Bimodal rheotactic behavior reflects flagellar beat asymmetry in human sperm cells

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Rheotaxis, the directed response to fluid velocity gradients, has been shown to facilitate stable upstream swimming of mammalian sperm cells along solid surfaces, suggesting a robust physical mechanism for long-distance navigation during fertilization. However, the dynamics by which a human sperm orients itself relative to an ambient flow is poorly understood. Here, we combine microfluidic experiments with mathematical modeling and 3D flagellar beat reconstruction to quantify the response of individual sperm cells in time-varying flow fields. Single-cell tracking reveals two kinematically distinct swimming states that entail opposite turning behaviors under flow reversal. We constrain an effective 2D model for the turning dynamics through systematic large-scale parameter scans, and find good quantitative agreement with experiments at different shear rates and viscosities. Using a 3D reconstruction algorithm to identify the flagellar beat patterns causing left or right turning, we present comprehensive 3D data demonstrating the rolling dynamics of freely swimming sperm cells around their longitudinal axis. Contrary to current beliefs, this 3D analysis uncovers ambidextrous flagellar waveforms and shows that the cell's turning direction is not defined by the rolling direction. Instead, the different rheotactic turning behaviors are linked to a broken mirror symmetry in the midpiece section, likely arising from a buckling instability. These results challenge current theoretical models of sperm locomotion.

sperm swimming | rheotaxis | fluid dynamics | microfluidics | simulations

Taxis, the directed kinematic response to external signals, is a defining feature of living things that affects their reproduction, foraging, migration, and survival strategies (1–4). Higher organisms rely on sophisticated networks of finely tuned sensory mechanisms to move efficiently in the presence of chemical or physical stimuli. However, various fundamental forms of taxis are already manifest at the unicellular level, ranging from chemotaxis in bacteria (5) and phototaxis in unicellular green algae (2) to the mechanical response (durotaxis) of fibroblasts (6) and rheotaxis (7, 8) in spermatozoa (3, 9–12). Over the last few decades, much progress has been made in deciphering chemotactic, phototactic, and durotactic pathways in prokaryotic and eukaryotic model systems. In contrast, comparatively little is known about the physical mechanisms that enable flow gradient sensing in sperm cells (3, 9–13). Recent studies (3, 12) suggest that mammalian sperm use rheotaxis for long-distance navigation, but it remains unclear how shear flows alter flagellar beat patterns in the vicinity of surfaces and, in particular, how such changes in the beat dynamics affect the steering process. Answering these questions will be essential for evaluating the importance of chemical (14) and physical (4) signals during mammalian fertilization (15–17).

A necessary requirement for any form of directed kinematic response is the ability to change the direction of locomotion. Multiflagellate bacteria achieve this feat by varying their motor activity, resulting in alternating phases of entangled and disentangled flagellar dynamics that give rise to run-and-tumble motion (5). A similar mechanism was recently discovered in the biflagellate eukaryote *Chlamydomonas reinhardtii* (18). This unicellular green alga actively redirects its swimming motion through occasional desynchronization of its two cilia (19), although it is still debated whether this effect is of mechanical (20) or hydrodynamic (21, 22) origin. Experiments (23) show that the alga's reorientation dynamics can lead to localization in shear flow (24, 25), with potentially profound implications in marine ecology. In contrast to taxis in multiflagellate organisms (2, 5, 18, 26, 27), the navigation strategies of uniflagellate cells are less well understood. For instance, it was discovered only recently that uniflagellate marine bacteria, such as *Vibrio alginolyticus* and *Pseudoalteromonas haloplanktis*, use a buckling instability in their lone flagellum to change their swimming direction (28). However, as passive prokaryotic flagella differ fundamentally from their active eukaryotic counterparts, it is unclear to what extent such insights translate to spermatozoa.

Earlier studies of human sperm locomotion have identified several potential steering and transport mechanisms, including thermotaxis (4), uterine peristalsis (29, 30), and chemotaxis (14, 16, 31), but their relative importance has yet to be quantified. Recent experiments (3, 32, 33) demonstrate that rheotaxis, combined with steric surface alignment (12, 34), enables robust long-distance navigation by turning sperm cells preferentially against an externally imposed flow direction (9, 10), but how exactly this realignment process happens is unknown. It has been suggested (32, 35, 36) that the intrinsic curvature or chiral beat dynamics (37, 38) of the flagellum could play an essential role in rheotactic steering, but this remains to be confirmed in experiments. Similarly, an increasing number of theoretical models (36, 39–47) still await empirical validation, because 3D data for the beat pattern of sperm swimming close to surfaces has been lacking.

