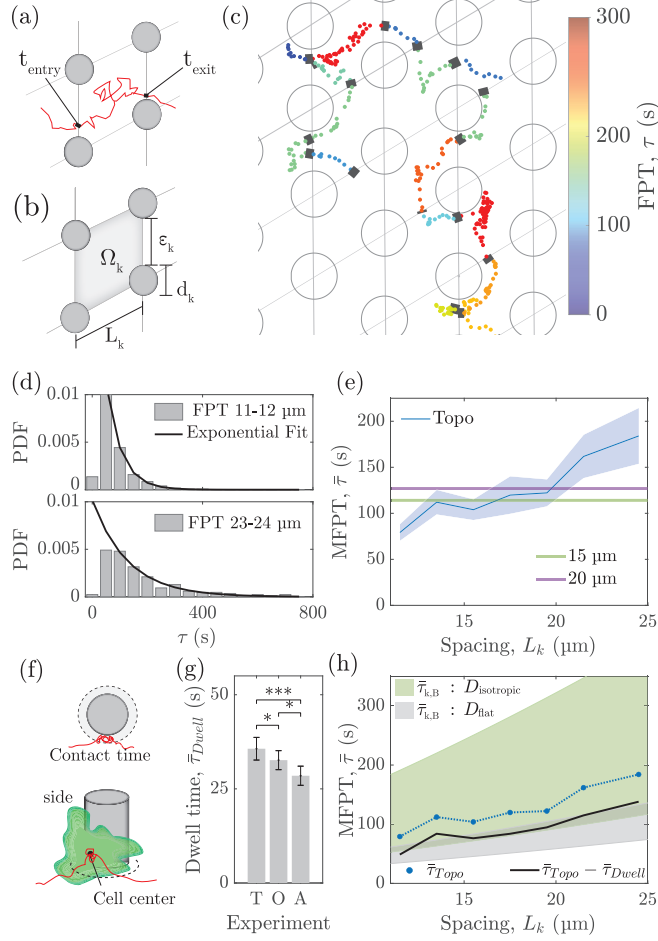


FIG. 5. MFPT analysis connects cell motion to the geometry of pillar fields. (a) The first-passage time (FPT, τ) is approximated as the time between entry and exit of a pillar domain. (b) Each pillar-delimited domain has an associated spacing L_k , surface area Ω_k , pore size ϵ_k , and pillar diameter d_k . (c) Example FPT measurement of a cell on the $15\ \mu\text{m}$ pillar field. Pillar positions are shown as gray circles and pillar-delimited domains are shown using gray lines. Each trajectory position is represented by a colored dot and domain transitions are marked by small blocks (dark gray). All trajectory points are colored according to the duration of passage (see color bar). (d) PDF with exponential fit of passage times measured on the T pillar field for small ($23\text{--}24\ \mu\text{m}$, MFPT = 79 s, Fit = 55 s) and large ($23\text{--}24\ \mu\text{m}$, MFPT = 184 s, Fit = 129 s) spacings. (e) All MFPTs measured on the topotaxis fields as a function of spacing L_k with isotropic fields $15\ \mu\text{m}$ (green) and $20\ \mu\text{m}$ (purple) overlaid (all no cAMP). (f) When the cell enters a subdiffusive state ($\alpha < 0.5$ using 1-MSD analysis), or the cell center is very close to the pillar wall (within a shell of $1\ \mu\text{m}$ away from wall), the duration of the state is recorded. The total dwell time adds all contact and subdiffusive states $\tau_{\text{dwell}} = \tau_{\text{pillarcontact}} + \tau_{\text{subdiffusive}}$. (g) Average dwell time $\langle \tau_{\text{dwell}} \rangle$ per domain passage for the three principal experiments. (h) The measured MFPT on the topotaxis field (dashed blue line) compared to the Brownian model $\bar{\tau}_{k,B}$ using 95% CI of diffusion constants on flat PDMS D_{flat} (gray) and on isotropic pillar fields $D_{\text{isotropic}}$ (green). When the measured MFPTs are adjusted using the $\bar{\tau}_{\text{dwell}}(k)$ per spacing (black line), the flat PDMS Brownian model is approximated.

of that domain is known as the first-passage time (FPT) τ . Here, we approximate the FPT as the duration between the entry t_{entry} and exit t_{exit} into and out of a pillar domain [Fig. 5(a)].

To measure the FPT times, the edges of each pillar domain were identified by finding the center of each pillar using bright-field image analysis and then computationally linking all the pillar centers. All cell passages through pillar domains were registered and used to calculate the passage times, $\tau = t_{\text{exit}} - t_{\text{entry}}$ (Fig. 5(a) and Movie S4 of Supplemental Material [49]). An example FPT calculation is shown in Fig. 5(c) for a cell moving through the isotropic $L = 15\ \mu\text{m}$ field. The FPT-PDFs of two different pillar spacings on the topotaxis field are shown in Fig. 5(d). Note that the distribution tails are well fit by a single exponential decay; this suggests that the escape from each domain is a Poisson-like process, an observation conserved over the entire pillar field. Figure 5(e) shows the MFPT, $\bar{\tau} = \langle \tau \rangle$, as a function of spacing for all experiments performed without a chemotactic gradient. The MFPT through a domain takes longer for larger spacings, and the MFPTs of the isotropic pillar fields (green, purple lines) are close to those of the topotaxis experiment (blue) at the same spacing.

It is instructive to compare the measured MFPTs $\bar{\tau}_k$ to the theoretical expected MFPTs if the cell were a purely Brownian particle. The persistence length of a migrating cell is of similar size to a pillar domain (Sec. II D) and passage through a domain is therefore around or beyond the typical onset of randomness. The MFPT $\bar{\tau}_{k,B}$ of a Brownian point particle escaping through (funneled) openings from a domain delimited by four pillars is [58]

$$\bar{\tau}_{k,B} = \frac{\pi |\Omega_k|}{4D\sqrt{2\epsilon_k/d}} \left(1 - O\left(\sqrt{\frac{2\epsilon_k}{d}}\right) \right) \approx \frac{\pi d^2 [(\sqrt{3}(L_k/d)^2 - \frac{1}{2}\pi)]}{8\sqrt{2}D\sqrt{L_k/d - 1}}. \quad (1)$$

Here, D is the diffusion constant of the particle and ϵ_k is the pore size between pillars of diameter d that delimit the surface area $|\Omega_k|$ in the k th domain centered at x_k [Fig. 5(b)]. As with the measured MFPTs, the theoretical MFPTs increase as pillars are spaced further apart, $L_k = \epsilon_k + d$, and the pores and pillar domain area $|\Omega_k| \sim L_k^2$ widen. Note that, as ϵ_k and $|\Omega_k|$ are fully defined by the lattice, the diffusion constant D is the only free parameter in case of fully Brownian motion.

The MFPTs $\bar{\tau}_{k,B}$, predicted by Eq. (1) and found by replacing the area and pore size with the pillar spacing L_k , are shown in Fig. 5(h) (gray) for the 95% CI of diffusion constants $D_{F,\text{eff}} = 1.7 \pm 0.5\ \mu\text{m}^2\ \text{s}^{-1}$ measured on flat PDMS (Fig. 8) and for the range of $D_{\text{Iso,eff}} = 0.9 \pm 5.0\ \mu\text{m}^2\ \text{s}^{-1}$ (green) measured isotropic pillar fields without cAMP (Table I). It is not surprising that the topotaxis MFPTs (dashed blue line) are longer than the $D_{F,\text{eff}}$ MFPTs (gray), as the cells interact with pillars in every domain, and the theory behind Eq. (1) assumes simple particle-pillar reflection inside the pillar domain. Moreover, the results in Sec. II D already showed that pillars can induce more complex long cell-pillar interactions or other subdiffusive states (Appendix B and Movie S5 in Supplemental Material [49]). The measured MFPTs on the

topotaxis assay lie on the faster side of the range of MFPTs when $D_{\text{Iso,eff}}$ are plugged in. Note that the cell MFPTs increase faster than the Brownian MFPTs.

To investigate whether the measured and $D_{F,\text{eff}}$ MFPTs agree better without taking into account contact and subdiffusive states, a “dwell time” τ_{dwell} was determined. The total dwell time was approximated as all time spent by the cell in either a subdiffusive state anywhere inside a pillar domain, or when in very close contact with a pillar [Fig. 5(f)]. Thereby, the total dwell time τ_{dwell} can be used to correct for the long cell-pillar interactions or stretched split-decision events observed during experiments (Appendix B and Fig. 9). The average τ_{dwell} per passage of the topotaxis (T), and multicue experiments (O and A), are shown in Fig. 5(g). Interestingly, when the cell is presented with a clear chemical signal (O and A), dwell times decrease and less time is spent in protracted “indecisive states.” Dwell-time-adjusted topotaxis MFPTs [Fig. 5(h), black line] approach the flat surface MFPTs (gray). The next section will investigate whether cells transitioning between pillar domains can be approximated as a Poissonian process, to assess the suitability of describing topotactic drift across the pillar field as a passage or jump process between zones of differing pillar spacing (and density).

F. First-passage times from single-pillar domains have memory

The measured MFPTs [Fig. 5(d)], presented in the previous section, exhibit exponentially decaying tails and suggest a memoryless process. As we will later model cell movement across the pillar field as a jump process between pillar domains, the absence of memory effects between domain passages is important in order to satisfy the Markov assumption of a jump process. The high degree of cell-to-cell and intertrajectory variability (Sec. II D) can obscure a true lack of memory in the measured MFPTs. To rigorously study memory effects and minimize ambiguity caused by cell-to-cell and intertrajectory variability, each FPT is normalized ($\tilde{\tau}$), using the averaged instantaneous velocity $\langle v_{0,i} \rangle$ during a passage i , and the spacing L_k to which the passage belongs:

$$\tilde{\tau}_i(k) = \tau \cdot \langle v_{0,i} \rangle / L_k. \quad (2)$$

The advantage of the dimensionless FPT is twofold; on the one hand, it provides a normalization to the continuously changing speeds caused by cell-to-cell and inter-trajectory variability, and on the other hand, it provides a way to compare relative passage times at different spacings through a connection to the pillar field.

