INTRODUCTION: Conventional nonequilibrium systems are composed of inanimate components whose dynamics is powered by the external input of energy. For example, in a turbulent fluid, energy cascades down many length scales before being dissipated. In comparison, diverse nonequilibrium processes in living organisms are powered at the microscopic scale by energy-transducing molecular processes. Energy injected at the smallest scales cascades up many levels of structural organization, collectively driving dynamics of subcellular organelles, cells, tissues, and entire organisms. However, the fundamental principles by which animate components self-organize into active materials and machines capable of producing macroscopic work remain unknown. Elucidating these rules would not only provide insight into organization processes that take place in living matter but might lay the foundation for the engineering of self-organized machines composed of energy-consuming animate components that are capable of mimicking the properties of the living matter.

METHODS: We studied isotropic active fluids composed of filamentous microtubules, clusters of kinesin molecular motors, and depleting polymers. The polymer bundles microtubules, whereas the adenosine triphosphate (ATP)-fueled motion of kinesin clusters powers their extension. The extensile bundles consist of oppositely aligned polar microtubules and thus have quadrupolar (nematic) symmetry. They generate local active stresses that collectively drive mesoscale turbulent-like dynamics of bulk active fluids. Upon ATP depletion, the motion of microscopic motors grinds to a halt; the turbulent-like dynamics of active fluids ceases, and one recovers the behavior of conventional gels. We confined such active isotropic fluids into three-dimensional (3D) toroids, disks, and other complex geometries whose dimensions range from micrometers to meters and studied their self-organized dynamics. Using particle tracking and image analysis, we simultaneously quantified the flow of the background fluid and the structure of the active microtubule network that drives such fluid flows.

RESULTS: We demonstrate that 3D confinements and boundaries robustly transform turbulent-like dynamics of bulk active fluids into self-organized coherent macroscopic flows that persist on length scales ranging from micrometers to meters and time scales of hours. The transition from turbulent to a coherently circulating state is not determined by an inherent length scale of the active fluid but is rather controlled by a universal criterion that is related to the aspect ratio of the confining channel. Coherent flows robustly form in channels with square-like profiles and disappear as the confining channels become too thin and wide or too tall and narrow. Consequently, this transition to coherent flows is an intrinsically 3D phenomenon that is impossible in systems with reduced dimensionality. For toroids whose channel width is much smaller than the outer radius, the coherent flows assume a Poiseuille-like velocity profile. As the channel width becomes comparable with that of the toroid outer diameter, the time-averaged flow velocity profile becomes increasingly asymmetric.

DISCUSSION: Thousands of nanometer-sized molecular motors collectively generate a gradient in active stress, which powers fluid flow over meter scales. Our findings illustrate the essential role of boundaries in organizing the dynamics of active matter. In contrast to equilibrium systems in which boundaries are a local perturbation, in microtubule-based active fluid the influence of boundaries propagates across the entire system, regardless of its size. Our experiments also demonstrate that active isotropic fluids with apolar symmetry can generate large-scale motion and flows. From a technology perspective, self-pumping active fluids set the stage for the engineering of soft self-organized machines that directly transform chemical energy into mechanical work. From a biology perspective, our results provide insight into collective many-body cellular phenomena such as cytoplasmic streaming, in which molecular motors generate local active stresses that power coherent flows of the entire cytoplasm, enhancing the nutrient transport that is essential for the development and survival of many organisms.∞

Increasing the height of the annulus induces a transition from locally turbulent to globally coherent flows of a confined active isotropic fluid. The left and right half-plane of each annulus illustrate the instantaneous and time-averaged flow and vorticity map of the self-organized flows. The transition to coherent flows is an intrinsically 3D phenomenon that is controlled by the aspect ratio of the channel cross section and vanishes for channels that are either too shallow or too thin.

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Transition from turbulent to coherent flows in confined three-dimensional active fluids

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Transport of fluid through a pipe is essential for the operation of macroscale machines and microfluidic devices. Conventional fluids only flow in response to external pressure. We demonstrate that an active isotropic fluid, composed of microtubules and molecular motors, autonomously flows through meter-long three-dimensional channels. We establish control over the magnitude, velocity profile, and direction of the self-organized flows and correlate these to the structure of the extensile microtubule bundles. The inherently three-dimensional transition from bulk-turbulent to confined-coherent flows occurs concomitantly with a transition in the bundle orientational order near the surface and is controlled by a scale-invariant criterion related to the channel profile. The nonequilibrium transition of confined isotropic active fluids can be used to engineer soft machines.