To examine the dynamics of human sperm rheotaxis quantitatively, we here combine microfluidic experiments with mathematical modeling and 3D flagellar beat reconstruction. Single-cell tracking

Significance

Successful sperm navigation is essential for sexual reproduction, yet we still understand relatively little about how sperm cells are able to adapt their swimming motion in response to chemical and physical cues. This lack of knowledge is owed to the fact that it has been difficult to observe directly the full 3D dynamics of the whip-like flagellum that propels the cell through the fluid. To overcome this deficiency, we apply a new algorithm to reconstruct the 3D beat patterns of human sperm cells in experiments under varying flow conditions. Our analysis reveals that the swimming strokes of human sperm are considerably more complex than previously thought, and that sperm may use their heads as rudders to turn right or left.

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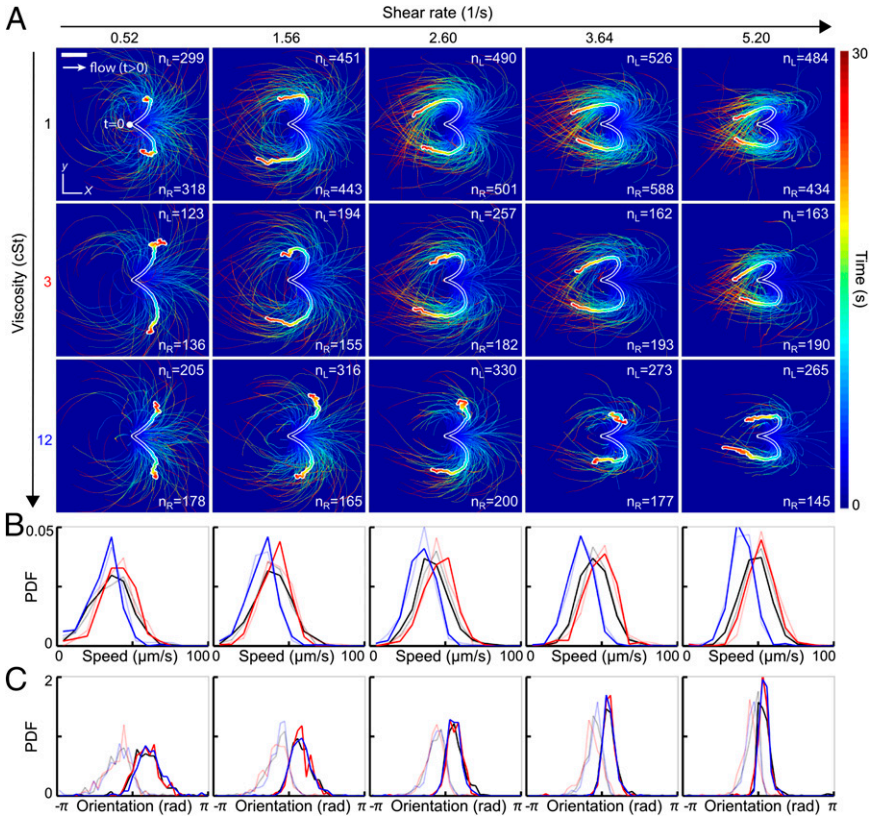

Fig. 1. Turning behavior of human sperm under flow reversal reveals two kinematically distinct swimming states. (*A*) Trajectories of individual sperm cells swimming close to the channel boundary in the (x, y) plane, with initial positions superimposed at time $t = 0$ (viewed from inside the channel). Equal-time trajectory averages for left- and right-turning cells are shown as thick white-shaded lines. Flow was reversed at $t = 0$, pointing in positive x direction for $t > 0$ (white arrow). The shear velocity increases linearly in z direction. Color encodes time. (Scale bar, 200 μm.) (*B*) Normalized speed distributions before flow reversal at time $t = 0$. Faint lines indicate left-turning cells, and the other lines indicate right-turning cells. (*C*) Distribution of the orientation angles $\varphi(0)$, measured relative to the x axis before flow reversal at time $t = 0$, signals two kinematically distinct cell populations. Colors in *B* and *C* indicate different viscosities (black, 1 cSt; red, 3 cSt; blue, 12 cSt).