The PDF of $\tilde{\tau}$ reveals a doubly peaked structure for all spacings, which indicates two characteristic timescales, as shown by an example distribution for a pillar field spaced $15 \mu\text{m}$ [Fig. 6(a)]. The first peak is formed by undercounting very short FPTs (<1) caused by experimental limitations in measuring very fast $<\delta t$ or short escapes $<0.1L_k$ (Sec. III E). The origin of the second peak is revealed by breaking down FPTs by their history of entry into the domain [Fig. 6(a), colors]. The FPT distribution actually consists of three separate contributions, related to the point of entry. Passages that

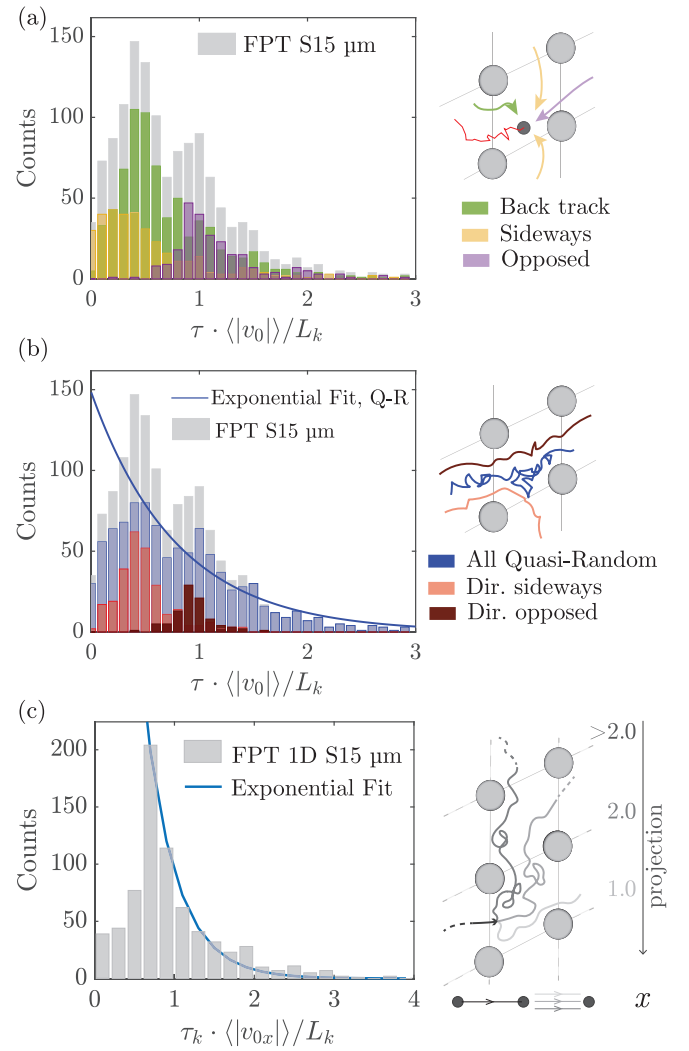


FIG. 6. Normalized FPTs reveal hysteresis in trajectory passage time analysis. (a) Histogram of all first-passage times (gray) on the isotropic $15 \mu\text{m}$ pillar field. Every passage recorded is multiplied by the average instantaneous velocity $\langle v_0 \rangle$ during the passage and divided by the pillar spacing (L_k) connected with the passage. The scaling reveals additional structure in the histogram that can be associated with the point of entry into the unit cell, which is the same (green), to the side (yellow), or opposed (purple) to the escape route. (b) Separating the normalized MFPT for predominantly directed (red) or quasirandom (blue) passages reveals the secondary peak, and thereby hysteresis, to be largely attributable to sideways (light red, $\langle \dots \rangle = 0.53$) and opposed (dark red, $\langle \dots \rangle = 1.2$) directed passages. The collection of predominantly quasirandom passages (blue, $\langle \dots \rangle = 1.0$) approaches a Markov process with a Poissonian probability function as, shown by the exponential fit (blue line, $\tilde{\tau}_k = 0.78$). (c) Normalized MFPTs of trajectories projected on the x axis for the $15 \mu\text{m}$ pillar field (gray, $\langle \dots \rangle = 1.7$). Peaks found in the MFPT distribution for single-pillar domains disappear for the x projection. 1D-passage times are often compounded from multiple passages in 2D (see schematic). Values below 0.5 are underrepresented due to technical limitations (frame rate, analysis resolution) and the influence of directed runs. The tail of the τ distribution shows good agreement with an exponential fit (blue line, $\tilde{\tau}_k = 0.40$).

backtrack (yellow) or go sideways (green), as measured from point of entry, make up the first peak (and its tail) of the $\tilde{\tau}$ distribution. Note that the *sideways* distribution itself also has a small second peak, caused by a difference in distances to the side of entry. However, FPTs measured for passage to the opposite side (purple) are the major contributor to the second peak.

Further categorization of the FPTs by dominance of either directed or quasirandom motion states during passage reveals that the peaks can be attributed to passages dominated by directed motion in opposed and sideways destinations, as is apparent from Fig. 6(b). This is expected, as for a directed run the point of origin and initial direction, i.e., the history of the trajectory, determines the most likely exit from a pillar domain. An example trajectory with long directed states that traverse multiple pillar domains was presented in Fig. 4(a). When directed contributions are subtracted, however, the remaining quasi-random FPT distribution is near featureless and shows a smooth exponential decay, indicating an almost Poissonian, memoryless process for these escapes [blue line, Fig. 6(b)]. There are more quasirandom than directed motion-dominated passages through domains, hence series of subsequent passages through a couple of pillar domains is more likely to present a process without memory.

If domain passages are projected on the one-dimensional (1D) axis of the spatial gradient, each passage in 1D may consist of multiple passages in 2D. This is illustrated in Fig. 6(c); a 1D escape may involve multiple passages perpendicular to the axis of projection. Moreover, as multiple passages are involved, memory effects in 1D-FPT distributions decrease. Figure 6(c) shows the 1D FPTs for a spacing of 15 μm , and indeed, the secondary structure has mostly disappeared. Therefore, it is reasonable to approximate series of passages projected on the 1D axis of the spatial gradient by a Markov process. In the next section, we exploit this finding and introduce a Markov model to predict the observed topotactic drift generated by the gradient pillar fields.

G. Modeling topographical guidance as a jump process

Thus far, we showed that highly motile, persistent-random amoeboid migration in an anisotropic pillar field results in topographical guidance (Sec. IIB). This process is conserved during persistent-random chemotaxis, suggesting long-range topotaxis is an independent physical effect (Sec. IIC). We then showed that the experimentally obtained MFPT increases for pillar-delimited domains as a function of pillar spacing (Sec. IIE). Furthermore, the pillar field induces additional randomness in cell movement (Sec. IID), making passage through pillar domains of all spacings close to a process without memory (Fig. 6). This means that on a slightly larger scale, e.g., on the scale of multiple pillar domains, sequences of transitions can be approximated by a Markov process. This scale is realized when domain passages are projected on the one-dimensional direction of the gradient [Sec. IIF and Fig. 6(c)].

Long-range topotaxis is a result of the combination of cell movement and the supercellular anisotropy, formed by a change in spacing of cell-sized pillars. We build an analytical model based on three observations: (1) the MFPTs

increase as a function of pillar spacing, (2) the MFPTs can be used to empirically determine transition rates, and (3) the transitions between stripes of pillar domain passages can be approximated by a Markov process. Departing from these observations, topographic drift is modeled by coarse-graining cell motion as a jump process across zones m of constant pillar spacing L_k [Fig. 7(a)]. The pillar zones m consist of multiple quadrilateral domains k . Therefore, in order to connect motion within pillar domains to a jump process between zones m , cell motion across the pillar field is coarse-grained twice. First, cell motion is coarse-grained as a jump process between zones m , with varying passage rates λ_m . Then, movement within each zone is connected to the motion inside single-pillar domains by a second coarse-grained jump process over four individual domains [Fig. 7(a)]. In the y direction, adjacent quadrilateral domains are always identical and no drift is expected.

The probability $p_m(t)$ that a moving cell is in pillar zone m at time t satisfies and is described using the master equation of a (possibly biased) random walk. Either the cell moved into pillar zone m from zone $m-1$ during Δt , with associated rate $\lambda_{m-1 \rightarrow m} = \lambda_{m-1}$, from the zone $m+1$ with rate $\lambda_{k+1 \rightarrow k}$, or the cell did not transition $1 - \lambda_m \Delta t$ out of zone m . Then, the master equation for the transition probability density function of a random walk on the gradient with exponentially distributed waiting times in and out of each zone is

$$\dot{p}_m(t) = \lambda_{m-1} p_{m-1}(t) + \lambda_{m+1} p_{m+1}(t) - \lambda_m p_m(t). \quad (3)$$

To derive a coarse-grained equation on the topotaxis lattice, the probability density is expanded in space $p_m(t) = p(x_m, t)$ using a Taylor expansion (Appendix C). Using Eq. (3) and remembering $\lambda_m = 1/(2\tau_m)$, the coarse-grained Fokker-Planck equation on the lattice points x_m is obtained,

$$\begin{aligned} \dot{p}_m(x_m, t) = & -a(L_m, \tau_m) \frac{\partial p(x_m, t)}{\partial x} \\ & + D(L_m, \tau_m) \frac{\partial^2 p(x_m, t)}{\partial x^2}, \end{aligned} \quad (4)$$

where L_m , L_{m+1} , and L_{m-1} are the respective pillar zone widths, $D(L_m, \tau_m)$ is the diffusion tensor, and the drift $a(L_m, \tau_m)$ is given by

$$a(L_m, \tau_m) = \frac{1}{2} \left[\frac{L_m + L_{m-1}}{2\tau_{m-1}} - \frac{L_m + L_{m+1}}{2\tau_{m+1}} \right]. \quad (5)$$

Hence, the drift in pillar zone m is a function of the difference in waiting (passage) times (τ_{m-1} and τ_{m+1}), and in width between adjacent pillar zones (L_{m-1} and L_{m+1}). When $(L_m + L_{m-1})/2\tau_{m-1} > (L_m + L_{m+1})/2\tau_{m+1}$, as measured on the topotaxis field (Sec. IIE), there is drift in the positive x direction toward the side of the field with an increase in pillar spacing.