Conventional nonequilibrium pattern-forming systems, such as Rayleigh-Benard convection, are powered by continuous injection of energy through macroscopic external boundaries (1). In comparison, hierarchically organized living matter is driven away from equilibrium by the motion of microscopic energy-transducing animate constituents. Starting with molecular motors and continuing up to subcellular organelles, cells, tissues, and entire organisms, each biological element is constructed from energy-consuming components. At each level of hierarchy, these animate elements push and pull on each other, thus generating local active stresses. Collectively, these stresses enable diverse functionalities that are essential for survival of living organisms, such as cell division and motility (2–4). Under geometrical confinement, active building blocks such as motile cells, tissues, and organisms can also organize into a coherent moving network of nonequilibrium states (9–17). Creating active matter systems from the bottom up that mimic the remarkable properties of living matter remains a fundamental challenge (18–20). Recent studies have revealed the emergence of diverse complex patterns in synthetic systems of active matter (21–26). The next step is to elucidate conditions that transform chaotic dynamics of these systems into coherent long-ranged motion that can be used to harvest energy and thus power various micromachines (27–32).

Here, we study three-dimensional (3D) active fluids and demonstrate an essential difference with their conventional counterparts. The Navier-Stokes equations dictate that a conventional fluid composed of inanimate constituents will flow only in response to an externally imposed body force, or stress and pressure gradients (33). This is no longer true for active fluids. In living organisms, the entire cellular interior can assume large-scale coherent flows in absence of any externally imposed stresses, a phenomenon known as cytoplasmic streaming (34–36). Despite recent advances using living bacterial suspensions (12, 13, 17, 37), creating tunable synthetic active fluids that exhibit autonomous long-ranged flows on length scales large compared with those of constituent units remains a challenge. We use a 3D microtubule-based isotropic active fluid whose bulk turbulent flows are driven by continuous injection of energy through the linear motion of the constituent kinesin motors (24, 38). We found that confinement robustly transforms locally turbulent dynamics of such active fluids into globally coherent flows that persist on meter scales. Our experiments demonstrate that nonequilibrium transitions of synthetic active materials can be used to engineer self-organized machines in which nanometer-sized molecular motors collectively propel fluid on macroscale.

**Microtubule-based active isotropic fluids**

The active fluid we studied comprises microtubule filaments, kinesin motor clusters, and depleting polymer (Fig. 1A) (24, 38). Kinesin motors are bound into synthetic clusters with tetrameric streptavidin (39, 40). The depleting polymer induces microtubule bundling (41), while the kinesin clusters simultaneously bind to and move along the neighboring filaments. Formed aligned microtubules with opposite polarity, kinesin clusters generate interfilament sliding that drives the flow of the background fluid (Fig. 1A) (42). At finite concentrations (0.5 mg/mL), active microtubule bundles form a self-rearranging 3D isotropic network, whose dynamics comprise repeating cycles of bundles extending, buckling, fracturing, and annealing. Such dynamics effectively drive the flow of the background fluid that is mostly composed of an aqueous buffer (~99.9%). In bulk suspensions, microtubule-based active networks generate turbulent-like flows that exhibit neither long-range order nor net material transport. The structure of these flows and the associated vorticity fields are visualized by doping active fluids with fluorescent tracer particles (Fig. 1D and Movie 1). Such analyses reveal a characteristic vortex size of ~10 μm, with an average lifetime of ~3 s.

Energy-efficient kinesin motors allow for assembly of bulk 3D active fluids that maintain steady-state turbulent flows for up to 10 hours. This feature allows us to quantify the time-average of various steady flows. Furthermore, filamentous microtubules effectively couple to the background fluid. Therefore, even at volume fractions as low as 0.05%, the microtubule network drives the bulk turbulent flows. At such low concentrations, the network has a very large pore size (~10 μm); thus, its structure can be visualized and quantitatively analyzed with optical microscopy. This property allows us to correlate the
properties of the emergent flows to the structure of the underlying active network.