reveals the existence of two kinematically distinct swimming states that result in opposite turning behaviors under flow reversal. We quantify this effect for a range of viscosities and shear rates, and use these comprehensive data to constrain an effective 2D model through a systematic large-scale scan (>6,000 parameter combinations). To identify the details of the flagellar beat dynamics during rheotaxis, we developed an algorithm that translates 2D intensity profiles into 3D positional data. Our 3D analysis confirms that human sperm perform a rolling motion (48), characterized by weakly nonplanar beat patterns and a rotating beat plane. However, contrary to current beliefs, we find that neither the rolling direction nor beat helicity determine the turning direction after flow reversal. Instead, the rheotactic turning behavior correlates with a previously unrecognized asymmetry in the midpiece, likely caused by a buckling instability. These findings call for a revision and extension of current models (36, 39–44, 46).

Results

Reorientation Dynamics After Flow Reversal. Understanding how efficiently a sperm cell can react to directional changes in ambient fluid flows is a first step toward evaluating the importance of uterine peristalsis on sperm transport during reproduction (29, 30). To examine the response of human sperm after a sudden flow reversal, we tracked individual cells in microfluidic channels (*Experimental Details*) at three different kinematic viscosities $\nu = 1$ centi-Stokes (cSt), 3 cSt, and 12 cSt, and five different shear rates, $\dot{\gamma} = 0.52\ s^{-1}$, $1.56\ s^{-1}$, $2.60\ s^{-1}$, $3.64\ s^{-1}$, and $5.20\ s^{-1}$ (Fig. 1). Hydrodynamic and steric forces cause sperm to accumulate near solid boundaries (12), where they remain trapped for several minutes while being exposed to a locally linear normal flow gradient. In our experiments, cells generally accumulated at distances of <10 μm from the wall. We therefore fixed the focal plane parallel to the upper wall of the microfluidic chamber, using a large depth of field to track all cells within distance 10 μm from the wall. The results presented below are thus integrated measurements over this accumulation layer. At time $t < 0$, a constant external flow field was applied in negative x direction, causing the cells to align preferentially in positive x direction (3, 32). At $t = 0$, the flow direction was rapidly reversed (switching time $\lesssim 1$ s), and the motions of 300 to 1,000 randomly selected sperm cells were tracked for a period of >30 s for each parameter pair $(\nu, \dot{\gamma})$.

Trajectory analysis shows that approximately half of the tracked cells respond to flow reversal by making a right turn whereas the other half pursue a left turn (Fig. 1*A* and Movies S1–S6). In both cases, the majority of cells perform a complete U-turn, provided the shear rate is sufficiently large $\dot{\gamma} > 1.56\ s^{-1}$. As the value of $\dot{\gamma}$ is increased, the characteristic curvature of the U-turns also increases, and the spread around the mean trajectories, obtained by averaging positions at equal time $t > 0$, is reduced (thick white-shaded lines in Fig. 1*A*).

The initial speed distributions, measured at the moment of the flow switch $t = 0$, show little variation between left-turning and right-turning cells (Fig. 1*B*). As expected, the maximum of the speed distribution is shifted to a lower value at high viscosity (blue curves in Fig. 1*B*). Strikingly, the initial offset angles $\varphi(0)$ of left-turning and right-turning individuals are bimodally distributed, suggesting that exposure to constant flow for $t < 0$ separates two different alignment modes that become magnified during a flow reversal (Fig. 1*C*).

To characterize the typical distance scale associated with the turning process, we consider the downstream persistence length Λ, defined as the maximum of the x component of a given mean trajectory in Fig. 1*A*. In our experiments, Λ is found to increase slightly with viscosity ν while showing a weak systematic decrease with the shear rate $\dot{\gamma}$ (Fig. 2*A*). The downstream persistence length Λ defines a characteristic turning time $T > 0$, corresponding to the time after which a given average trajectory in Fig. 1*A* begins to point against the flow. Assuming a passive response to the flow reversal, basic dimensional arguments suggest $T \propto 1/\dot{\gamma}$. This trend is confirmed in our experiments (Fig. 2*B*).