The next step is to connect the passage times from each pillar zone m , and thereby any net drift, to transitions of cells between quadrilateral pillar domains k . Within each pillar zone, cell movement projected onto the one-dimensional gradient direction is a jump process between four pillar domains, as the spacing L_k changes after every fourth pillar column [Fig. 7(a), box m]. As the pillar domains are identical within a zone, all transition rates from the domains are the

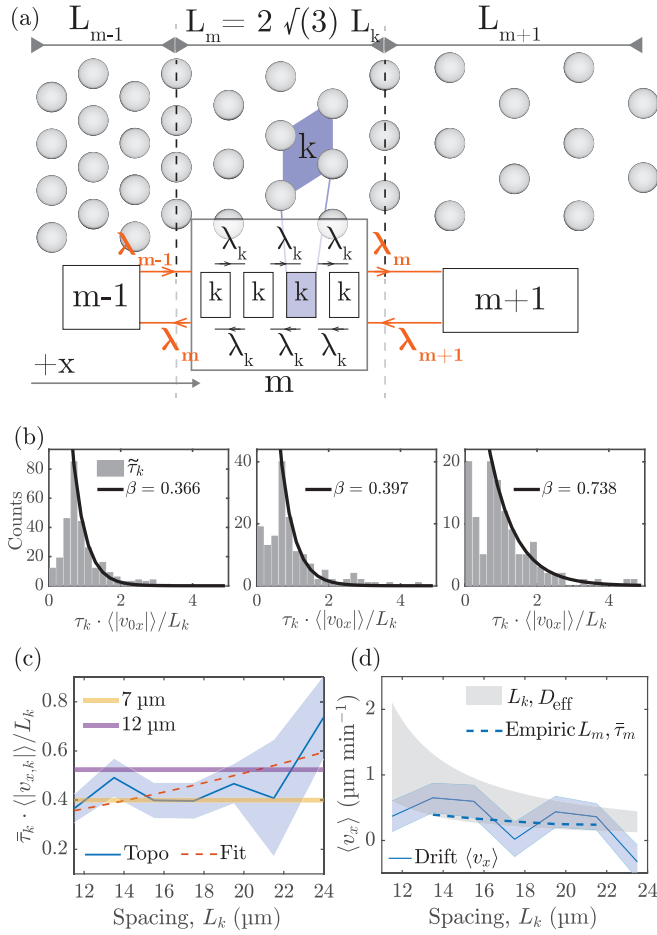


FIG. 7. Modeling topotactic drift using a jump process. (a) Cell motility in the pillar gradient is coarse-grained twice. First, as a jump process in 1D between bands of equal interpillar spacing L_k , by projecting cell motion between quadrilateral pillar domains k in 2D, as a series of passages in 1D (small boxes k) with passage time (τ_k) and rate (λ_k). Then, cell motion between pillar domains is coarse-grained a second time to a 1D jump process between zones m of width L_m (dissected by dashed lines), with a passage time (τ_m) and rate (λ_m). (b) 1D-projected and dimensionless MFPTs are longer for increased pillar spacing, as the example distributions show for spacings 13–14, 17–18, and 21–22 μm on the T field. Small $\tilde{\tau} = 0$ –0.5 are underrepresented in the histogram due to technical limitations, like frame rate and image resolution. Fit parameters of exponential fits (black line) are inset in the histograms. (c) MFPTs measured on the T field (blue) as a function of pillar spacing, with a smoothed approximation as a function of pillar spacing (red). The MFPTs of isotropic 15 (yellow) and 20 μm (purple) fields are shown for comparison. (d) Drift velocity on the T field (blue) overlaid by drift of empirical model (dashed blue line) using $\tilde{\tau}$. Drift for different values of MFPTs of diffusive escapes from domains, estimated using a wide range of effective diffusion constants $D_{\text{eff}} = 0.9(5) \mu\text{m}^2 \text{s}^{-1}$ (gray), and Eq. (1).

same $\lambda_k = 1/2\tau_k$. Using a continuous Markov model with four states and one absorbing state, and in the limit of an initial uniform distribution over the states (assuming large population statistics), the MFPT from a pillar zone m in terms

of a pillar domain k , is given by (Appendix C)

$$\bar{\tau}_m = \frac{\bar{\tau}_{\text{out}}}{2} = \frac{15}{4\lambda_k} = \frac{15\bar{\tau}_{k,1D}}{2}. \quad (6)$$

Here, $\bar{\tau}_{k,1D}$ is the MFPT from a column of quadrilateral domains k projected on the x axis [Fig. 6(c)], and $\bar{\tau}_m = \bar{\tau}_{\text{out}}/2$ is due to the symmetry of either escaping to the left or right of m . Finally, we can now directly relate the drift $a(L_m, \tau_m)$ in zones of equal spacing [Eq. (5)] to the pillar spacing L_k and the diffusion constant D of the cells. By substituting the zone width with the pillar spacing $L_m = 2\sqrt{3}L_k$, assuming a constant gradient strength ($\Delta = L_k - L_{k-1} = L_{k+1} - L_k$), and using the MFPT from a zone [Eq. (6)], we find the drift of cells along the topotactic gradient to be

$$a(L_k, \Delta, \tau_k) = \frac{\sqrt{3}}{15} \left[\frac{2L_k - \Delta}{\bar{\tau}_{k-1}(L_k, \Delta)} - \frac{2L_k + \Delta}{\bar{\tau}_{k+1}(L_k, \Delta)} \right]. \quad (7)$$

The drift expression $a(L_k, \bar{\tau}_k)$ in Eq. (7) predicts topotactic drift of a population of cells along the gradient direction of a pillar field with increasing spacing if MFPTs are observed to increase. Equation (7) is also useful for identifying the main parameters influencing the drift on the level of a single-pillar domain, as it depends on both the gradient strength Δ and the difference in MFPTs between the pillar domains to the less spacious side $\bar{\tau}_{k-1}$, and to the more spacious side $\bar{\tau}_{k+1}$. The difference between these two MFPTs is likely nontrivial, as the passage of cells through domains is a complex interaction between factors like the cell morphology, cell-surface adhesion, motility, directional persistence, and possible biological functions influenced by the size of the domain (e.g., self-chemotaxis [59]). The next section will examine the validity of our model by comparing the measured topotactic drift per pillar spacing to predictions of the drift using empirical MFPTs.

H. Empirical prediction of topotactic drift by MFPTs analysis

The model introduced in the previous section provides a method of predicting the net topotactic drift from MFPT statistics (Sec. II G and Appendix C), as the drift can be predicted by relying on the Markov assumption for transitions between pillar domains and using the associated drift [Eq. (5)]. The pillar zone MFPTs can be approximated by substituting the experimentally determined individual pillar domain MFPTs $\bar{\tau}_{k,1D}$ [Eq. (6)], which need to be determined for all interpillar spacings to obtain a smooth drift approximation. This section will first develop a smooth estimation of $\bar{\tau}_{k,1D}$ per-pillar spacing k , based on cell trajectory measurements on the pillar field, and then compare the empirical drift predictions to the measured drift.

The previously introduced dimensionless FPT distributions will be the basis of developing a smooth approximation of all $\bar{\tau}_{k,1D}$, and are shown in Fig. 7(b) for three regimes measured on the T field (13–14, 17–18, and 21–22 μm). The tails of these dimensionless $\tilde{\tau}_{k,1D}$ distributions were fit to an exponential decay $f(\tau_k) = A \cdot e^{1/\beta \cdot \tau_k}$ (black line), where β is the rate parameter used to estimate $\tilde{\tau}_k$. The duration of the dimensionless FPT distributions increases as a function of pillar spacing L_k . All MFPTs $\tilde{\tau}_k$ found for the topotaxis experiments are plotted in Fig. 7(c) (blue), as well as the

results for the two isotropic pillar fields (green and purple). In order to get a smooth approximation for the empiric MFPTs on the topotaxis field as a function of pillar spacing, the binned values per two pillar spacings were fit by $f(L_k) = A \cdot L_k^{1.5} + B$ (red dashed line). The validity of the estimation scheme is supported by the intersection of the fit (red) with the measured MFPTs for the isotropic fields at the appropriate spacings of 15 and 20 μm , as these measurements have the richest statistics.

The measured topotactic drift $\langle v_x(k) \rangle_T$ can now be compared to drift predictions via the MFPT model using the smooth approximation from the previous paragraph. Using the MFPT per pillar spacing approximated in the last paragraph [Fig. 7(d), dashed line], and the width per pillar zone L_m , the expression for the drift $a(L_m, \tau_m)$ from Eq. (5) can be compared to $\langle v_x(k) \rangle_T$. The drift predicted by $a(L_m, \tau_m)$ [Fig. 7(d), dashed line] over- and underestimates the drift for some spacings, mostly keeping the middle ground across the entire field, as such, it demonstrates the validity of an MFPT description of pillar gradient-generated topotaxis. It is also instructive to compute the drift if individual MFPTs $\bar{\tau}_{k,1D}$ from pillar domains would be determined by a range of differing effective diffusion constants $D = 0.9 \pm 5.0 \mu\text{m}^2 \text{s}^{-1}$ [gray, Fig. 7(d)]. This description approximates the drift reasonably well for a range of spacings, given that movement of the starved *Dd* amoeba is notably non-Brownian, and given the complex cell-pillar interactions that take place within each pillar domain (Secs. IID and IIE).