**Confinement-induced coherent flows**

We found that 3D toroidal confinements can effectively transform directionless turbulent flows of bulk fluids into coherent circular currents capable of transporting materials over macroscopic scales (Fig. 1, B and E). Similar phenomena were also observed for cylindrical confinements, demonstrating that toroidal geometry is not an essential requirement for the formation of coherent flows (Fig. 1F). The autonomous circular currents persisted for hours and ceased only after the available chemical energy [adenosine triphosphate (ATP)] is depleted (Fig. 1C). We measured $F \approx 0.6$, and the magnitude of the order parameter remained fairly constant over the entire sample lifetime (Fig. 1C).

**Fig. 1. Coherent macroscopic flows of confined active fluids.** (A) The three main constituents of an active fluid: microtubules; kinesin clusters, which power interfilament sliding; and pluronic micelles, which act as a depleting agent. (B) Schematic of a coherent flow that emerges when active fluids are confined in a toroid. (C) Time evolution of the averaged azimuthal velocity (blue) and the circulation order parameter (red) demonstrates that circular flows cease upon ATP depletion. (D) A flow map demonstrates that bulk active fluids exhibit turbulent flows that do not transport material. The vector field indicates the local flow velocity normalized by the mean flow speed. The color map represents the normalized vorticity distribution, with blue and red tones representing CCW and CW vorticities, respectively. Left and right halves are instant and time-averaged plots, respectively. (E) An active fluid in a toroidal confinement exhibits persistent circular flows. (F) Coherent flows in a cylindrical confinement. Confinement heights are 1.3 mm. (D) to (F) share the same scale, velocity, and color bars.

**Movie 2. Coherent and incoherent flows in toroids and cylinders.** Flows of active fluids in toroidal and cylindrical confinements that support and suppress coherent flows. Increasing confinement height induces a transition from incoherent to coherent flows. Heights of taller toroid (top left) and cylinder (top right) are 1.3 mm, and heights of shorter ones (bottom left and right) are 0.33 mm. Black vectors and color maps (left half in each panel) represent fluid velocity fields and vorticity distributions extracted from the motion of tracer beads (right half). Time stamp is hour: minute: second.

Not all geometries support coherent flows. For example, reducing the height of a toroid or a cylinder below a critical value completely suppressed coherent flows and yielded turbulent-like dynamics (Movie 2 and fig. S1). To determine geometries that support self-organized circular
Fig. 2. Phase diagram and velocity profiles of coherent flows in toroids and cylinders. (A) A phase diagram indicating confinement geometries that support circular flows. The phase diagram is limited to thin confining geometries, \( h \leq 2w \). Circles and dots represent toroidal and cylindrical confinements, respectively. (B) The circulation order parameter, \( \Phi \), as a function of the confinement aspect ratio, \( \alpha = (h - w)/\text{Max}(h, w) \). \( \alpha = 0 \) represents a square cross section. Blue and red dots represent toroids and cylinders, respectively. For \( \alpha < 0.5 \), \( \Phi \) was measured at midplane. For \( \alpha > 0.5 \), \( \Phi \) was measured at a quarter plane because of the finite working distance of the microscope objective. (C) Flow velocity profiles in various toroidal confinements taken at a midplane. Outer diameters of toroids for blue, teal, and green curves are 4300 \( \mu \text{m} \) and for olive and red curves are 2000 \( \mu \text{m} \). (Inset) Flow profiles of toroids with width \( \leq 300 \mu \text{m} \). (D) Midplane flow profiles in various cylindrical confinements. (Inset) Flow profiles in cylinders with radius \( \leq 300 \mu \text{m} \). The color bar on the right indicates the confinement heights.

