Supplementary Information

Title: Fight the Flow: The Role of Shear in Artificial Rheotaxis for Individual and Collective

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Theory Supplement

Simulations without Wall-Induced Tilting

To model the chemical kinetics and resultant motility of a single nanorod, Moran and Posner¹ formulated a set of nonlinear coupled equations that take into account the fluid motion, reactioninduced creation and transformation of different charged species, consequent dynamic electric field and migration of the species in the resultant electric field. The fluid motion is described by the Stokes equation, the electric field is accounted by the Poisson equation, and the constitutive equations for all the chemical species are captured by a set of Nernst-Planck equations.¹

Here, we consider a system of many interacting nanorods. Instead of solving the full set of equations that describe the electrophoretic behavior, we take advantage of the scaling argument given by Moran and Posner that establishes the relationship between the velocity of the self-propelling rod and the concentration of fuel in the surrounding fluid.¹ Their analysis shows that for low fuel concentrations, the velocity of a rod, \mathbf{V}_{sp} , scales linearly with local reagent concentration C_{i} i.e., $\mathbf{V}_{sp} = \beta C(\mathbf{r})\hat{\mathbf{p}}$; here, $\hat{\mathbf{p}}$ denotes the orientation vector of a rod.¹

Incorporating this dependence in our model (see SI), we assume that a nanorod consumes H₂O₂ equally at both ends and is propelled with a speed proportional to the local fuel concentration C around the body at the position Γ in the direction given by its orientation $\hat{\mathbf{p}}$. The proportionality constant β depends on many system parameters (such as solution conductivity, ionic strength of the solution and surface flux of charged species, which are not included in our model) and was assigned to match the velocities of self-propelling nanorods observed in our experiments. The velocity \mathbf{V}_{sp} in our simulations is on the order of $10\mu m/s$, as observed experimentally.

The catalytic reactions on the two halves of the bimetallic nanorod produce chemical intermediates before the final end products.^{2,3} The rates of formation of these intermediates determine the rate of the catalytic decomposition of the reagent, i.e., hydrogen peroxide. The decomposition reactions' rates show saturation as the concentration of reagent increases and are typically modeled by the Michaelis-Menten-like kinetics⁴. Therefore, the assumed rate of fuel decomposition over the rod

surface is $K_d(C) = \frac{r_{\text{max}}C}{K_M + C}$. Here the concentration of hydrogen peroxide is *C* and the effective surface reaction rate r_{max} (mol s⁻¹m⁻²) characterizes catalytic decomposition of the fuel per unit surface area of the rod. Additionally, K_M plays a role similar to the Michaelis constant in the Michaelis-Menten equation. We set $r_{\text{max}} = 1.4 \times 10^{-4}$ mol m⁻²s⁻¹ and $K_M = 6.8 \times 10^{-3}$ M; this choice of the maximal reaction rate and the Michaelis-like constant are close to the known experimental values.^{2,3} For simplicity, these values of r_{max} and K_M characterize both halves of the rod.

To understand the effect of tilting ($\phi_{eq} > 0$ and $\phi_{eq} > 0$) on the rod dynamics, we perform simulations in the absence of wall-induced alignment, i.e., $\phi_{eq} = \phi_{eq} = 0$ (Figure 2). When confined

within a rectangular channel, many self-propelling rods undergo rheotaxis by forming an acute angle ($\varphi < 90^{\circ}$) with the imposed flow while remaining in the equilibrium plane. For background fluid velocities in the range $0 < V_f < 2V_{sp}$, the rheotaxis occurs via "swinging" trajectories of rods about the center line (in the equilibrium plane) of the channel, as can be seen in all panels of Figure S1 (SI Video SV5).