I. Discussion and conclusion

We investigated long-range topotaxis in triangular micropillar fields for highly motile amoeboid migration, using starved *D. discoideum* as a model organism. The (subtle) topotactic cue was generated by a change in pillar spacing after every fourth pillar in the gradient direction, resulting in spatially dependent differences in enclosure and pore size. This type of topotaxis acts at long range, as changes in pillar spacing occur at a length scale much larger than the cell body. In such a system, the cell body lacks continuous environmental feedback on the gradient, as it is not always in contact with the topographical asymmetry. The gradient was found to generate a topotactic drift v_{topo} of around 5% of overall motility (quantified by $\langle v_0 \rangle$), which leads to an average directed displacement of around one cell body per half an hour.

Recent *in vitro* studies on the topographical guidance of eukaryotic cells can be divided into two categories, based on the typical length scales of the anisotropy (or local asymmetry) under consideration. The first category consists of systems that model the topography of collagen and fibronectin fibers and fibrils, by presenting asymmetries at the (sub)micron scale to cells, like experiments with nanoposts [22,23] or micron-sized grooves and ridges [20,21,24]. The second category is made up of systems with topographical asymmetries of around cell size, like skewed hills or ratchets [7,17,25], which mimic the large-scale topographical structures cells encounter in nature, like the differences in tissue density encountered by patrolling macrophages [31], or cancer cells migrating from a tumoroid to a blood vessel [34,46]. Our pillar assay falls at the very end of the second category, with pillars of cell size,

and a supercellular asymmetry on a length scale 1–2 orders of magnitude larger than systems aiming to model differences in fiber topography. Recent work on ratchetaxis studied a similar length scale, but instead of giving occasional topotactic feedback to the cells, provided uninterrupted environmental bias to cells using cell-sized ratchet units, resulting in local directional memory [10]. In future work, it would be interesting to explore the potential of ratchetaxis at a much larger scale, by studying cell trajectories across series of gradient pillar fields and the transition dynamics at the joining edges.

Surprisingly, long-range topotaxis for highly motile cells was found to be conserved in a multicue environment. This was shown by recording cell migration through the (gradient) micropillar fields while exposing cells to a gradient in cAMP, a strong chemoattractant for *D. discoideum*. We aligned and opposed the chemotactic gradient to the topotactic pillar fields, which resulted in a higher and lower drift than measured for the same chemotactic gradient on isotropic pillar assays. This result was investigated further by calculating the differences in drift response between the aligned and opposed assays as a function of pillar spacing, which gave an independent reconstruction of the topotactic drift measured without a cAMP gradient present. This comparison showed that the topotactic and chemotactic drifts add linearly, indicating that subtle topotactic guidance effects can also play a role in complex physicochemical environments (e.g., *in vivo*) for highly motile cell migration.

Relatively few *in vitro* cell migration experiments have been performed with two well-controlled simultaneous stimuli [2,17,38,60–62]. Additionally, these studies focused on cell-palpable multicue environments, combining haptotaxis with ratchetaxis [17], or durotaxis [2,62]. Although not explicitly discussed in their study, the migratory data presented in Comelles *et al.*, consisting of both ratchets and a fibronectin gradient, also seemed to indicate linear addition of cues, similar to our findings. In another study, Maout *et al.* also reported antagonistic effects between chemotactic and ratchetaxis [61]. It is important to note that the assay of Comelles *et al.* also does not confine cell motion to one direction, similar to our assay, unlike many other forms of topographical guidance studied [7,15,24,61,63]. It would be worthwhile to investigate whether other types of topographical guidance also add linearly to competing cues like chemotaxis, in order to better understand their influence in complex *in vivo* environments.

After analyzing the measured drifts, we investigated the origin of the topotactic response by using FPT analysis. This analysis examines cell migration across the pillar field as sequences of escapes from enclosures (domains) of varying pore size and surface area. The pillar field was computationally dissected into quadrilateral domains, and the MFPT of cells migrating between domains was measured as a function of pillar spacing L_k . The MFPT was found to increase as a function of pillar spacing ($\sim L_k^{1.5}$). Taking inspiration from this result, topotaxis in the gradient pillar field was modeled as a jump process between zones (and domains) of equal pillar spacing. This MFPT model successfully predicts the topotactic drift as a function of pillar distance and gradient strength. Moreover, using an approximation of the MFPT of a Brownian particle and different effective diffusion constants for motion inside the pillar domains also resulted in a drift.

However, this result does not automatically equate to drift for a population of Brownian particles in the gradient pillar field, as this is dependent on the boundary conditions on the edges of field.

The empirical model coarse-grained topotactic drift on the level of single passages (or escapes) from differing quadrilateral pillar domains. The exact interplay of factors that determine motion within single domains is likely dependent on many interacting cell and substrate properties that may or may not change as a function of pillar spacing, like cell morphology, cell-pillar adhesion, overall motility, directional persistence, long-lived cytoskeletal asymmetries [64], and possible biological processes influenced by the size of the domain (e.g., self-chemotaxis [59]). The sudden drop in migratory drift on the topotaxis field at the crossover length scale could be an example of the influence of biological processes, inducing a preference of cells for average cell-diameter-sized enclosures, as the social amoeba in the *D. discoideum* in the starved state is driven to find neighboring cells and form multicellular aggregate mounds to prepare for sporulation [29,65].

Since the publication of the preprint of this paper [66], we have investigated three factors numerically (persistence length, cell body deformation, and actin-polymerization disruption) that influence passage times within pillar domains, i.e., at a finer granulation, to complement the empirical model presented here. Using active Brownian particles, we showed that topographical pillar gradients introduce a spatial modulation of the particles' persistence, leading to directed motion toward regions of higher persistence [67]. Furthermore, using cellular-Potts modeling we showed that pillar-mediated cell body deformation and collision-induced reorientation contribute to higher topotactic drifts [68]. This numerical work also supports the hypothesis that topotactic drift over long distances in the gradient field is generated by positionally dependent effective diffusion, as suggested by experimental results on isotropic triangular pillar fields here, where $D_{\text{eff}, 15 \mu\text{m}} < D_{\text{eff}, 20 \mu\text{m}}$ (Table II). Drift in stochastic motion caused by positional variations in particle diffusion is a well-known effect, and has been used to develop models on different types of taxis, like run-and-tumble bacterial chemotaxis [69], durotaxis [70], or topotaxis [67,71]. The strong dependence of the topotactic drift on many random interactions with a locally changing topography, and some effective diffusion constant over long times, also explains why the drift is conserved for (relatively shallow [39,40]) cAMP chemotaxis. If a chemotactic response would eliminate all stochasticity in cellular motion, leading to fully ballistic trajectories toward the source, any long-range topotactic drift would be unlikely (beyond some lateral displacement, see, for example, deterministic lateral displacement [72]).

Concluding, we showed that subtle, long-range topographic anisotropies can significantly influence the chemotactic response of cells, due to linear addition of the effects of both stimuli. This observation is not only relevant for *in vitro* applications, like tissue engineering [73] and cell sorting [74], but also when studying highly motile amoeboid migration *in vivo*, like leukocyte migration [31] and tumor cell detachments [41]. While studies *in vivo* are usually focused on chemotaxis, our results suggest that the tissue

topography can play a role as well, and that it might even be necessary to correct for its effects when studying cell migration.

III. MATERIALS AND METHODS

A. Cell culture and imaging

Axenic *D. discoideum* (Ax2) with a cytoplasmic green fluorescent protein (GFP) insertion (strain HG1694, obtained from Dr. G. Gerisch, MPI for Biochemistry, Germany) was used for experiments. Cells were grown at 20 °C in HL5 medium, supplemented with 20 $\mu\text{g ml}^{-1}$ gentamicin (Gentamycin solution, Merck, Netherlands) as a selection antibiotic. The cells were cultured in 100 mm Petri dishes (100 mm TC-treated culture dish, Corning, USA), and confluency was kept below 70% during culturing.

To prepare for imaging, cells were harvested and centrifuged at 1500 rpm for 3 min followed by three successive washing steps of the cellular pellet with 17 mM K-Na phosphate-buffered saline (PBS), adjusted to pH = 6.0 (Sigma Aldrich, Steinheim, Germany). After resuspension in PBS, the cells were placed in a conical tube in a shaker for 1 h to settle. Then, to induce cAR1 expression, cells were pulsed with 150 nM cAMP applied in 6 min intervals over 4 h while shaking. After pulsation, any residual cAMP was removed by centrifugation and following subsequent resuspension, cells were left to shake in a conical tube with PBS for another 30 min.

Postpulsing, cells were inserted into a microfluidic observation chamber. Cell suspension was added to the chamber (sticky Slide I 0.8 Luer: sterilized, Ibidi, Gräfelfing, Germany) until a concentration of 5–10 cells per 360 by 360 μm (camera field of view). Imaging was started 1 h after cell seeding. In case of a combined gradient, cAMP was pumped (Tubing: BIO-rad, Hercules, USA. Syringe: 710 LT, Hamilton, Bonaduz, Switzerland) into the diffusion reservoir of the microfluidic channel and let to diffuse (see Appendix E), establishing a chemical gradient.

Cells were imaged every 8–10 s for 1–2 h on a Nikon Eclipse Ti microscope equipped with a Yokogawa confocal spinning disk unit operated at 10 000 rpm and a motorized stage (Nikon, Tokyo, Japan). GFP was excited by a 488 solid state diode laser (Coherent, Santa Clara, CA, USA), supported in an Agilent MLC4 unit (Agilent Technologies, Santa Clara, CA, USA). Emission was filtered by a quadband fluorescence filter (TR-F440-521-607-700, IDEX LLC, Rochester, New York, USA). Images were captured by an Andor iXon Ultra 897 high speed EM-CCD camera (Andor Technology, Belfast, Northern Ireland), through an NA 0.75 20x air objective (CFI Plan Fluor, Nikon, Tokyo, Japan) and exported to a .tif format using NIKON software (NIS Elements 4.6, NIKON, Tokyo, Japan).

The two scanning electron microscopy (SEM) images [Figs. 1(c) and 1(d)] of cells on the pillar field were imaged with a nanoSEM (FEI/Thermo Fisher, Eindhoven, The Netherlands) at 10 kV with a spot size of 4.0. Before imaging, cells were seeded as described above and fixed according to a previously published protocol [75] and critically point dried.