Fig. 3. Controlling flow of coherent handedness. (A) The handedness of the circular flows is controlled by decorating the toroid’s outer surface with counterclockwise or clockwise ratchets. The vector and color maps represent the local flow velocity and vorticity. (B) Velocity field and vorticity maps demonstrate that each notch induces a stationary vortex. (C) Midplane flow profiles in CCW and CW ratchets with varying notch numbers. Backflow from ratchets occurs near the outer edge, radius \( r \) = 2300 \( \mu \text{m} \). All channel heights are 1.3 \( \mu \text{m} \). (D) The circulation order parameter as a function of the number of notches.
flows do not occur (Fig. 2B). In other words, the scale-invariant criterion does not exist in two dimensions because there is only one channel dimension \(w\) perpendicular to the flow. In contrast, in 3D systems, the cross section has two dimensions \(h, w\), making it possible to construct a dimensionless aspect ratio that controls the onset of the coherent flows.

**Structure of the coherent flows**

To quantify the spatial structure of the self-organized circular flows, we measured time-averaged azimuthal velocity profiles \(v_\theta(r)\) in both toroidal and cylindrical geometries. For small toroidal widths \((w < 1000 \mu m)\), coherent flows exhibited Poiseuille-like symmetric profiles, with a peak velocity at the channel midpoint and decreasing to zero at the boundary because of the no-slip condition (Fig. 2C). The peak velocity increased with channel height. For a 330-\(\mu m\)-high and 650-\(\mu m\)-wide toroid, the maximum velocity was \(~4 \mu m/s\). Increasing the height to 1300 \(\mu m\), for the same width, increased the peak velocity to \(~10 \mu m/s\). For the largest toroids studied, peak velocities reached \(~17 \mu m/s\), whereas for smallest micrometer-sized ones, peak velocities were as small as \(~1 \mu m/s\) (Fig. 2C, inset). The asymmetry of the time-averaged velocity profile increased with increasing toroid width. The asymmetry became especially pronounced for cylindrical geometries in which the velocity increased from zero at the disk center, growing linearly to a maximum value before decreasing rapidly to zero at the outer edge (Fig. 2D). Such a distinguishing feature is universal for both micrometer- and millimeter-sized cylinders, indicating again that the flow structure is independent of the confinement size.

**Controlling handedness of coherent flows**

In mirror-symmetric toroids and cylinders, coherent flows are equally likely to be either counterclockwise

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**Fig. 4. Circulating flows in closed tracks.** (A) Velocity profiles of coherent flows in racetrack geometries are independent of the length of the straight segment (solid curves). (B) Coherent flow in a 200-mm-long racetrack. Because of limited observation windows, we only observed the blue and red segments whose flow profiles are dashed same-color curves in (A). The fluids in these segments flow as fast as in an equivalent toroid whose \(w = 1 \text{ mm}\) and \(h = 1.3 \text{ mm}\) [dashed black curve in (A)]. (C) Self-organized flows can solve a 2D maze consisting of a closed loop (brown) and 10 dead-end branches (black). We observed only the blue and red segments in the close loop and the green segment in one branch. Their flow profiles are the dotted same-color curves in (A). Dead-end branches inhibit net transport, whereas the closed loop supports a persistent circulating flow. All channel heights are 1.3 mm.

**Movie 3. Coherent flows in 12- and 1-tooth CCW and CW ratchets.** Decorating the toroid outer surface with notches induced coherent flows in the direction of same handedness. Even one notch is sufficient to control flow direction. The notch sizes are \(~300 \mu m\). Top two ratchets (left, CCW; right, CW) have 12 teeth, and bottom ones (left, CCW; right, CW) have only 1 tooth. Heights are 1.3 mm. Time stamp is hour: minute: second.

**Movie 4. Coherent flows in concentric micrometer-sized toroids.** Toroids’ outer radii range from 175 to 1525 \(\mu m\). Varying the radii does not alter flow rates, implying that coherent flow is independent of channel curvature. Tracks are shaded in blue and red for CCW and CW coherent flows. Channel heights are 60 \(\mu m\). Time stamp is hour: minute: second.

**Movie 5. Coherent flows in racetracks with varying circumferences and an equivalent toroid.** Varying circumferences does not alter flow rate, implying that coherent flow is independent of channel length. The lengths of straight paths from left to right are 17,200, 12,900, 4300, and 0 \(\mu m\), respectively. Heights and widths in the racetracks and toroid are both 1.3 mm. Time stamp is hour: minute: second.
Coherent flows in complex confinements. (A and B) Two distinct flow configurations in a pair of partially overlapping toroids. Confinement heights are ~60 μm. (C and D) Flow paths in three partially overlapping toroids. Confinement heights are ~60 μm. (E) Coherent flows in a square-like confinement. Channel height is ~75 μm.