Effective 2D Model Captures Turning Dynamics. To capture the experimentally observed turning dynamics quantitatively in a mathematical model, we assume that the quasi-2D locomotion of a sperm cell near the boundary can be effectively described in terms of a position vector $\boldsymbol{X}(t) \in \mathbb{R}^2$ and a unit orientation vector $\boldsymbol{N}(t) = (\cos\varphi(t), \sin\varphi(t)) \in \mathbb{S}^1$. Considering flow along the x axis, the translation dynamics is governed by

$$\frac{d\boldsymbol{X}}{dt} = V\boldsymbol{N} + \sigma U \boldsymbol{e}_x \qquad [1]$$

where $V > 0$ is the self-swimming speed, $\sigma(t) \in \{\pm 1\}$ is the flow direction, and $U > 0$ is the mean advective flow speed experienced by the cell. The reorientation dynamics $d\boldsymbol{N}/dt = (-\sin\varphi(t), \cos\varphi(t))\, d\varphi/dt$ is determined by an Adler-type equation

$$\frac{d\varphi}{dt} = \frac{\sigma}{\tau_R}\sin\varphi + \frac{\chi}{\tau_C} \qquad [2]$$

where $\tau_R > 0$ is the rheotactic realignment time scale and $\tau_C > 0$ is an intrinsic turning time, with $\chi = \pm 1$ accounting effectively for a preferential turning behavior. As in the experiments, we assume that the cells are viewed from inside the microchannel so that, in the absence of external flow (corresponding to $\sigma = 0$), the parameter choice $\chi = +1$ corresponds to a left-turning cell. A microscopic physical mechanism underlying χ will be identified below. For the values of the shear-rate $\dot{\gamma}$ probed in our experiments, the rheotactic response is faster than the intrinsic circling period, $\tau_R < \tau_C$. Instead of adding rotational noise (32) in Eq. **2**, we sampled the turning times from Gaussian distributions (*Parameter Scans*), which seems more realistic because sperms cells experience only weak rotational diffusion due to their large size but may exhibit systematically different beat patterns across a range of individuals.

To determine how the model parameters $(U, \tau_C \pm \Delta\tau_C, \tau_R \pm \Delta\tau_R)$ depend on the experimental control parameters $(\nu, \dot{\gamma})$, we simulated Eqs. **1** and **2** for >6,000 parameter combinations (*Parameter Scans*). Distribution parameters of the self-swimming speed V were directly estimated from experiments performed at $\dot{\gamma} = 0$ (Fig. S1 and *Parameter Scans*). For each parameter pair $(\nu, \dot{\gamma})$, a best-fit model was determined by identifying the simulation run that most accurately reproduced the experimentally measured ensemble mean trajectories (*Parameter Scans*).

The best-fit simulation runs and the experimentally measured mean trajectories are compared in Fig. S1. Although the underlying mathematical model is relatively basic, the numerically obtained mean trajectories generally agree well with their experimental counterparts (thick white-shaded lines in Fig. S1*A*). Note that the satisfactory quantitative agreement holds not only for the mean trajectory shape but also for the color-coded temporal progression. One can therefore conclude that the effective model defined by Eqs. **1** and **2** provides an adequate quantitative minimal representation of the rheotactic alignment process.

The dependencies between the best-fit model parameters and experimental control parameters $(\nu, \dot{\gamma})$ are summarized in Fig. S1*B*: At high viscosity, the typical free-swimming speed V is significantly reduced. The mean advection speed U grows linearly with the shear rate $\dot{\gamma}$. The rheotactic realignment time scale

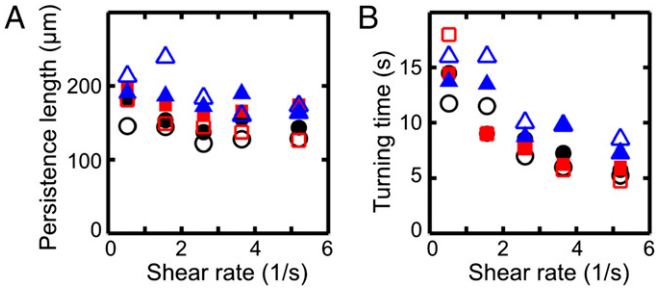

Fig. 2. Characteristics of the turning process for the cell trajectories in Fig. 1, using the same color coding for viscosities, with filled (unfilled) symbols indicating right-turning (left-turning) trajectories. (*A*) The persistence length Λ, defined as the maximum of the x component of each mean trajectory in Fig. 1*A*, shows weak variation with shear rate $\dot{\gamma}$. (*B*) The mean turning time T, defined as the time after which the x component of a mean trajectory reaches its maximum, is approximately inversely proportional to the shear rate, $T \propto 1/\dot{\gamma}$.

