Supporting Online Material for

Molecular Sorting by Electrical Steering of Microtubules in Kinesin-Coated Channels
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Materials and methods

Nanofabrication. Nanofluidic channels were fabricated in 500-µm-thick fused-silica substrates of 13 x 19 mm, containing a 2 x 3 array of 1-mm diameter entrance holes separated by 5 mm. The chips with holes (created by powder blasting) were purchased from PlanOptik (Unter den Eichen, Germany). Substrates were cleaned by subsequent sonications of 5 min in acetone, fuming nitric acid (HNO₃), and iso-propanol alcohol (IPA). Then, a 35-nm-thick layer of chromium (Cr) was deposited by sputter deposition, functioning as an electron sink during electron-beam (e-beam) lithography. A ~800-nm-thick e-beam-sensitive resist polymethylmethacrylate (PMMA) was spincoated on the sample, followed by a 15-min bake on a hotplate at 170 °C. Channel structures were defined with a Leica electron-beam pattern-generator with a dose of 1200 µC/cm². The samples were developed by a 75-s immersion in a mixture (1:3) of methyl-isobutyl ketone and IPA and a subsequent immersion of 45 s in IPA to stop the development. A 1-min immersion in Cr-etchant (Merck) removed the Cr layer in the structures, exposing the fused silica. The channels
were wet etched ~800 nm into the substrates using ammonium-fluorid etchant (Merck 7:1). After etching, the resist and chromium were removed by a 15-min sonication of the substrates in PRS3000 (Merck) to strip the PMMA, and then a 1-min submersion in cr-etchant.

The channel structures were sealed by a 170-µm-thick microscope cover slip using a silica bonding procedure (S1). To this end, the fused-silica substrates and the cover slips were thoroughly cleaned by a 15-min sonication in PRS3000 and a 10-min sonication in HNO3. Then, the substrates were exposed to a 0.5 % solution of hydro-fluoric acid for 1 min. A 2 % sodium-silicate solution (Sigma) was spin-coated (6000 rpm, 1 min) onto the cover slip, immediately followed by pressing the sodium-silicate surface to the patterned channel surface. Finally, the devices were cured for 2 hours at 90 °C.

**Polymerization of microtubules.** Microtubules were polymerized from 5 µL of bovine brain tubulin (4 mg/mL, Cytoskeleton, Denver, CO). For the red-labeled microtubules we used a stoichiometry of one rhodamine-labeled unit and three unlabeled units of tubulin. For the green-labeled microtubules, we used a stoichiometry of one fluorescein-labeled unit and 2 unlabeled units, to match the intensity of red and green fluorescence in our setup. Polymerization occurred in the presence of 4 mM MgCl2, 1 mM GTP and 5% DMSO in BRB80 buffer (37 °C for 45 min). Then the microtubules were stabilized and 40x diluted in BRB80 containing 100 µM Taxol (BRB80T).

**Motility protocol.** Microtubule motility was reconstituted in fluidic channels by flushing in the protein constituents using a pressure-driven flow. First, channels were filled with BRB80 buffer solution (80 mM Pipes, 1 mM MgCl2, 1 mM EGTA, pH=6.9), supplemented with 100 µM paclitaxel (Taxol), 5 mM ATP, and 2 % beta-mercaptoethanol (BME) and flushed to remove air
bubbles. The resistance of the channel was measured to check the integrity of a channel. Second, a 0.9 mg/mL casein solution in BRB80 was added to the entrance reservoir and flushed through the channel using a pressure difference of 5 kPa. This flow was maintained for a time sufficiently long to coat the entire channel structure with casein (~30 min, depending on geometry of the channel). Third, a kinesin solution \([70 \mu g/mL \text{ his-tagged full-length } Drosophila \text{ conventional kinesin (S2), 0.9 mg/mL casein, 9 mM ATP in BRB80}]\) was flown through the channels with the same pressure.

After coating the channels with kinesin, the entrance reservoir was emptied and a motility solution was added. The motility solution contained microtubules (~200 nM tubulin) in BRB80, supplemented with 100 µM Taxol, 6 mM ATP and an antifade cocktail (120 mM D-Glucose, 0.12 mg/mL glucose-oxidase, 0.05 mg/µL catalase and 2% BME) to prevent photobleaching. Subsequently, microtubules are propelled by kinesin towards the entrance of the channel structures.