(CCW) or clockwise (CW). To control the flow handedness, we decorated the toroid outer edge with sawtooth notches, finding that a CCW geometry induces flows of same handedness and vice versa (Fig. 3A and Movie 3) (43, 44). In contrast, decorating inner edges with the same pattern leads to flows with opposite handedness (fig. S2, A and B). Coherent flows could be completely suppressed by decorating both inner and outer surfaces with notches of the same chirality (fig. S2, C and D). Decreasing the number of notches increased the circulation order parameter, Φ, and even a single notch controlled the flow direction (Fig. 3D). Flow maps reveal that each notch creates a stationary vortex (Fig. 3B), and the back-flow associated with this vortex decreases the overall flow coherency (Fig. 3C).

We explored how the self-organized flow rates scale with the channel curvature and length, by either changing the toroid radius or stretching toroids into shapes resembling racetracks while maintaining the same cross section. The toroidal radius (curvature) does not affect the properties of the self-organized flows (Movie 4 and fig. S3). For racetrack geometries, we varied the total circumference length from 14 to 48 mm (Fig. 4A and Movie 5). Flow profiles in these geometries were nearly identical, demonstrating not only that the flow rate is independent of the pipe length but also that the straight and curved segments of a racetrack provide equal driving power. To further confirm this finding, we designed a 20-cm-long track (Fig. 4B). Coherent flow persisted in such a lengthy track; its rate remained nearly the same as that in a toroid of equivalent cross-section but shorter circumference (Fig. 4A).

Coherent flows in complex confinements

The self-organized coherent flows are remarkably robust self-organized patterns. To determine limits of their stability, we explored emergent dynamics in geometries of increasing complexities. First, coherent flows developed not only in simple toroids but also in a complex maze-like geometry comprising a closed loop and many dead-end branches (Fig. 4C). Upon filling such geometry with active fluid, coherent flows developed in the closed loop while remaining turbulent in the dead-end branches. However, unlike racetrack geometries, the flow rate was ~50% slower than in an equivalent toroid, indicating that the dead-end branches disturb the self-organized flows (Fig. 4A). Second, we explored flows that emerge in two or three adjoining toroids and found that such geometries support multiple self-organized states (Fig. 5 and Movie 6) (45). For example, in one state, flows in the two adjoining toroids had opposite handedness and were thus reinforced in the mutually adjoining region (Fig. 5A). In another state, the flow organized along the outer edge of the toroid doublet, therefore suppressing coherence in the adjoining region (Fig. 5B). Similarly, in triplet geometry we observed either flow circulating along the outer edge, leaving the center quiescent (Fig. 5C), or CW flow in one toroid and CCW flow in the other two (Fig. 5D). Third, coherent flows also developed in confinements with sharp corners, such as a square-like geometry (Fig. 5E). However, sharp corners induced formation of stationary vortices, which generated back-flow, thus decreasing the circulation order parameter, when compared with a toroid with an equivalent cross section.

Last, we found that the self-organized flows are not limited to microfluidic channels. For example, active fluids exhibited macroscopic flows both within a torus inscribed within a finite yield-stress elastomer (fig. S4) (46), as well as a 1.1-m-long plastic tube (Fig. 6).