τ_R decays with $\dot{\gamma}$, and so does the intrinsic circling time scale τ_C, consistent with the experimental results in Fig. 2*B*.

The model defined in Eqs. **1** and **2** postulates an intrinsic preference for left/right turning, encoded by the parameter $\chi = \pm 1$ and required to reproduce the experimentally observed turning dynamics. To understand how the flagellar beat dynamics determines the sign of χ, we next reconstructed the 3D flagellar pattern of individual sperm cells swimming freely near a surface in shear flow.

Three-Dimensional Beat Reconstruction. To identify the microscopic origin of the two distinct rheotactic responses, we developed a two-step algorithm that reconstructs the vertical beat component from 2D bright-field high-speed [450–600 frames per second (fps)] microscopy images (Fig. 3 *A* and *B* and Movie S7). The algorithm first identifies the projected 2D shape of the flagellum, based on the pixel intensity levels. Subsequently, the z coordinate is estimated by analyzing the intensity profile along cross sections normal to the flagellum (*Beat Reconstruction* and Fig. S2). As in Rayleigh–Sommerfeld back-propagation (49, 50), this reconstruction method exploits that an object located behind the focal plane appears bright with a dark halo, whereas a point source in front of the focal plane appears dark with a bright halo (51). The algorithm robustly recovers the 3D beat dynamics of the anterior ~70% of the flagellum (Movie S7). The tail resolution is limited by the frame rate. The necessity of a full 3D analysis becomes evident when one compares the dynamics of the 2D projected (52) tangent angle $\alpha(s_*)$ at $s_* \approx 15$ μm (Fig. 3) with the corresponding 3D angle (Fig. S3*A*). Although $\alpha(s_*)$ is periodic in time (Fig. S3*B*), it exhibits a spurious mode (36) that vanishes in the 3D signal (Fig. S3 *B* and *C*).

Three-Dimensional Rolling Motion and Beat Planarity. At low viscosities, human sperm cells swim in a rolling mode, characterized by a rotation of the flagellum around its longitudinal axis (35, 53). In our experiments, almost all cells perform rolling, as evident from the rotation of the sperm head (Movie S7). The 3D conical rolling beat pattern, thought to be linked to a calcium signaling pathway, leads to strong rheotactic alignment (3). Hydrodynamic models predict that the rolling motion and rheotaxis are caused by an out-of-plane component in the flagellar beat (40, 42, 46), but dynamics and geometry of this beat mode have yet to be confirmed through 3D measurements on freely swimming cells. To test the robustness of our reconstruction algorithm, we first analyzed the 3D rolling motion. When viewed from head on, we found that the flagellum beats almost always in a counterclockwise rolling motion as indicated in Fig. 3*C*, whereas the normal vector angle θ of the corresponding beat plane rotates in the opposite direction (Fig. 3 *C* and *D*; details and additional examples in Figs. S4 and S5). The rotation of the beat plane is synchronized with the beat dynamics such that the flagellum typically performs half a beat in a plane of fixed orientation, but then

