

Micro- and Nanorobots Swimming in Heterogeneous Liquids

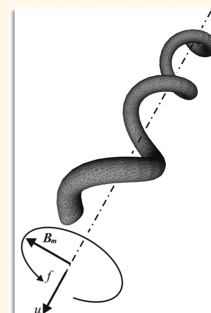
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ABSTRACT Essentially all experimental investigations of swimming micro- and nanorobots have focused on swimming in homogeneous Newtonian liquids. In this issue of *ACS Nano*, Schamel *et al.* investigate the actuation of “nanopropellers” in a viscoelastic biological gel that illustrates the importance of the size of the nanostructure relative to the gel mesh size. In this Perspective, we shed further light on the swimming performance of larger microrobots swimming in heterogeneous liquids. One of the interesting results of our work is that earlier findings on the swimming performance of motile bacteria in heterogeneous liquids agree, in principle, with our results. We also discuss future research directions that should be pursued in this fascinating interdisciplinary field.



One of the joys of making and experimenting with micro- and nanorobots that swim in Newtonian liquids at low Reynolds numbers is how well they obey the laws of fluid dynamics that predominate at those scales. Unlike turbulence, with its chaotic and unstable nature, low Reynolds number flow is analytically tractable, particularly when Brownian motion exhibits a minimal effect. Whenever we do find deviations from what we expect, we tend to discover something interesting. An example is when we discovered that simple nanowires can be rotated perpendicular to a surface to create mobile fluidic traps, that is, mobile microvortices that “walk” near the surface despite their reciprocal motion.^{1,2} This, in turn, led to the “RodBot”, a magnetically driven microrobot that delicately traps tiny protein crystals and transports them to an X-ray crystallography loop for cryopreservation and future analysis in high-energy X-ray beams.^{3,4}

Since one of the main applications envisioned for future microrobots is for targeted drug delivery or minimally invasive surgery,^{5–7} we need to reconsider the low Reynolds number assumptions placed on the medium in which the microrobots will swim. Within the human body, this

environment is often a heterogeneous fluid that exhibits non-Newtonian fluid dynamics, and the physics becomes more complicated but, fortunately, more interesting as well. There are many different fluid environments in the body we can consider, such as the cerebrospinal fluid, the vitreous humor, or blood, to name just a few (Figure 1). Body fluids contain molecules and cells that fulfill important functions, such as oxygen transport by red blood cells (RBCs). These fibers and microparticles influence the properties of the fluid. For example, they can increase the viscosity or change the shear response such that the fluid exhibits non-Newtonian characteristics. The challenge is to be able to provide the forces and torques to actuate microrobots in these fluid environments. Microrobots must overcome the increased viscosity and the additional interactions between the microrobotic agent and the fibers (see Figure 1b inset).

In 1979, Berg and Turner showed that *Escherichia coli* and other types of bacteria can swim more efficiently in high-viscosity gel-forming fluids than in water.⁸ They tested *E. coli* bacteria in solutions of methyl cellulose (MC) (Figure 2), a long unbranched polymer, which is a nontoxic substance with

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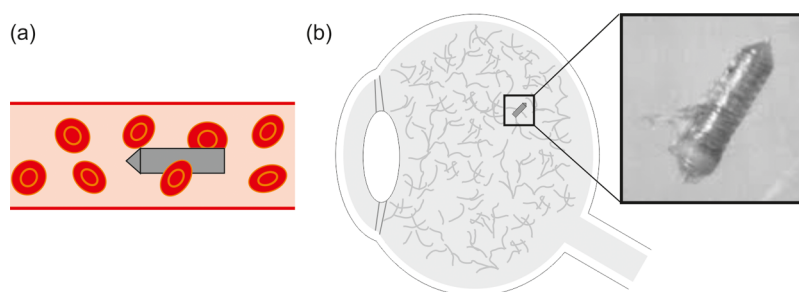


Figure 1. Challenges for microrobots to be employed in the human body. (a) Schematic of a robot inside the bloodstream. Blood cells can obstruct the propulsion and could potentially become damaged. (b) Schematic of an eye indicating the fibers inside the vitreous humor. Inset shows a 2.4 mm long screw-type microrobot inside porcine vitreous humor. After several rotations, large collagen fibers wrap around the microrobot. Reprinted with permission from ref 21. Copyright 2012 IEEE.

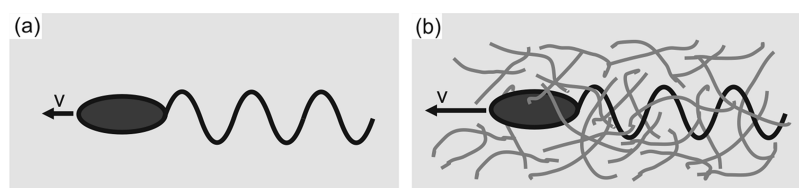


Figure 2. Schematic representations of an *E. coli* bacterium swimming in (a) water and (b) gel-forming fluids. A bacterium can exploit the fiber structures inside the gel-type fluid and swim more efficiently than in water. Reprinted with permission from ref 21. Copyright 2012 IEEE.

many uses in the food industry, cosmetic industry, and even in clinical applications. The long MC molecule chains create a heterogeneous fluid that remains Newtonian for low concentrations in water (<0.5%) and becomes non-Newtonian for larger concentrations. Their interesting findings of increased swimming efficiency of microorganisms presents the question of whether microrobots such as our artificial bacterial flagella (ABFs)⁹ are not only able to move in fibrous environments but may possibly perform better than in unstructured environments.