B. Fabrication of pillar gradient structures

The pillar molds were made using two different lithography techniques. The pillar gradient field molds were made as previously described in Ref. [43] using standard photolithography techniques. Instead, the molds of isotropic pillar fields were produced by two-photon lithography using a nanoscribe photonic professional GT and IP-S resin (Nanoscribe GmbH, Eggenstein-Leopoldshafen, Germany), as described in Ref. [55]. The nanoscribe molds were developed in polyglycidyl-methacrylate (PGMA), according to the standard Nanoscribe protocol.

Similar to the protocol in Ref. [43], the casts were made with PDMS (Sylgard 184 Silicon Elastomer Kit, Dow Corning, USA) mixed 1:10, spin coated to a thickness of approximately 250 μm , and baked 4 h at 110 $^{\circ}\text{C}$. Before PDMS coating, wafers with (and without) pillar molds were silanized by trichloro(1H,1H,2H,2H-perfluorooctyl) silane deposition under vacuum (50 mbar) for 1 h to ensure proper PDMS detachment. Flat PDMS surfaces were made by spin coating PDMS on test-grade wafers (Siegert Wafer GmbH, Aachen, Germany) to a thickness of 50–200 μm . Finally, to prepare for cell seeding, PDMS surfaces were hydrophilicized by 10 min of UV/ozone exposure (UVO-42, Jelight Company, Irvine, CA, USA), thrice washed with ethanol (70%) and PBS. The PDMS surfaces and pillar fields were placed at a distance of 20 ± 0.25 mm from the inlet of the microfluidic channel (0.8 Sticky-Slide I Luer, Ibidi, Martinsried, Germany).

C. Establishing the chemotactic gradient

The PDMS pillar fields were arranged either in parallel or antiparallel to the direction of a cAMP gradient inside a microfluidic chamber. To achieve an optimal chemotactic response from the cells, the cAMP gradient (∇c) was kept between 0.001 and 10 nM μm^{-1} during experimentation. The gradient was set up by the injecting 40 μl of 0.1 mM cAMP in PBS into the diffusion reservoir on the appropriate side of the microfluidic. Subsequently, cAMP was let to diffuse for a set, predetermined time. The precise volume and diffusion time needed were calibrated using a fluorescent dye (Alexa fluor 488 Hydrazide, Thermo-Fisher, Waltham, USA), which has a similar molecular weight to cAMP, 570.48–329.20 Da, respectively [Figs. 12(a)–12(d)]. Additionally, finite element simulations were performed with COMSOL MultiPhysics (COMSOL BV, Zoetermeer, The Netherlands) to investigate the stability of the gradient over time during experiments, to determine suitable injection concentrations, and to explore any effects of the pillar geometries on setting up and maintaining a chemotactic gradient by diffusion [Figs. 12(e)–12(h)].

D. Cell tracking and trajectory analysis

Cell movement was extracted from microscopy measurement data by a number of image analysis steps. First, each exported TIF image (NIS Elements, Nikon Instruments, Amstelveen, The Netherlands) from the acquired time series (with δt time between frames) was prepared for the CellEvaluator plugin [76] by intensity thresholding, despeckling, and a Gaussian blur using FIJI [77]. Then, CellEvaluator was used to determine and link center-of-mass xy coordinates of each

cell for each frame and thereby create cell trajectories $R(t) = (x(t), y(t))$. The trajectories were further analyzed with an in-house MATLAB code (version 2021b, The Mathworks, Inc., Natick, MA, USA) to couple the recorded displacements $\bar{r}(t_i) = \bar{R}(t_i + \delta t) - \bar{R}(t_i)$ to registered imaging times t_i , and to calculate general motion statistics, like instantaneous velocities v_{in} , and TAMSD. A TAMSD selection criterion was applied to select for trajectories belonging to starved cells, and prevent biased results by including still vegetative, dying, PBS shocked [29], or otherwise immotile cells in the dataset. All cell trajectories with a TAMSD of $\langle \Delta R^2(\tau_{\text{crit}}) \rangle \leq (5 \mu\text{m})^2 \pi$ at a lag time of $\tau_{\text{crit}} = 200$ s, i.e., escaping the surface area of a cell [50], were deemed too passive to be of interest for this motility study and discarded.

The migratory drifts were computed using the mean velocity $\langle v_{x,y} \rangle$ in either the x or y direction,

$$\langle v_{x,y} \rangle = \frac{1}{n-1} \sum_{i=1}^{n-1} (\bar{R}(t_i + \delta t) - \bar{R}(t_i)), \quad (8)$$

where n is the total number of displacements measured. The drifts were either averaged for all displacements of all trajectories measured per experimental configuration [Fig. 2(c)], or averaged per pillar zone m and pillar spacing L_k [Figs. 1(m)–1(o)]. Error estimates were based on 95% CIs using a t distribution, $x \pm t \cdot s/\sqrt{n}$. Here, t is the upper critical value for t distribution [$t = F^{-1}(p|v)$], where $p = 0.025$ and 0.0975 was used, and degrees of freedom were set at $v = n - 1$, where n is the sample size. Drift distributions were found to be non-normal by Kolmogorov-Smirnov and Lilliefors testing; as such, the Kruskal-Wallis test by ranks ($\alpha = 0.05$) was used to determine whether samples originated from different distributions. To determine pairwise statistical significance, a *post hoc* Dunn's test was used on independent groups.

For this study, the l-MSD analysis was adapted for cell trajectories belonging to highly motile cells imaged at low frame rate. L-msd analysis was previously applied to the motility of vegetative *D. discoideum* [43], where the time between frames (δt) was short relative to changes in motion states of the trajectory, allowing for a sliding window with size $M = 30$. Starved *D. discoideum* have a speed at least three times higher than vegetative cells [42,43], but ΔT needs to stay similar to experiments with vegetative cells in order to prevent cytotoxicity, the l-MSD algorithm was adapted.

As a quick summary, for every time point t_i in the trajectory, representing the center of a rolling time window ($T = M\delta t$), the local MSD is fit to a local lag time:

$$\Delta R^2(t_i, \tau_k) = A \cdot \tau_k^{\alpha_i}. \quad (9)$$

The exponent in Eq. (9) characterizes the migratory state of the cell at time t_i . When $\alpha_i \leq 2 - \sigma_\alpha$, the cell is marked to be in a quasirandom motion state, whereas between $\alpha_i > 2 - \sigma_\alpha$ it is marked to be in a directed state. Following previous work [43,57], the uncertainty in motion state was set to $\sigma_\alpha = 0.3$. The prefactor A is either an effective diffusion coefficient analog when in a quasirandom motion state, or can be interpreted as the velocity in the case of directed motion. Contrary to Refs. [43,57], no additional persistence functions were applied in combination with Eq. (9). Additionally, as starved

D. discoideum changed motion states more often than vegetative cells, and have a higher v_{in} , the size of the sliding window was reduced to $M = 12$. The lag time was rescaled proportionally $\tau_i = 4\delta t$, to maintain an optimal τ_i to M relation [78].

E. Cell motion as a function of topography

To analyze the behavior of cells as a function of topography, the pillar field was either dissected into columns of equal pillar spacing [Fig. 3(b)] or divided into quadrilateral domains as defined by the pillar lattice [Fig. 5(c)]. For both methods, the position of each pillar was identified using bright field microscopy, enabled by the increase in intensity of each pillar by light-guiding effects through the pillar axis. Pillar images were recorded immediately before and after each live cell experiment to also check for any measurement drift. After processing the images (binarizing and applying a Gaussian blur $\sigma = 2$), pillars were identified using appropriate Sobel filters by a custom matlab algorithm.

For pillar fields with a gradient in spacing, the design was fit to the pillars found, in order to label each part of the field of view by the appropriate spacing. For 1D FPTs and transition probabilities, the imaging positions were dissected into vertical pillar columns of equal spacing. The pillar columns were formed by linking all pillar centers in the y direction by a linear fit. Pillar columns (in $\pm y$) were also used to correct for any small misalignments between the sample (lab frame) and the x -gradient direction, by rotating images before cell tracking. The 2D-FPT analysis required sectioning the imaging positions in quadrilateral domains, achieved by computationally linking all pillar centers to their four nearest neighbors, thereby dividing the pillar field into domains delimited by pillars. A transition between pillar domains or columns was recorded if the cell center crossed the dividing line by at least $0.1 \cdot L_k$. As the topotaxis fields change spacing, lattice defects are formed during quadrilateral sectioning. These defects lead to irregularly shaped small and large pillar domains; any passages from such regions were discarded from the analysis.

For transition probabilities between different pillar spacings [Figs. 3(d)–3(f)], all transitions of cells from pillar column to column were counted. Transitions were then separated in going toward the more spacious ($+x$, $S_{k,spacious}$) or narrower ($-x$, $S_{k,narrow}$) part of the array. The transition probabilities were then calculated by $P_+ = S_{k,spacious}/S_{total}$ or $P_- = S_{k,narrow}/S_{total}$ as a function of pillar spacing L_k . The difference between these probabilities is a measure for the topotactic response, $\Delta P_T = P_{+,T} - P_{-,T}$, and chemotactic response $\Delta P_{cAMP} = P_{+,cAMP} - P_{-,cAMP}$, of the cells. These probability differences can then be used to find an expression for the multicue response,

$$\Delta P_O = \frac{\alpha}{2}(\Delta P_T + 1) - \frac{1-\alpha}{2}(\Delta P_{cAMP} + 1), \quad (10)$$

$$\Delta P_A = \frac{\alpha}{2}(\Delta P_T + 1) + \frac{1-\alpha}{2}(\Delta P_{cAMP} + 1). \quad (11)$$

Here, the expression for ΔP_T and ΔP_{cAMP} were mapped onto $[0, 1]$ by the prefactors to keep those differences between $[-1, 1]$ and easily comparable. The factor α allows for a

difference in contribution of both gradients in these multicue expressions and was found to be $\alpha = 0.47$.

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DATA AVAILABILITY

The data are available from the corresponding author upon reasonable request.