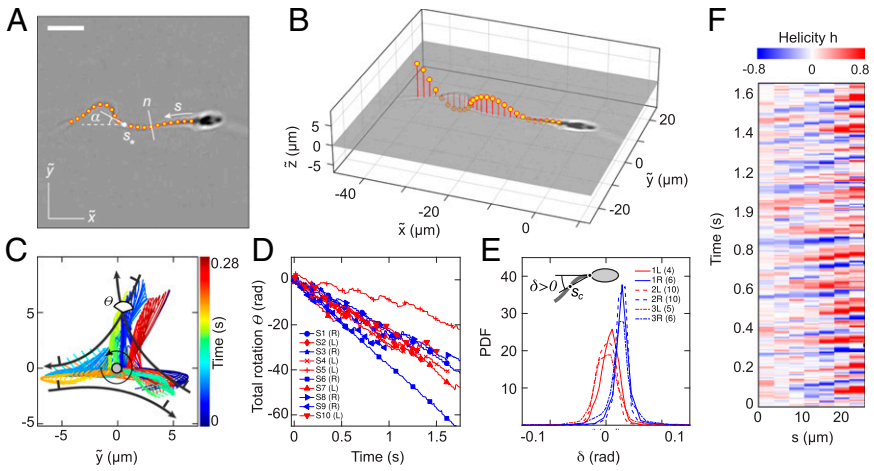

Fig. 3. A 3D flagellar beat reconstruction reveals that a mirror symmetry breaking in the midpiece curvature separates left-turning from right-turning sperm. (*A*) A 2D bright-field image and tracked flagellum in the head-centered comoving frame, with arc-length s and normal line n. (Scale bar, 10 μm.) (*B*) A 3D beat reconstruction in the head-centered frame (*Beat Reconstruction* and Movie S7). (*C*) Typical 3D beat plane rotation for a single sperm, seen from head-on, with beat period of ∼0.08 s. The circular arrow indicates rolling direction of the flagellum. (*D*) The cumulative beat plane rotation θ, shown for 10 typical samples of left-turning (L) and right-turning (R) cells, implies that the rolling and turning direction are not correlated. (*E*) Midpiece curvature, quantified by the bend angle δ between the tangent at $s=0$ and the secant through $s_c \approx 4$ μm, correlates strongly with the turning direction (three different donors, sample size in brackets). (*F*) A 3D reconstruction reveals ambidextrous helicity in the first ∼70% of the flagellum.

completes the second half in another plane. The beat envelope is not symmetric about the vertical axis, due to the presence of the wall, which is located below the cell in the body-centered frame (Fig. 3*C*). A large number of beats are performed parallel to the wall, reflecting inhibition of rolling by hydrodynamic and steric interactions between wall and flagellum, as also observed in previous experiments (48) and simulations (42).

We quantify the beat planarity through the length ratio $P = |\boldsymbol{r}_-|/|\boldsymbol{r}_+|$ of the two minor axis vectors $\boldsymbol{r}_\pm$ of the flagellar inertial ellipsoid (Fig. S6). The shortest axis $\boldsymbol{r}_-$ is normal to the best-fit plane through the flagellum, and $P=0$ for planar curves. We find that the flagellum remains mostly planar (Fig. S7), with a sample mean of $\langle P\rangle \approx 0.2$, in excellent agreement with estimates from previous 2D orthogonal measurements (35). Tracking a single point at arc length $s* \approx 15$ μm from the head, we obtain flagelloid curves similar to those observed in head-fixated mouse spermatozoa (Fig. S5) (48) and recent hydrodynamic simulations (40), corroborating the accuracy of the 3D reconstruction.

Turning Behavior Is Independent of Rolling. To test if the rolling motion causes different turning directions, we track the normal vector $\boldsymbol{n} = (\tilde{n}_x, \tilde{n}_y, \tilde{n}_z)$ of the best-fit plane through the flagellum in the head-centered frame (Fig. 3*C*). The projected orientation angle $\theta(t) = \tan^{-1}(\tilde{n}_z/\tilde{n}_y)$ is found to undergo persistent clockwise rotation, interrupted by short periods of counterclockwise rotation (Fig. 3*D*). These results reconcile seemingly contradicting earlier reports of purely unidirectional (54) and bidirectional (35) rolling motion in human sperm. Importantly, however, our data show no correlation between rolling and rheotactic turning direction of the sperm cells (Fig. 3*D*).

Turning Behavior Is Independent of Beat Chirality. It has been suggested that the beat patterns of human sperm flagella resemble spirals of well-defined helicity (32, 35, 36). If true, then the different turning behaviors could be caused by a chiral mechanism. Even though helicoidal models of human sperm swimming are widely used in theoretical studies (32, 40, 46), the helicity of the beat patterns has never been measured directly in experiments. Using our 3D data, we can determine the local helicity of the flagellum shape $\boldsymbol{\Gamma}(s)$ at time t from the binormal vector $\boldsymbol{b}(t,s) = \boldsymbol{\Gamma}'(s)\times\boldsymbol{\Gamma}''(s)/|\boldsymbol{\Gamma}'(s)\times\boldsymbol{\Gamma}''(s)|$. In the head-centered frame, a helicoidal flagellum winding in counterclockwise direction when viewed from the front has local helicity $h(t,s) = \boldsymbol{b}(t,s)\cdot\boldsymbol{e}_x > 0$, whereas $h(t,s) < 0$ if the winding is clockwise. Plotting $h(t,s)$ along the flagellum as a function of time, we find no persistent helicity (Fig. 3*F*). Instead, the flagellar dynamics is dominated by helicity waves of either handedness (Fig. 3*F*). The mean helicity $H(t) = \frac{1}{L}\int_0^L h(t,s)\,ds$ fluctuates around zero, showing no discernible difference between left-turning and right-turning sperm (Figs. S8–S10).