Structure of the microtubule network

To elucidate the microscopic mechanism that drives coherent flows, we imaged the microtubule network structure of active fluids in both turbulent and coherent states and extracted the orientational distribution function (ODF) of the constituent bundle segments (Fig. 7A and Movies 7 and 8) (47). In the channel center, the measured ODFs were isotropic in both the coherent and turbulent states (indistinguishable from ODFs of bulk active fluids) (Fig. 7B). Emergence of the coherent flows was correlated with the change in the thickness of the nematic layer that wets the surfaces. We measured the spatial variation of the nematic order parameter in the direction perpendicular to the confining wall (Fig. 7C). In turbulent states, the nematic order decays away from the boundaries with a characteristic length.
scale ($l \sim 30 \, \mu m$). Transition to coherent flows is accompanied with an increase in the thickness of the surface-induced nematic layer ($l \sim 100 \, \mu m$). Similar to fluid flow profiles (Fig. 2C), lateral profiles of nematic order were asymmetric for circulating toroids with wide cross sections, whereas they were symmetric for narrow ones (Fig. 7C, inset), implying that the shear rates generated by coherent flows drive the filament alignment. The angle between the confining surface and the self-organized nematic layer had a finite nonzero value of ~20° (Fig. 7B), suggesting that active stresses generated by the spatially distorted wall-bounded nematic layer push against the no-slip boundary and thus propel the fluid forward (49).

**Discussion**

We demonstrated that confining boundaries transform turbulent dynamics of bulk isotropic active fluids into long-ranged coherent flows. Our results suggest a mechanism by which this transition takes place. In conventional isotropic liquid crystals, the bounding surface breaks the local symmetry and locally aligns the constituent particles (49). In active systems, turbulent flows enhance this effect, magnifying and extending the wall-induced nematic order. We suggest that the nematic-wetting layer aligns to the surface at an oblique angle, thus generating an active surface stress that induces further flows. In turn, these flows further enhance and stabilize the orientation of the wetting layer. This positive feedback loop yields a cooperative nonequilibrium transition to globally coherent flows, reminiscent of a phenomenon known as “backflow” in conventional liquid crystals, in which mutually reinforcing coupling between the reorienting nematic director and the fluid velocity leads to emergent periodic structures (50, 51).

Emergence of coherent flows is determined by a universal scale-invariant criterion, which is related to the aspect ratio of the confining channel cross section rather than the overall size of the confining geometry. In particular, coherent flows take place when the channel cross section is relatively symmetric—that is, the width and height of the channel are within a factor of 3 of each other. Understanding the onset of large-scale flows is a central focus of active matter research. One class of theoretical models predicts an active Fréedericksz transition from quiescent to coherent flows for monodomain active liquid crystals (27, 28, 52, 53). There are several notable differences between our observations and the active Fréedericksz transition. First, our phenomenon is inherently 3D. That is, the aspect ratio alone, and not the absolute channel size, controls the onset of coherent flows forbids this transition from taking place in systems with lower dimensionality. This restriction does not hold for the active Fréedericksz transition, which can occur in two dimensions. Second, in our experiments the coherent flows form in an active isotropic fluid, instead of a liquid crystalline one. Third, the flows we observed also emerged from the turbulent state, whereas the active Fréedericksz transition predicts the formation of such flows from a quiescent, single-domain nematic.
The boundary-generated active stresses power coherent flows. This bears similarity to electro-osmosis, in which an external electric field creates coherent flows. This bears similarity to electro-osmotic plug flow is independent of solid body, resemble such plug flows. However, there are also notable differences. The velocity of electroosmotic plug flow is independent of channel size when the boundary layer is small. In contrast, we observed that the coherent active flow velocity varies with channel size. Additionally, active flows in tori resemble Poisuelle flow, indicating pressure-driven flow.

The possible relevance of our mechanism to cell biology still has to be ascertained. In particular, coherent cytoplasmic flows in living cells are powered by cytoskeletal filaments that are permanently affixed at the cellular cortex (34–36), whereas in our system, the active elements are dispersed throughout the sample interior and dynamically self-organize at the solid boundaries that confine the fluid.

We demonstrate that nonequilibrium transitions in active matter can be used for the robust assembly of self-organized machines that produce useful work by powering large-scale fluid flows. These machines are fueled by the collective motion of the constituent nanometer-sized motors and are thus evocative of living organisms. However, the goals of active matter research are not only to recreate materials found in nature but additionally are to discover and elucidate the full range of dynamical organization that is possible in living and nonliving systems alike. Toward this end, explaining the scale-invariant criterion for the onset of coherent flows and their velocity profiles represents a challenge for active matter theory.