APPENDIX A: MOTION OF STARVED *D. DISCOIDEUM* ON FLAT PDMS SURFACES

To benchmark cell movement through anisotropic pillar fields embedded in chemotactic gradients, the motility of *D. discoideum* was first characterized on flat PDMS surfaces. Recent studies of *D. discoideum* motility have mostly focused on the vegetative state of the amoeba [43,79]. However, in the starved state, cells move markedly different in their search for cAMP gradients [40]. Like previous work studying starved cells on non-PDMS surfaces [42], we also measured an increase in both speed and persistence of the cells relative to vegetative *D. discoideum*.

When in the starved state, cells chemotax toward cAMP sources, but when devoid of such a cue, migrate randomly to pick up on a signal. As a consequence of their search strategy, cell trajectory statistics (at low cell density) closely resemble those of persistent-random walkers (PRW). The MSD of a PRW is [80]

$$\langle r(t)^2 \rangle = 2v_c^2 \tau_p^2 (t/\tau_p + e^{-t/\tau_p} - 1), \quad (A1)$$

where v_c is a constant velocity and τ_p is the persistence time of the walker. On short times ($t \ll \tau_p$), Eq. (A1) simplifies to $\langle r(t)^2 \rangle = (v_c t)^2$ and at long times ($t \gg \tau_p$) to $\langle r(t)^2 \rangle = 2v_c^2 \tau_p t$. In other words, it describes purely ballistic motion at short large lag time and purely diffusive behavior at long timescales.

The TAMSD measured on flat PDMS [Fig. 8(c)] resembles the curve of a persistent-random walker [81]. However, a power-law fit ($\langle r(t)^2 \rangle = At^\alpha$) reveals that on short timescales (<100 s), the motion is not purely ballistic ($\alpha \approx 1.7$ versus $\alpha = 2$). The ballisticlike regime is followed by a crossover region (100–200 s, for which $\alpha \neq 1$) until the purely diffusive regime is reached (>200 s). A linear fit of the diffusive regime returns an effective diffusion constant of $D_{eff} = 106 \pm 31 \mu m^2 s^{-1}$. All error margins are found by a fit to the minimum and maximum of 95%-confidence bounds of the averaged TAMSD [gray, Figs. 8(a) and 8(b)]. For long times, the measured TAMSDs grow linearly with time, congruent to a PRW. Although qualitatively the TAMSDs indicate persistent-random motion, the cell centers do not perfectly follow a PRW, similar to other cell types [82–84]. It is instructive to estimate the effective persistence length of the PRW-like

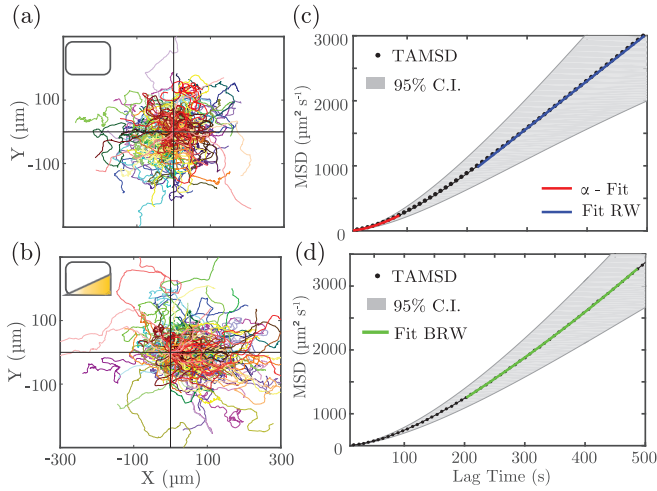


FIG. 8. Cell migration of starved *D. discoideum* on flat PDMS with and without a cAMP gradient. (a) Cell trajectories measured on flat PDMS without a chemotactic cAMP gradient. (b) Cell trajectories measured on flat PDMS with a cAMP gradient (source at $+x$). (c) TAMSD of 159 cell trajectories on flat PDMS. A power-law fit as a function of time is shown in red (up to 80 s) and a linear fit to extract the diffusion constant is shown in blue (from 250 s onward). (d) MSD of 186 cell trajectories on flat PDMS with a chemotactic cAMP gradient. A fit to BRW model is shown in green.

cell motion on flat PDMS in order to compare with other characteristic lengths, like the pillar spacing L_k or pillar zone width L_m . As the TAMSD becomes linear around 100–200 s, and multiplying with the average instantaneous velocities, the persistence length averages between 20–40 μm , about two to four cell lengths [50].

Then, to compare cell response in a multicue environment to chemotaxis on flat PDMS, cell trajectories were recorded in a cAMP gradient on a flat surface [Fig. 8(b)]. The cell trajectories show that the cAMP gradient in our setup (Secs. III C and III E) has a clear chemotactic response, but still lies in the intermediate signal-to-noise regime [45] of what is best achievable for *D. discoideum* cAMP chemotaxis. The TAMSDs of chemotaxing cells show a similar ballisticlike regime at short timescales, evolving in a diffusive regime at long times. However, chemotaxing cells experience a drift v_d through biased motion, as the TAMSD curve is not linear for long times (>250 s). Thus, the TAMSD resembles a biased random walk (BRW) at long lag times, which is given by

$$\langle r(t)^2 \rangle = (v_d t)^2 + 4Dt. \quad (\text{A2})$$

A fit to Eq. (A2) returns an effective diffusion constant of $D_{\text{eff}} = 71.4 \pm 11.4 \mu\text{m}^2 \text{min}^{-1}$ and drift velocity of $v_d = 3.48 \pm 0.90 \mu\text{m min}^{-1}$. Finally, the instantaneous velocities of starved *D. discoideum* moving on flat PDMS measure $\langle v_{\text{in}} \rangle = 10.4 \pm 0.1 \mu\text{m min}^{-1}$ and $\langle v_{\text{in}} \rangle = 11.9 \pm 0.1 \mu\text{m min}^{-1}$ with and without a cAMP gradient, respectively. The drop in speed for cells exposed to a cAMP gradient has been previously reported [53].

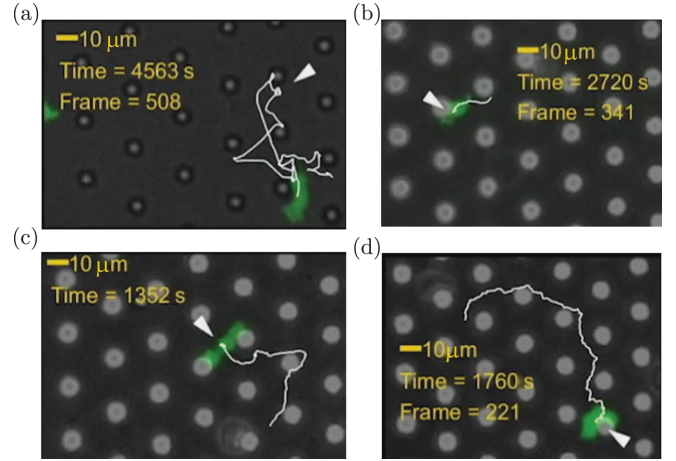


FIG. 9. Examples of long-duration cell-pillar interactions. (a), (b), (d) Cells predominantly slide past or bounce from pillars (shown in the figures by smooth trajectories), but sporadically spend a longer time exploring vertical areas of pillars, here highlighted by white arrows, where cell trajectories have many data points within a small area [as shown in Figs. 4(a) and 4(b)]. (c) Cells engaged in a “split-decision event” are in contact with multiple pillars and remain static for an extended period of time. The cell trajectories do not advance from the center of a pillar unit cell.

APPENDIX B: CONTACT TIME OF EXTENDED CELL-PILLAR INTERACTIONS

The cells predominantly brush past pillars, which leads to short-lived quasirandom motion states induced by the topography [as shown in Figs. 4(a) and 4(b)]. The example trajectory in Fig. 1 (Movie S1 in Supplemental Material [49]) shows some of these short-lived states, a result of the cell sliding or bouncing off a pillar. Additionally, cells have sporadic, much longer interactions with pillars (Movie S5 in Supplemental Material [49]). For example, the cells occasionally explore the vertical pillar surfaces with the leading pseudopod [Figs. 9(a) and 9(b)], fully wrap around pillars [Fig. 9(d)], or cope with a “split decision” when in contact with two pillars at once [Fig. 9(c)]. Vertical pillar interactions or “split decisions” contribute to overall lower migration speeds in the lattice, when compared to migration on a flat surface [43].

APPENDIX C: MODELING TOPOTAXIS ON AN ANISOTROPIC PILLAR FIELD IN 2D AS A JUMP PROCESS IN 1D

In this Appendix, we derive a minimal model, inspired by Biess *et al.* [85], to explain and predict long-range topotaxis generated by a gradient in pillar spacing. The pillar field changes spacing after each fourth pillar column in the $\pm x$ direction [Figs. 10(a) and 10(b)], giving rise to a topotactic gradient. Adjacent quadrilateral domains are always identical in the y direction and no drift is expected. As such, the field can be divided into zones m of equal pillar spacing L_k in the x direction, which in turn, can be subdivided into quadrilateral domains k with free space Ω_k delimited by pillars [Fig. 10(c)]. In order to capture the dynamics between the zones and single quadrilateral domains within each zone, we shall coarse-grain