This swinging motion is caused by a non-uniform shear imposed by the Poiseuille flow and first analyzed by Zottle et al⁵. Namely, the shear varies from low at the centerline to high next to the side walls. This shear profile reorients the head of the rod toward the center line of the channel and, thus, causes the swinging motion. During this motion, the rod's trajectory repeatedly passes from one half of the channel to the other half. If the amplitude of this swinging motion is sufficiently large, the rod can reach the opposite walls. Along such trajectories, the rods spend some portions of time rheotaxing along the side walls and through the bulk between the walls. The rheotaxis along the side wall, however, is unstable due to the high shear imposed by the Poiseuille flow. Note that the rods' rotational diffusion breaks the symmetry of the trajectories, producing unequal trajectory loops, as seen in the top and middle panels in Figure S1. In addition, occasional collisions between the rods also randomize their trajectories.

At a certain value of the background fluid velocity $V_{sp} < V_f < 2V_{sp}$, the rheotaxis changes from positive to negative, as illustrated by the change in the direction of the red arrow in the bottom panel of Figure S1. At higher fluid velocities, the rods spend less time next to the walls due to increased shear rates. Therefore, the size of the trajectory loops near the walls progressively decreases as the flow velocity increases. Also, the relative randomizing influence of the rotational diffusion of rods becomes smaller (relative to the shear); therefore, neighboring loops along the rod trajectory (shown in blue in the bottom panel of Figure S1) become similar and the trajectory comes closer to a periodic one.

Unlike the rheotaxing rods, the trajectories of rods self-propelling along the flow (moving down stream) are stabilized next to side walls by the imposed shear. These non-rheotaxing trajectories are shown with red dashed lines in all three panels of Figure S1.



Figure S1. Representative trajectories from simulations of ten rods at different velocities of imposed flow V_f . A bimetallic rod, represented with connected segments of black (head) and yellow (tail), is propelled autonomously towards the black end. As shown by the direction of red arrows, at slow flow velocity, $V_f < V_{sp}$ (top panel), rods rheotax positively (blue trajectories) throughout the domain, at moderate velocities (middle panel) they rheotax positively due to migration next to the corners (blue trajectory) where fluid velocity is low, and at high $V_f \ge V_{sp}$ (bottom panel) rods rheotax negatively (blue trajectory). The rheotaxis along the corner regions is not stable due to flow induced high shear and, therefore, produces loops. The time a rod spends at the vicinity of the corner decreases progressively as the imposed flow increases. Trajectories of particles moving with the flow through the corner regions are shown with a red dashed line.

A separation of positive and negative electric charges within the bimetallic rod and the surrounding protonic cloud generates an electric dipole associated with each rod. Consequently, similar ends of two nearby rods repel each other and opposite ends of nearby rods attract each other. While in the absence of the electrostatic interaction, the linear clusters of rods assemble via the hydrodynamic interactions only, the presence of the electrostatic interaction affects the internal structure of the clusters.

To model the electrostatic interaction between the rods, each rod, together with the surrounding protonic cloud, is associated with an electric dipole which is constructed by assigning positive and negative charges to 11 elastically connected nodes representing the rod. To preserve the electrical neutrality of the rod, 5 nodes are given positive, q_m , and 5 negative, $-q_m$, charges with the middle node having zero charge. The force acting between two constituent beads (of different rods) separated by the distance r is calculated via the screened Coulomb potential, $V(r) = k_e \frac{q_m}{\varepsilon_f r} \exp\left(-\frac{r}{\lambda}\right)$. Here, $k_e \approx 9 \cdot 10^9$ Nm²C⁻² is the Coulomb's constant, $\varepsilon_f \approx 1.8$ is the

relative permittivity of the aqueous solution, $q_m = 0.19e$ is the effective charge associated with

each constituent bead, and $\lambda = 1.5 \mu m$ is the characteristic range of screening. Note that, the value of the length of electrostatic screening, λ , is suggested by the work of Moran and Posner and the value of q_m is an *ad-hoc* tuning parameter used in the simulations to match the dynamics of cluster to the experimental observations. The electrostatic interaction between the rods controls the structure of the clusters of rods but does not affect the dynamics of the cluster aggregation significantly shown in Figures S2 and Figure 8B for cases without and with the electrostatic interactions.