Materials and methods

Assembling kinesin clusters

We used kinesin motors dimers comprising a 401-amino acid N-terminal domain derived from Drosophila melanogaster kinesin labeled with a 6-his tag and a biotin tag. Kinesin motors were purified according to a previously published protocol (55). An isolated kinesin moves along a single microtubule protofilament toward its plus end. Linking kinesins into multimotor clusters enables them to bind multiple microtubules and drive interfilament sliding. The biotin-labeled motors were assembled into multimotor clusters using streptavidin tetramers. To assemble kinesin clusters, we mixed 1.5 \( \mu \)M kinesin motors with 1.8 \( \mu \)M streptavidin (Invitrogen, S-888) at a 1:1.2 ratio in microtubule M2B buffer (M2B: 80 mM PIPES, pH 6.8, 1 mM EGTA, 2 mM MgCl\(_2\) containing 120 mM dithiothreitol (DTT)). The mixture was then incubated for 30 min at 4°C, before being aliquoted and stored long-term at -80°C.

Microtubule polymerization

Tubulin monomers were purified from bovine brains with two cycles of polymerizations and depolymerizations in a high salt buffer (1 M PIPES buffer), followed with one cycle in M2B buffer (56). To induce polymerization, the tubulin suspension (8 mg/mL in M2B) was mixed with 600 \( \mu \)M GTP (guanosine-5’-[(\(\alpha,\beta\)-méthylthio]triphosphate, Jena Biosciences, NU-4056) and 1 mM DTT. For fluorescence imaging, 3% of tubulin monomers were labeled with Alexa-Flour 647 (Invitrogen, A-20006). The tubulin mixture was first incubated for 30 min at 37°C, followed by an annealing step at room temperature for six hours. The resulting microtubules have an average length of ~1 \( \mu \)m (24). The polymerized microtubules were stored at -80°C.

Microtubule-based active fluids

To assemble active fluids, 1.3 mg/mL microtubule suspension was mixed with kinesin-streptavidin motor clusters in a high-salt M2B (M2B + 3.9 mM MgCl\(_2\)) (24). Kinesin motors hydrolyze adenosine triphosphate (ATP) into an adenosine diphosphate (ADP) while stepping toward the microtubule plus end. To fuel such motion we added 1.4 mM ATP. To maintain constant ATP concentration and steady dynamics in active fluids, we have incorporated an ATP regeneration system comprising 26 mM phosphoenol pyruvate (PEP) and 28% v/v stock pyruvate kinase/lactate dehydrogenase enzymes (PK/LDH) (Sigma, P-0294) (57). PK consumes PEP and converts ADP back to ATP, thus maintaining steady ATP concentration. To reduce photo-bleaching effects during fluorescence imaging, we added 2 mM trolox (Sigma, 238813) and an oxygen scavenging enzyme mixture comprised of 0.22 mg/mL glucose oxidase (Sigma, G2133), 0.038 mg/mL catalase (Sigma, C40), and 3.3 mg/mL glucose. To stabilize proteins we added 5.5 mM DTT as a reducing agent. As the depletion agent we used 2% w/w Pluronic F127, which assembles into ~20 nm micelles (58). Finally, to track the fluid induced by active fluid, we added ~0.0004% v/v Alexa 488-labeled 3-\(\mu\)m polystyrene particles, stabilized by Pluronic F127. Upon mixing all of the above ingredients, nonequilibrium dynamics of active fluids can last for hours, until both ATP and PEP are depleted.

Engineering microfluidic chips

Millimeter-scale (\(\geq 0.3\) mm) devices were manufactured by directly milling a 2-mm-thick COC plaque (TOPAS Advanced Polymers) with a CNC end-milling machine, which reads G-code translated from CAD with Cut2D (Vector). Micron-scale devices were manufactured by embossing COC plaques (thermoplastic polymers) with patterned polydimethylsiloxane (PDMS, thermosetting polydimethylsiloxane) master molds by casting uncured PDMS resin (1:10 ratio of 10-cst PDMS oil (Sigma-Aldrich, 378321-250ML) and 74% w/w elastomer (Dow Corning 9041 silicone elastomer blend). To inscribe a torso, we inscribed a 27-gauge blunt needle (SAI Infusion Technologies, B27-150) into the elastomer, followed by rotating the elastomer while pumping active fluids through the needle. The pumped fluid formed a torus after one full revolution. The torus radius could be tuned by changing the distance between the needle tip and the elastomer rotation axis; the torus thickness by controlling the pumped fluid volume. Subsequently, the needle was extracted from the elastomer, yielding an isolated torso sealed within the elastomer (Fig. S4). Active fluid contained Pluronic.
that coated the inner torus surface, preventing microtubules adsorption. Unlike COC, the elastomer contains PDMS oil so the surface of the torus confinement is fluid.