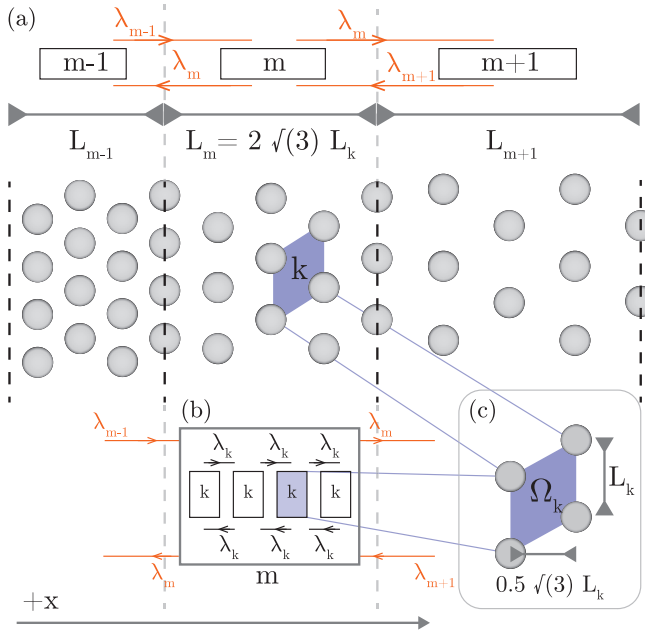


FIG. 10. Modeling topotactic drift using a jump process. (a) On the pillar field, cells move through series of pillar zones m each consisting of four columns of pillars with constant spacing L_k . When projected onto the gradient direction x , the cell effectively jumps through series of pillar zones, $\dots \leftrightarrow m-1 \leftrightarrow m \leftrightarrow m+1 \leftrightarrow \dots$, with an associated escape rate λ_m (b) In the x direction, pillar zones m consist of four equally spaced quadrilateral domains k . The cell must move through these domains $k-1$ to the domains with the next highest spacing $k+1$. (c) Equally spaced quadrilateral domains k within the same pillar zones m_k have the same escape rate λ_k , as they are equal in area Ω_k and pore size $\epsilon_k = L_k - d$.

cell motion across the pillar field twice. First, we coarse-grain cell motion as a jump process between zones of equal pillar spacing. Then, we connect movement within an entire zone to the scale of single quadrilateral pillar domains (as studied in Secs. II E and II F) by a second coarse-graining process.

1. Jump process between zones

Cell motion on the anisotropic pillar field is coarse-grained as a one-dimensional jump process between zones m of equal pillar spacing in the direction of the topotactic gradient. With each jump from a zone m , there is an associated escape rate λ_m to the left $m-1$ (resp. right $m+1$) zone [Fig. 10(a)]. The MFPT $\bar{\tau}_m$ from a pillar zone m is the reciprocal of the escape rate,

$$\lambda_{m \rightarrow m+1} = \lambda_{m \rightarrow m-1} = \frac{1}{2\bar{\tau}_m}. \quad (\text{C1})$$

Here, the factor 2 accounts for the fact that half of the population can return. In the Markov approximation, the escape time distributions associated with Eq. (C1) are Poissonian. The goal of the remaining section is to derive an effective stochastic equation for the coarse-grained motion of a cell across pillar zone m .

To coarse-grain the dynamics into a random walk between the centres of adjacent pillar zones m , we approximate the

master equation for the transition probability density function of the random walk by a one-dimensional convection-diffusion equation [86]. We project the equation on the x axis and recall that the mean exit time from a zone is long, and the waiting times are exponentially distributed with rates λ_m .

The probability $p_m(t)$ that a moving cell is in zone m at time t satisfies the master equation. Indeed, using the transition that occurred from the left zone with rate $\lambda_{m-1 \rightarrow m} = \lambda_{m-1}$ with probability $p_{m-1}(t - \Delta t)$, or from the right with rate $\lambda_{m+1 \rightarrow m}$ and probability $p_{m+1}(t - \Delta t)$, and the probability of no transition out of m during Δt is $1 - \lambda_m \Delta t$, we get

$$\dot{p}_m(t) = \lambda_{m-1}p_{m-1}(t) + \lambda_{m+1}p_{m+1}(t) - \lambda_m p_m(t). \quad (\text{C2})$$

To derive a coarse-grained equation, we expand in space the density $p_m(t) = p(x_m, t)$ using a Taylor expansion with $x_{m+1} = x_m + \frac{1}{2}(L_m + L_{m+1})$ and $x_{m-1} = x_m - \frac{1}{2}(L_m + L_{m-1})$, where L_m , L_{m+1} , and L_{m-1} are the pillar zone widths, yielding,

$$p(x_{m+1}, t) = p(x_m, t) + \frac{1}{2}(L_m + L_{m+1})\frac{\partial p}{\partial x}(x_m, t) + \frac{1}{4}(L_m + L_{m+1})^2\frac{\partial^2 p}{\partial x^2}(x_m, t) + \mathcal{O}(L_{m+1}^3), \quad (\text{C3})$$

$$p(x_{m-1}, t) = p(x_m, t) - \frac{1}{2}(L_m + L_{m-1})\frac{\partial p}{\partial x}(x_m, t) + \frac{1}{4}(L_m + L_{m-1})^2\frac{\partial^2 p}{\partial x^2}(x_m, t) + \mathcal{O}(L_{m-1}^3). \quad (\text{C4})$$

Using Eq. (C2) and remembering $\lambda_m = 1/(2\bar{\tau}_m)$, we obtain the coarse-grained Fokker-Planck equation on the lattice points x_m ,

$$\dot{p}_m(x_m, t) = -a(L_m, \tau_m)\frac{\partial p(x_m, t)}{\partial x} + D(L_m, \tau_m)\frac{\partial^2 p(x_m, t)}{\partial x^2}, \quad (\text{C5})$$

where we have used that $\lambda_{m-1} + \lambda_{m+1} - \lambda_m = 0$, as there is no accumulation of cells at a pillar zone. From Eqs. (C2) (C5), we can identify the effective drift $a(x)$ and diffusion tensor $D(x)$ as

$$a(L_m, \tau_m) = \frac{1}{2} \left[\frac{L_m + L_{m-1}}{2\bar{\tau}_{m-1}} - \frac{L_m + L_{m+1}}{2\bar{\tau}_{m+1}} \right] \quad \text{and} \quad D(L_m, \tau_m) = \frac{1}{4} \left[\frac{(L_m + L_{m-1})^2}{2\bar{\tau}_{m-1}} + \frac{(L_m + L_{m+1})^2}{2\bar{\tau}_{m+1}} \right]. \quad (\text{C6})$$

This allows us to propose a coarse-grained stochastic description of cell movement,

$$\dot{X} = a(X) + \sqrt{2D(X)}\dot{w}, \quad (\text{C7})$$

where w is the Wiener process, which is valid at a spatial scale much larger than the space between two neighboring pillar zones. Note that the drift $a(X) = a(L_m, \bar{\tau}_m)$ and diffusion constant $D(x_m) = D(L_m, \bar{\tau}_m)$ of the jump process between pillar zones m depends directly on the pillar spacing L_k and quadrilateral domains, through $L_m(L_k)$ and $\bar{\tau}_m(\bar{\tau}_k)$.

2. The MFPT out of a single-pillar zone

We now present a second coarse-grained reduction of cell movement *within* a pillar zone by reducing the motion across

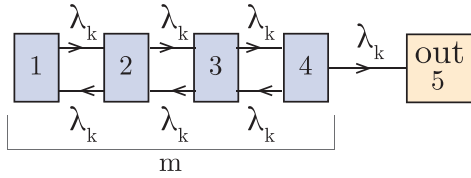


FIG. 11. 1D-Markov chain description of a cell moving across a pillar zone. The four states (violet) represent single quadrilateral domains k projected onto the x axis and are connected to an absorbing state (yellow). The absorbing state “5” represents the transition from this pillar zone to the next one.

quadrilateral domains [the effective free space between pillars, Fig. 10(b)] to a jump process. For that goal, we project cell movement onto the x axis, giving rise to a series of four possible jumps (see Fig. 11). The escape rates between the identical quadrilateral domains are all equal to λ_k , and are related to escape time as $\lambda_k = 1/(2\bar{\tau}_k)$. The escape rate from any of the pillar domains on the border of a pillar zone m , into a domain of an adjacent pillar zone to the left $m-1$ or right $m+1$, is also λ_k [Figs. 10(b) and 10(c) and state “out 5” in Fig. 11]. We shall now compute the escape rate in the limit where cells are uniformly distributed (yielding the initial state probability vector $p_0 = \{\frac{1}{4}, \frac{1}{4}, \frac{1}{4}, \frac{1}{4}, 0\}$) using a continuous time Markov model (Fig. 11). The transition rate matrix Q where we have an absorbing state is given by

$$Q = \begin{bmatrix} -\lambda_k & \lambda_k & 0 & 0 & 0 \\ \lambda_k & -2\lambda_k & \lambda_k & 0 & 0 \\ 0 & \lambda_k & -2\lambda_k & \lambda_k & 0 \\ 0 & 0 & \lambda_k & -2\lambda_k & \lambda_k \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}. \quad (\text{C8})$$

Using a continuous Markov model [Eq. (C8)], $\lambda_k = 1/(2\bar{\tau}_k)$ and p_0 , a direct computation of the MFPT $\bar{\tau}_m$ out of a pillar zone m as a function of the escape rate from a domain λ_k is

$$\bar{\tau}_m = \frac{\bar{\tau}_5}{2} = \frac{15}{4\lambda_k} = \frac{15\bar{\tau}_{k,1D}}{2}, \quad (\text{C9})$$

where $\bar{\tau}_{k,1D}$ is the MFPT from a column of quadrilateral domains k projected on the x axis, and $\bar{\tau}_m = \bar{\tau}_5/2$ is due to the symmetry of either escaping from m left or right.