Figure S2. The formation of the linear clusters of nanorods (without electrostatics) with time along the corner of the channel is demonstrated for three representative clusters. The hydrodynamic attraction between the rods promotes their collision and formation larger clusters with time.

The rods initially distributed along the corner of the chamber assemble into the linear clusters as shown in Figure 8A. Unlike the linear clusters assembled by the hydrodynamic interactions (discussed above), the liner clusters assemble by the electrostatic interaction have quite distinctive structure. In particular, the cathode (yellow) half of each rod almost exactly overlaps with the anode (black) half of the neighboring rod within the cluster. In case of the hydrodynamically assembled linear cluster this overlap is less precise and not as prominent.

The rods assembling away from the side walls can form "disordered" clusters shown in Figure 8B-C. The structure of the clusters is not linear but the electrostatic interaction dictates that positive and negative ends of the rods are connected or overlapped within the clusters. With time, the clusters can attach to one of the side wall and eventually form a structure shown in Fig 14d. Note also, that in the absence of electrostatic interaction, the "disordered" clusters are not observed in the simulations.

Experimental Supplement

In this work, we used Au-Pt bimetallic nanorods with the dimensions being 2 μ m long, 500 nm diameter. The Au-Pt nanorods are synthesized via electrochemical deposition. Before deposition, an additional layer of sacrificial layer of sliver is first deposited into the pre-made silver-backed template. Next equal amounts of gold and then platinum is deposited until a total amount of 2 μ m of material has been deposited. Following deposition, the silver is dissolved in 5 M nitric acid leading to the release of the bimetallic nanorods into solution. After concentrating the solution, the bimetallic nanorods are washed in Millipore water three times. To achieve an ideal oxidation state of the two metals and ensure all the acid has been neutralized, the bimetallic nanorods undergo a 5 M sodium hydroxide bath. A final Millipore water washing is carried out to clean the nanomotors.

Micro-nozzles are fabricated *via* a soft lithography process. Both positive and negative photoresists can be used with their respective polarized masks, allowing flexibility in fabrication. An adhesion layer of hexamethyldisilazane (HMDS) is spin coated onto a 4 inch silicon wafer, regardless of positive or negative photoresist. For a positive photoresist, approximately 3 mL of SPR-955 is spun onto the wafer at 3000 rpm for 45 seconds. The wafer is transferred to a hot plate for a pre-exposure bake at 95 C for 2 minutes to relax the photoresist. Next the wafer is exposed under hard contact mode, 100 μ m gap, for 12 seconds. This imprints the pattern onto the photoresist. Following exposure, the wafer undergoes a post-exposure bake at 95 C for 2 minutes to release any surface tension that might distort the final pattern. Then the wafer is developed in Microchem CD-26 for 6 minutes to remove the polymerized photoresist. A final post-develop bake at 95 C for 2 minutes is employed to further help the pattern set. The pattern is then etched onto the wafer to create 100 um deep features using a Silicon Dry Etch instrument. Finally, the remaining photoresist is removed by immersing the wafer into a NanoPGRemover for 20 minutes at 80 C inside a sonicating bath. The positive Si-etched wafer is now ready to be used as a mold.

For a negative photoresist mold, Microchem SU-8 100 is spun onto the wafer at 2900 rpm for 35 seconds to create an approximately 100 µm deep film. The wafer undergoes several pre-exposure bakes to relax the photoresist, starting at 60 C for 10 minutes, 95 C for 20 minutes, 60 C for 2 minutes, and finally a cool plate for 1 minute. The gradual increase in temperature is necessary to prevent distortions in the film caused by high surface tensions. Next, the wafer is exposed under hard contact with a 100 m depth for 15 seconds for 7 cycles with a resting period of 30 seconds between exposures. A post-exposure bake is carried out to help relieve any lingering surface tensions that might distort the pattern. As with the pre-exposure bake, the wafer starts at 60 C for 2 minutes, 95 C for 2 minutes, 60 C for 1 minute, and a cooling plate for 1 minute. Then the wafer is developed for 10 minutes in Microchem SU-8 Developer to remove any unpolymerized photoresist. After developing, the wafer is washed with isopropyl alcohol (IPA) to remove the developer. Finally, the wafer is subjected to a post-develop bake to further polymerize the remaining photoresist. Incremental heating steps are necessary to prevent cracking or deformations from occurring in the pattern. The post-exposure bake times are as follows in order: 60 C for 1