**Tube confinements**

To investigate the broader applicability of our findings we loaded active fluids into a plastic tube [Scientific Commodities Inc., BB31696-PE/5, (Fig. 6)]. The tube length was 1,060 mm; its outer and inner diameters were 1.32 and 0.86 mm, respectively. Tube ends were joined with a 50-mm long 20-gauge metallic needle (SAI infusion technologies, B20-150). The needle outer and inner diameters were 0.91 and 0.60 mm, respectively. To reduce evaporation from gaps between the needle and tube, we applied vacuum grease (Dow Corning). For imaging purpose the tube was fixed on a glass slide with tape. The tube cross-sectional profile was circular, distorting tracer images due to index of refraction mismatch. To reduce such distortions, the tube was immersed in uncured UV glue.

**Analyzing structure of active networks**

To correlate fluid flow with underlying active gel structure we z-scan microtubule networks with confocal microscopy when in coherent and turbulent states. From the midplane slice (thickness = 10 μm) we use a filament tracking algorithm to extract bundle structures, represented as winding snakes (magenta curves in Fig. S6A and Movie 8 (44)). Snakes are composed of unit-length bundle segments whose r-φ-plane orientations are given by angle θ, where −π/2 ⩽ θ ⩽ π/2. To measure time-averaged distributions of θ, the 3D confocal images of the networks structure were continuously acquired, and from these angles θ were extracted. The snake reconstruction algorithm has a bias that yields a slight peak around θ = 0 and π/2, even for suspensions that are known to be isotropic (blue curve in Fig. S6B). When the images were rotated by ~45° the peaks also shifted by ~45°, demonstrating the biased detection associated with the reconstruction algorithm. To remove this artificial bias we rotated the images from 0° to 360° by 30° (Fig. S6C). From these images, we extracted and stacked bundle orientations. The stacked orientations yielded a probability orientation distribution to within a few percent (gray curve). Repeating the same experiments with different active networks yielded similar distributions (black curve). This method was applied to eliminate the bias of the reconstruction algorithm, for both coherent and incoherent suspensions (Fig. 7, A and B).

To characterize bundle orientational order we measured the scalar nematic order parameter (NOP), the largest eigenvalue of the nematic order parameter tensor, Q_{ij} defined as Q_{ij} ≡ (δ_{ij} - n_i n_j)/(2), where $n = \cos(\theta)\hat{r} + \sin(\theta)\hat{\phi}$ is the director of a bundle segment, and $\delta_{ij}$ is Kronecker delta function (Fig. S6A). To characterize NOP spatial distribution we measure its radial profile, NOP (d_p), the corresponding tensor profile Q_{ij}(d_p) is measured by taking the average over r and φ: Q_{ij}(d_p) = <δ_{ij}>. In either coherent or incoherent states the NOP profile decays from boundaries. The decay length λ is defined by where NOP is reduced by a factor of three: NOP (distance J from a boundary) = NOP (at the boundary)/3 (Fig. 7C).

**REFERENCES AND NOTES**

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S6

Fig. S1. Figs. S1 to S6

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Go with the changing flow

The transport of ordinary fluids tends to be driven by pressure differentials, whereas for active or biological matter, transport may be isotropic or governed by the presence of specific chemical gradients. Wu et al. analyzed the emergence of spontaneous directional flows in active fluids containing a suspension of microtubules and clusters of the molecular motor kinesin, confined in a variety of microfluidic geometries (see the Perspective by Morozov). When confined in periodic toroidal channels and cylindrical domains, the flow was organized and persisted in a unidirectional motion, either clockwise or counterclockwise. Oddly, this behavior was independent of scale; as long as the aspect ratio of the geometry was chosen appropriately, flows were observed for a wide range of system dimensions.

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