Midpiece Asymmetry Determines Turning Direction. The midpiece connecting head and flagellar tail of a human sperm cell is ∼5 μm long, and its microstructure differs from that of the remaining flagellum (55). Our 3D data reveal that, unexpectedly, left-turning and right-turning sperm exhibit a notably different midpiece curvature. To quantify this effect, we measured the bend angle δ between the tangent at $s=0$ and the secant through $s_c \approx 4$ μm, and found that the bend angle distributions of left-turning cells are centered near zero, whereas right-turning cells exhibit a mean bend angle of $\bar{\delta} \approx 0.04$ rad for $\nu = 1$ cSt and $\dot{\gamma} = 2.56$ s^{-1} (Fig. 3*E* and Fig. S11).

Discussion

Structure of the 2D Model. The 2D trajectory data reveal two kinetically distinct swimming states, corresponding to $\chi = \pm 1$ in Eqs. **1** and **2**. More precisely, it is necessary to postulate an intrinsic preference for left turning ($\chi = +1$) or right turning ($\chi = -1$) in the effective 2D model because of the experimental observation that, after reversal of the flow direction, the majority of cells perform a complete U-turn (Fig. 1 and Movies S1–S6). To clarify this important detail, we may consider a hypothetical collection of cells without intrinsic turning preference, corresponding to $\chi = 0$. If the flow is along the negative x direction ($\sigma = -1$), the only stable fixpoint of Eq. **2** is $\varphi = 0$, corresponding to exact alignment against the flow direction. If we further assume that the cell orientations are approximately symmetrically distributed around this fixed point, then, after a flow reversal from $\sigma = -1$ to $\sigma = +1$, about 50% of the cells would turn left and right, respectively. However, each of those subpopulations would stop turning once they reach the new stable orientation fixed point $\varphi = \pi$. Thus, the resulting trajectory ensemble would trace out an open W shape instead of the experimentally observed "closed heart" shape (Fig. 1*A*).

Ambidextrous Beat Helicity. Our 3D analysis implies that neither rolling direction nor helicity controls the rheotactic turning

behavior of sperm cells, challenging the current paradigm of human sperm rheotaxis. Although it is, in principle, possible that helicity becomes biased near the posterior tip, which could not be reliably tracked due to the experimental frame rate limitations, such a scenario seems rather implausible, as the chiral waves should propagate through the whole flagellum. Moreover, a recent 3D analysis of the malaria parasite *Plasmodium berghei*, which has a 9+2 axoneme structure similar to human sperm, also challenges the picture of persistent helicity in flagellar propulsion (50).

Intrinsic Midpiece Curvature vs. Buckling. Our data demonstrate that the rheotactic turning behavior correlates with an asymmetry in the midpiece curvature distributions (Fig. 3*E* and Fig. S11). Recent hydrodynamic simulations show that midpiece curvature asymmetries strongly affect swimming trajectories, thus providing an effective long-range steering mechanism (42). Possible explanations for a curved midpiece are intrinsic curvature or symmetry breaking caused by a dynamic buckling instability. Intrinsic midpiece curvature is a known feature of rodent sperm (56) and has been linked to strongly asymmetric beat patterns (57). A less pronounced intrinsic curvature has been reported for human sperm, requiring artificial elevation of the intracellular calcium levels (58) absent in our experiments. An intrinsically curved midpiece per se does not explain the observed asymmetry in the distribution of the projected 2D bend angles (Fig. 3*E*), as the rolling motion would effectively symmetrize the distributions, and both left-turning and right-turning cells exhibit nearly identical rolling statistics (Fig. 3*D*). Therefore, a more plausible explanation is dynamic buckling, which is common in thin structures such as flagellar axonemes and cross-linked filament bundles (59). For sea urchin sperm, a buckling instability was suggested as an explanation for asymmetric compressed beat patterns at high viscosities (60). Nonlinear analysis and hydrodynamic simulations revealed that flagellar buckling arises generically from the interplay between elasticity and fluid forces (61), resulting in spontaneously broken symmetries of beat patterns and curved swimming trajectories. Although these buckling instabilities were predicted to affect the entire flagellum, localized buckling as observed in our experiments can be caused by inhomogeneities in the flagellar elastic stiffness. In human sperm cells, the midpiece carries the mitochondrial gyres and is therefore thicker than the tail of the flagellum (55); ergo, it is plausible that buckling occurs primarily at the transition point between midpiece and tail, triggered by perturbations that arise from the inhomogeneous material properties. In this picture, left-turning cells fluctuate symmetrically around the unbuckled state $\delta = 0$ whereas right-turning cells predominately occupy a buckled configuration with positive bend angle δ (Fig. 3*E*). The two distinct turning behaviors can then be inferred from basic force balance considerations (32, 33, 62): Approximating the flagellar beat envelope by a cone rotating counterclockwise around its symmetry axis, hydrodynamic interactions with the wall effectively result in a left-turning torque (Fig. 4). This rheotactic turning mechanism depends only on the flagellar rolling motion but not on the head and its geometry, in agreement with recent experiments demonstrating rheotaxis for headless mouse sperm (3). For right-turning cells trapped in a buckled state, the head acts as a tilted hydrofoil ("front rudder") that overcomes the rolling force. Thus, mirror symmetry is not just broken by the rolling motion but also by the midpiece bend.