**Experimental setup.** Microtubule motility was observed using fluorescence microscopy on an inverted IX81-olympus microscope using a 100x oil-immersion objective (Olympus, N.A. = 1.35) and a Hamamatsu CCD-camera. For most observations we used an ORCA ER B/W camera. For the molecular sorting experiments, we used a Hamamatsu C7780-10 color camera. All experiments were performed at room temperature.

**Electric field application.** Electric fields in the nanochannels were induced by applying voltages to platinum electrodes in the entrance reservoirs using a Keithley 6517A electrometer. At all times, the current through the channel was simultaneously measured using the electrometer. During experiments, the entrance reservoirs were exposed to atmospheric pressure, thus preventing a Poiseuille backflow due to EOF-induced pressure build-up. The buffer levels in the reservoirs
were equalized within an accuracy of <1 mm. The corresponding maximum hydrostatic pressure difference between the reservoirs of 10 Pa would induce a maximum fluid flux of \( \sim 10^{17} \text{ m}^3/\text{s} \). Given the geometry of our channels, the average fluid velocity due to unequal buffer levels is thus limited to <1 \( \mu\text{m/s} \), which is negligible to EOF-velocities at the field strengths used.

The effect of electric field application on the buffer temperature can be estimated as small. The dissipation per unit volume in a channel due to the current can be estimated as \( P_{diss} = \sigma E^2 \), where \( \sigma = 1.25 \text{ S/m} \) is the conductivity of the buffer solution, and \( E \) is the electric field. In steady state, the dissipation is balanced by thermal conduction through the glass. The thermal flux \( \Phi \) equals \(-\lambda \partial T/\partial z\), with \( \lambda \) the thermal conductivity of glass (\( \lambda = 1.38 \text{ W/Km} \)) and \( \partial T/\partial z \) the temperature gradient. In steady state the temperature gradient in the glass substrate is thus given as \( \partial T/\partial z = -\sigma E^2 h/\lambda = 10^5 \text{ K/m} \), for a channel of height \( h = 1 \mu\text{m} \) at the highest \( E = 100 \text{ kV/m} \).

Assuming linear gradients in the glass substrates of 170 \( \mu\text{m} \) and 500 \( \mu\text{m} \) thickness on both sides of the channel, the temperature increase in the channel due to the electric field is limited to 2 °C. An in-situ probe of the temperature is provided by the velocity of the motility in our channels. Motility speed is known to double for approximately every 10 °C increase in temperature (S3). The fact that we do not see any systematic increase in gliding velocity during field application can be taken as a indication that the temperature increase in our channels is much smaller than 10 °C.

The effect of electric field application on buffer composition is also negligible. The total buffer volume in each reservoir is \(~20 \mu\text{L} \). Measured currents in our experiments are \(~1 \mu\text{A} \), inducing an electron-transfer rate at each electrode of \(~ 10^{13} \text{ e/s} \). Assuming hydrolysis of water in the reservoirs, these currents induce a local change in the ion-concentration of \(~ 1 \mu\text{M/s} \). During the time span of experiments (\(~1 \text{ hour} \)), the current application creates \(~ \text{mM} \) of mainly \( \text{H}^+/\text{OH}^- \) ions, which is easily buffered in our 80 mM PIPES buffer.
Electrophoresis experiments. Observations of the electrophoretic velocity of microtubules were made at a rate of 15-20 Hz. Within this time resolution, Brownian motion of the microtubules can be neglected. We estimate the center-of-mass diffusion of a microtubule of length $L$ as $(\Delta r)^2 = 2k_BT(c_\perp^{-1} + c_\parallel^{-1})/L = 6k_BT/c_\perp L$. Here, we have used the Einstein relation and the longitudinal drag coefficient $c_\parallel = c_\perp/2$. Using the value of $c_\perp$ mentioned in the main text, we find that for a 5 µm long microtubule the Brownian movement between successive frames is limited to $\Delta r < 0.2$ µm.