3. The drift of cells moving on a pillar field with changing spacing

Finally, we can directly relate the drift $a(L_m, \bar{\tau}_m)$ of the jump process between pillar zones m to the pillar spacing L_k and MFPT of a single quadrilateral domain $\bar{\tau}_k$. Using Eqs. (C6) and (C9), $\bar{\tau}_k$, and the width of each pillar zone L_m , we can approximate the drift of cells between pillar zones m as

$$a(L_k, \tau_k) = \frac{\sqrt{3}}{15} \left[\frac{L_k + L_{k-1}}{\bar{\tau}_{k-1}} - \frac{L_k + L_{k+1}}{\bar{\tau}_{k+1}} \right]. \quad (\text{C10})$$

This expression for the drift depends on the spacing between pillars L_k , the difference in spacing toward the more spacious L_{k+1} and less spacious side L_{k-1} , and the passage times $\bar{\tau}_{k+1}$, $\bar{\tau}_{k-1}$ from the domains neighboring L_k . Introducing a variable Δ for a constant change in pillar spacing between subsequent

pillar zones, Eq. (C10) can be rewritten to

$$a(L_k, \Delta, \tau_k) = \frac{\sqrt{3}}{15} \left[\frac{2L_k - \Delta}{\bar{\tau}_{k-1}(L_k, \Delta)} - \frac{2L_k + \Delta}{\bar{\tau}_{k+1}(L_k, \Delta)} \right]. \quad (\text{C11})$$

APPENDIX D: EXPERIMENTAL OVERVIEW

Table II gives an overview of cell migration measurements performed on all types of experimental assays with associated average instantaneous velocities and diffusion constants. The diffusion constants were fit between a lag time of 250–350 s for all experimental configurations. Table II gives an overview of all the calculated transition probabilities and their difference according to the method in Sec. III E.

APPENDIX E: cAMP GRADIENT FORMATION IN A MICROFLUIDIC CHAMBER CONTAINING PDMS MICROPILLARS

To establish the cAMP gradient across micropillar fields, we used a microfluidic channel [Fig. 12(a)] with a diffusion reservoir. The diffusion through the channel can be modeled by solving Fick’s second law [87] in a semi-infinite bar [Fig. 12(b)], which has the solution

$$C(x, t) = \frac{B}{\sqrt{\pi Dt}} \exp \frac{-x^2}{4Dt}. \quad (\text{E1})$$

Here, B is the total amount of solute, D its diffusion coefficient, x the distance from the boundary of the reservoir, and t the time. The chemical gradient is set up controllably by very slowly pumping in a set amount B of cAMP-PBS solution C into a diffusion reservoir and waiting a set time t for the solute to diffuse through the channel. By placing the pillar field at the same particular distance from the reservoir, the same order of magnitude gradients can be established. Note that more sophisticated, dynamic mixing, flow-based setups exist, which we [40] and others [39,53] have used in previous work. However, a diffusion gradient was chosen over a mixing chamber, as the initiation of a gradient within the pillar field by diffusion is unaffected by pillars (Fig. 12), whereas mixing processes with active flow are perturbed by the presence of obstacles. Moreover, for this study a diffusion chamber also suffices, as *D. discoideum* exhibits chemotaxis in a very wide range of chemical gradients $\nabla C = 10^{-3}$ –10 nM (with a peak motility between $\nabla C = 0.01$ –0.1 nM) and over a similarly wide range of baseline concentrations ($\sim \mu\text{M}$) [39].

The appropriate solute concentration, volume, and waiting time for a cAMP gradient at a distance of 2 cm distance from the reservoir was benchmarked using the fluorescent dye Hydrazine 488. Different concentrations of dye were imaged inside the microfluidic channel [Fig. 12(c) shows the evolution of the hydrazine gradient as a function of time for the volume and concentration used in the experiments]. Hydrazine 488 was chosen as a suitable fluorescent substitute for cAMP, as it has a similar molecular weight to cAMP, 329.21 Da versus 570.48 Da, and thereby shares similar diffusive behavior. The pillar array was placed at $x = 2$ cm, and before each live cell experiment a volume of $B = 40 \mu\text{l}$ with a cAMP concentration of $C = 0.1 \mu\text{M}$ was pumped into the reservoirs slowly. Then,

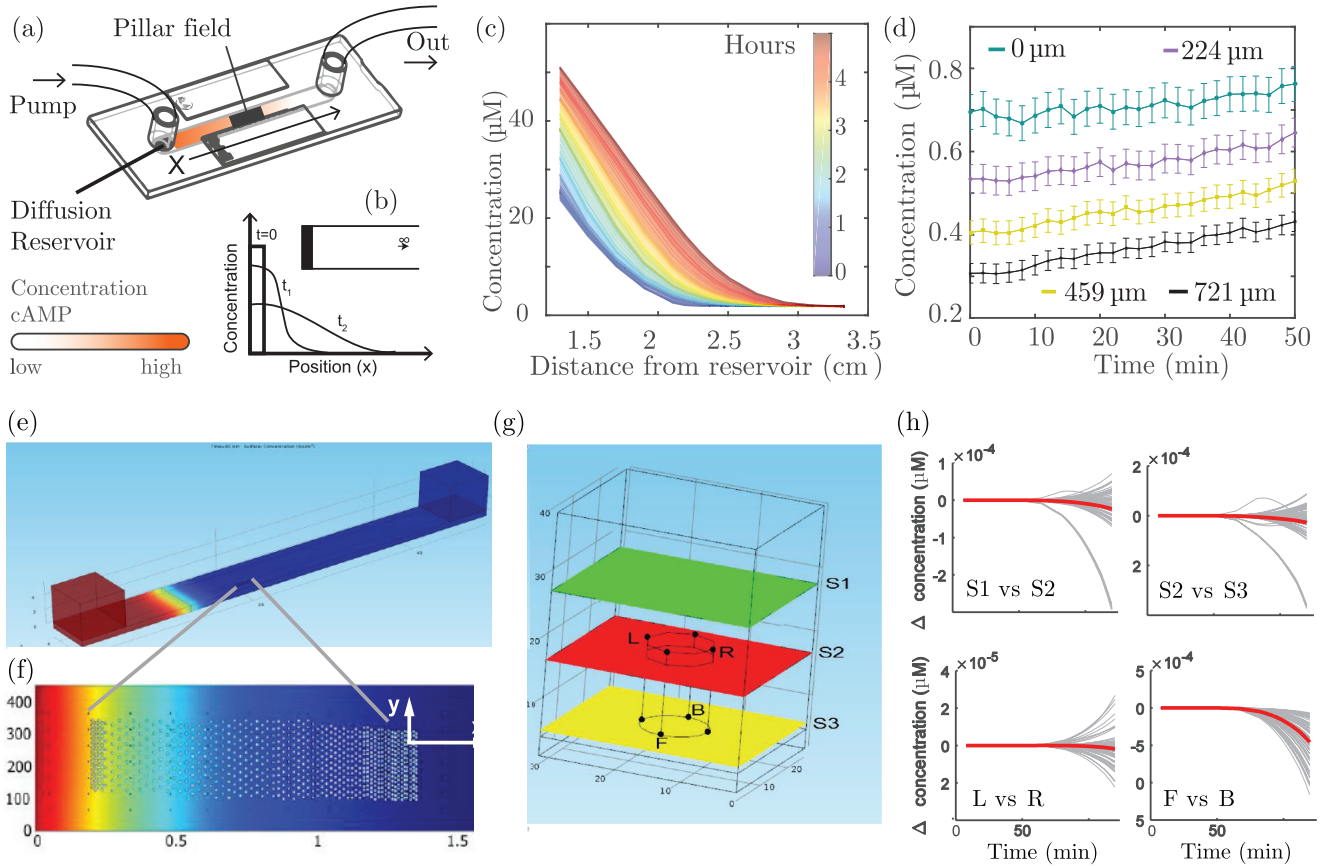


FIG. 12. Characterization of cAMP gradient inside the microfluidic device. (a) Schematic of microfluidic channel used with a chemotactic gradient and micropillar field inside. The pillar fields were placed 2 cm into the channel. (b) Qualitative sketch of solutions to Fick's second law at different times for a solute diffusing into a semi-infinite bar (inset). (c) Measurement of the diffusion of fluorescent Hydrazine 488 (similar molecular weight to cAMP) inside the microfluidic channel as a function of distance to the reservoir. (d) Concentration of dye along the pillar field at different distances into the array, 0 μm corresponds to $x = 2$ cm in the microfluidic channel. Error bars are standard deviation in measured fluorescence. For the duration of live cell experiments, base concentrations remained between ~ 0.1 – 1 μM . (e) Difference of concentrations is shown, showing the chemical gradient stays within the $\sim \text{nM}/\mu\text{m}$ range over the course of an experiment. (f) Visualization of the cAMP gradient along the channel of the microfluidic device, with pillar array inside. Red symbolizes high concentration and blue low. (g) Enlarge the concentration profile over the pillar array at the end of a simulation at $t = 120$ min. (h) The diffusion of a solute was investigated as a function of the front (F), back (B), left (L), and right (R) of representative pillars by finite element modeling. This was done to investigate any influence of the pillar geometry on the chemotactic gradient established by diffusion. (i) The difference in concentration 8 μm above (S1) and at the top (S2) of representative pillars (gray lines) along the pillar gradient, and average difference (red). Concentration differences remained between 0 and 0.05 nM for all pillars during live-cell experiments. (j) The difference in concentration at base (S3) and top (S2) pillars (gray lines) and their average (red) over the course of experiments. (k) The difference in concentration between the left (L) and right (R) of each representative pillar is investigated. Concentration differences remain between 0–0.02 nM for all pillars during a live-cell experiment. (l) The difference in concentration between the front (F) and back (B) of each representative pillar, the difference in concentration between these pillar sides is highest as expected, namely, between 0.01 and 0.1 nM.

live cell imaging was started after $t = 30$ min and with an established cAMP gradient of ~ 0.1 nM μm^{-1} [Fig. 12(d)].

To make sure the pillar field did not significantly influence the diffusion of cAMP, the initiation of the chemotactic gradient was investigated numerically using finite-element simulations [Figs. 12(e) and 12(f)]. Figure 12(g) shows a micropillar from inside the microfluidic channel; the concentration differences at various heights (S1, S2, and S3) and sides (front F, back B, left L, and right R) were compared

during diffusion simulations. The chemical gradient is not significantly different (< 0.1) at various heights inside and above the pillar field [Fig. 12(h), top row] over the course of a live-cell experiment ($t_{\text{tot}} = t_{\text{wait}} + t_{\text{exp}} = 120$ min). Differences in concentration are equally negligible between the left and right sides [Fig. 12(h), bottom left] of the array. As expected, there is a concentration difference between the front and back of each pillar [Fig. 12(h), bottom right], which confirms solute diffusion and the formation of a chemotactic gradient.

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