Conclusions

Our joint experimental and theoretical study focused on the dynamics of human sperm cells swimming under the influence of a time-dependent linear flow gradient near a solid surface, a situation that is common in a wide range of external and internal fertilization processes. We identified two kinematically distinct rheotactic turning behaviors and showed that an effective 2D

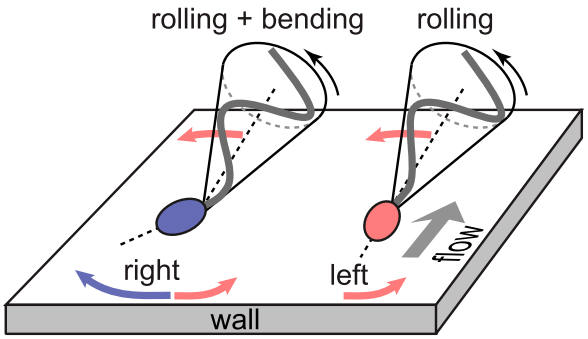

Fig. 4. Turning mechanisms implied by 3D data. Left-turning (red) and right-turning (blue) sperm cells roll their flagellum counterclockwise with a conical beat envelope, resulting in a left-turning torque. For right-turning sperm, this effect is counteracted by a larger opposing force due to the tilted head acting as a hydrofoil or "rudder."

mathematical model suffices to reproduce quantitatively the experimentally observed trajectory statistics. Building on, to our knowledge, the first systematic 3D beat reconstruction for freely swimming sperm cells, we found that human sperm flagella perform nearly planar beats in a stepwise-rotating plane. However, the rheotactic turning behaviors are independent of this rolling motion. Contrary to current opinion, the 3D beat patterns exhibit no persistent helicity but instead are composed of helical waves of either handedness. Interestingly, similar beating modes were reported recently for *Trypanosoma brucei* (63) and malaria parasites *P. berghei* (50). Taken together, these results suggest that ambidextrous beat patterns may be a common feature of eukaryotic uniflagellates that share the canonical 9+2 axoneme structure. Furthermore, our 3D beat pattern analysis reveals that rheotactic separation into left-turning and right-turning cells is related to a curved midpiece section. In the absence of evidence for intrinsic midpiece curvature in human sperm, a buckling instability combined with the wall-induced partial suppression of rolling can provide a plausible explanation for the observed bend angle asymmetry in right-turning cells. Recent experiments showed (28) that uniflagellate marine bacteria use buckling to change their swimming direction. Although passive prokaryotic and active eukaryotic flagella are built differently, buckling could provide a general physical mechanism for controlling reorientation in single-cell uniflagellates.

More generally, the above 3D observations call for a revision and extension of the currently prevailing helical models of flagellar propulsion. Future theoretical studies should focus on the interplay between rolling dynamics, wall interactions, and structural and elastic inhomogeneities in midpiece and flagellum. The basic ingredients for a bimodal rheotactic response—cell rolling and beat curvature asymmetry—are generic features of many sperm species (42, 48, 60, 61). It will thus be important to investigate experimentally if bimodal rheotactic response occurs in other species, in particular those featuring strong head asymmetries that may critically affect the proposed hydrofoil effect. Moreover, it will be interesting to study how calcium concentration (3), increased viscous load (36), and viscoelastic fluid environments affect the flagellar dynamics. The 3D beat reconstruction approach implemented here provides a promising starting point for such future studies.

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