Electro-osmotic flow measurements. Values of the electro-osmotic flow (EOF) mobility $\mu_{\text{EOF}}$ were measured by a current-monitoring method (S4). In these experiments we first coated the channels with casein and kinesin to create identical surface properties as in the motility experiments. The two reservoirs at both ends of the channels were filled with respectively 100% buffer and 90% buffer, diluted in ddH$_2$O. The reservoirs thus contain the same electrolyte, however, with a different conductivity. By applying a potential difference between the reservoirs, an EOF is induced. The polarity of the voltage determines the direction of EOF and thus which buffer solution will replace the channel volume. Depending on flow direction, the monitored channel resistance will linearly increase or decrease until the total channel is filled with solution from one of either reservoirs. This measurement was repeated 6 times by reversing polarity and the EOF velocity was obtained from dividing the channel length (5 mm) by the average time interval of the conductivity change.
Supporting text

Orientation-dependent electrophoretic velocity. The anisotropic mobilities of cylindrical colloidal particles for electrophoretic motion parallel ($\mu_{e,||}$) and perpendicular ($\mu_{e,\perp}$) to the axis lead to an orientation-dependent velocity. For a cylinder oriented under an angle $\theta$ with respect to the $x$-axis (see Fig. S1), and the electric field $E$ along the $y$-axis, the electric field can be decomposed into components parallel and perpendicular to the long axis. The resulting electrophoretic perpendicular ($v_\perp$) and parallel ($v_{||}$) velocities are then:

$$v_\perp = \mu_\perp E \cos \theta,$$

$$v_{||} = \mu_{||} E \sin \theta.$$  \hspace{1cm} (1)

Both $v_\perp$ and $v_{||}$ can be decomposed in their $x$ and $y$-components in the coordinate system defined in Fig. S1:

$$v_x = v_{||} \cos \theta - v_\perp \sin \theta,$$

$$v_y = v_{||} \sin \theta + v_\perp \cos \theta.$$  \hspace{1cm} (2)

Combining Eq. (1) and (2) and adding the EOF-velocity along the electric field, we obtain Eq. (3) in the main text.

Bending of microtubules under distributed load. The bending of a microtubule tip by the electric field induced force is equivalent to the deviation of a clamped beam with length $\delta$ under a distributed load $f_\perp$ (See Fig. S2A). For small deflections $y$ of the beam, the deflection at each position $x$ is related to the bending moment $M(x)$ and the flexural rigidity $EI$ of the beam:

$$\frac{\partial^2 y}{\partial x^2} = -\frac{M(x)}{EI}.$$  \hspace{1cm} (3)

In the situation depicted in Fig. S2A, the bending moment can be derived from equilibrium to be:
\[ M(x) = -\frac{f_x}{2}(\delta - x)^2. \quad (4) \]

Combining equations (3) and (4) and integrating using the boundary condition \( \partial y/\partial x=0 \) at \( x=0 \) yields for the deflection as a function of position (S5):

\[
y(x) = \frac{f_x}{24EI} \left((\delta - x)^4 + 4\delta^3 x - \delta^4\right). \quad (5)
\]

From this, we calculate the maximum deflection \( y_m \) at \( x=\delta \) to be:

\[
y_m = \frac{f_x \delta^4}{8EI}. \quad (6)
\]

We approximate the deflected beam as part of an arc segment with radius of curvature \( r \) (Fig. S2B). We relate the deflection \( y_m \) to \( r \) as \( y_m = r(1-\cos(\delta/r)) \). For small \( \delta/r \), this yields \( y_m \approx \delta^2/2r \). Substituting this relation in Eq. (6) and using \( EI = k_b TL_p \) results in equation (4) in the main text.
Supporting figures

**Fig. S1.** Electrophoretic velocity ($v$) of a cylinder under an externally applied field ($E$) depends on the orientation ($\theta$) of the filament with respect to $E$. (A) The anisotropic mobilities for movement parallel ($\mu_{c,//}$) and perpendicular ($\mu_{c,\perp}$) to the cylinder’s axis, with $\mu_{c,//} > \mu_{c,\perp}$, result in a relatively larger speed parallel ($v_{//}$) than perpendicular ($v_{\perp}$) to the axis. The velocity of the cylinder is not collinear with the electric field. (B) The velocity components $v_{//}$ and $v_{\perp}$ can be decomposed into velocities along the $x$ ($v_x$) and $y$ ($v_y$) directions. The EOF-velocity ($v_{EOF}$) acts in the $y$-direction.
**Fig. S2.** Deflection and induced curvature of a clamped beam of length $\delta$ under a load $f$. (A) The deflection of the beam $y(x)$ increases to a maximum value of $y_m = y(\delta)$. (B) For small deflections $y_m$ of the beam, the beam deflection can be approximated with as an arc segment with a radius of curvature $r$.

**Supporting references and notes**