minute, 95 C for 2 minutes, 175 C for 2 minutes, 95 C for 1 minute, 60 C for 1 minute, and a cooling plate for 1 minute. The negative photoresist wafer is now ready to be used as a mold.

Regardless of how the wafer was fabricated, both the positive photoresist and etched wafer and the negative photoresists SU-8 wafer can be utilized as a positive mold for fabricating micronozzles. A 5:1 mixture of Sylgard 184 polydimethylsiloxane (PDMS) to curing agent is thoroughly mixed. The mixture is then poured over the mold before being placed underneath vacuum for 2-4 hours. The PDMS and mold are then moved into a polymerization oven for 2-12 hours to speed curing time. After polymerization, the PDMS nozzles are cut away from the mold and the two inlets are hollowed out with a drill. The micro-nozzles are cleaned with an adhesion strip and then nitrogen gas to remove any debris from drilling. Next, the PDMS nozzles are ready for experiments.

While the horizontal angle of the rode with respect to the side wall (φ) can be easily determined from experimental images by the means of bright-field microscopy, the measurement of the vertical angle (ϕ) is challenging. The following approach was used. We tracked individual nanorods in the field of view and measured the projection length s(t) on the bottom. We assumed the length attains its maximal value s_m when the rod is nearly parallel to the bottom. Since the rods are localized near the bottom and do not leave the focal plane of the microscope, the change in the projection length is associated with a tilting of the nanorod. Then the vertical angle for an individual rod can be estimated as $\phi = \arccos(s(t)/s_m)$. From these measurements we constructed a histogram of the vertical angle (ϕ) (**Figure 2B**).

List of Videos

SV1: The video shows positive rheotaxis of a single Au-Pt nanorod along the corners the microfluidic channel. The video was captured at 56 frames per second (fps) and in reflectance mode.

SV2: The video shows a linear cluster of Au-Pt nanorods positively rheotaxing along the corner. The video was captured at 56 frames per second (fps) and in reflectance mode.

SV3: The video shows a three-particle disordered cluster of Au-Pt nanorods negatively rheotaxing along the corner, i.e. moving with the fluid flow. The video was captured at 56 frames per second (fps) and in reflectance mode.

SV4: The video shows rheotaxis motion of autonomously motile bimetallic rods above the bottom surface of the rectangular channel, in the presence of wall-induced alignment of rods ($\theta_1 > 0$ and $\theta_2 > 0$). The strength of the imposed background flow is $V_f = 15 \mu m/s$ and the self-propulsion velocity of the rod is $V_{sp} = 10 \mu m/s$. The wall-induced alignment, schematically shown in Fig. 9, prevents rods from departing the corners of the channel. As a result, trajectories along the corners (shown in green lines in Fig. 10) are stabilized.

SV5: The video shows "swinging" rheotaxis motion of autonomously motile bimetallic rods above the bottom surface of the rectangular channel, in the absence of wall-induced alignment of rods ($\theta_1 = \theta_2 = 0$). The strength of the imposed background flow is $V_f = 10 \,\mu m/s$ and the self-propulsion velocity of the rod is $V_{sp} = 10 \,\mu m/s$. High shear at the corner-regions of the channel, naturally provided by the Poiseuille flow, induce a hydrodynamic torque on the rods, leading to eventual departure from the wall. We note the absence of any rheotaxing trajectory along the corners.

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