In this issue of *ACS Nano*, Fischer's group gives an insightful example demonstrating how the size of the microrobot, or nanopropeller in their case, relative to the structure of the fluid, influences swimming performance.¹⁰ The focus of this Perspective sheds further light on this complex phenomenon by showing how our larger helical microrobots swim in a heterogeneous fluid and how this influences their effective pitch, their step-out frequency, and their general ability to swim. One of the interesting results of our work is that that earlier findings on the swimming performance of motile

bacteria in heterogeneous liquids⁸ agree, in principle, with our results.

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Viscous Environments. *Newtonian and Non-Newtonian Fluids.* The viscosity η [Pa·s] of a liquid represents the relationship between the shear stress τ_s [Pa] and shear rate $\partial u/\partial y$ [1/s] in a fluid (see Figure 3a):

$$\tau_s = \eta \frac{\partial u}{\partial y} \quad (1)$$

A liquid is considered Newtonian when η remains constant and

non-Newtonian if η changes. The viscosity can change in different ways, and different types of non-Newtonian fluids can be defined, such as “shear-thinning” and “shear-thickening” fluids or “viscoplastic” and “viscoelastic” fluids (see Figure 3b). Non-Newtonian behavior can be explained by the microscopic structure within the particular fluid. For example, fluids may contain polymer chains, large molecules, fibers, or microparticles. These structures can become deformed, stretched, or realigned and, hence, bias the response of the fluid under shear forces.¹¹ A simple means to measure viscosity is by applying a shear rate between two parallel plates and measuring the shear forces. In order to characterize viscoelastic fluids, oscillating measurements are required to determine both the plastic and elastic properties of such complex fluids.

Fluids in the Human Body. There are many areas in the human body where microrobots can be employed, such as in the spine and brain (cerebrospinal fluid), in the urinary tract, bloodstream, or human eye (vitreous humor).⁵ Body fluids often contain molecules and cells

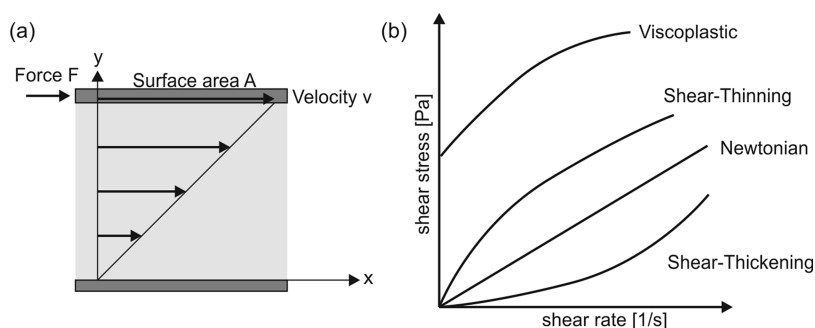


Figure 3. Viscosity of fluids. (a) Schematic of a simple shear flow between a stationary (bottom) and moving (top) plate. The shear stress τ is given by the force F applied over an area A . (b) Newtonian (linear) and non-Newtonian types of shear stress to shear rate relationships. Reprinted with permission from ref 21. Copyright 2012 IEEE.

that influence viscosity. The concentration of proteins and RBCs, for example, has been shown to influence the viscosity of cerebrospinal fluid¹² or the viscosity of the blood.¹³ Blood is an interesting fluid, as it can be modeled assuming Newtonian viscosity for flows in large arteries, but this approximation fails to hold for the flow in capillaries where the diameter of the blood vessel is similar in scale to the individual RBCs. Vitreous humor is an even more complex fluid, exhibiting viscoelastic properties and containing collagen fibers.^{6,14}

Locomotion in Viscous Environments.

Two types of swimming behavior in viscous environments have been observed and investigated. The first type of behavior is seen in viscoelastic (*i.e.*, non-Newtonian) fluids. Berg and Turner reported that *Leptospira*, a slender helical bacterium, can swim faster in non-Newtonian fluids than in Newtonian fluids.⁸ Liu *et al.* showed that the period of the helix rotation relative to the relaxation time of the fluid plays an important role and can be tuned to achieve higher velocities than in Newtonian fluids.¹⁵ The enhanced propulsion of other swimmers, such as a flexible sheet, in non-Newtonian fluid has been modeled by Lauga.¹⁶ The second type of swim behavior is observed in structured, Newtonian fluids. A fluid containing “structures”, such as filaments or polymer chains, may keep its Newtonian property if the (macroscopic) relationship between the shear rate and shear stress remains linear. In the case of MC solutions, this situation occurs

for MC concentration of less than 0.5% in water. It is not the non-Newtonian qualities but the structures present in the fluid that can influence the swim behavior of microorganisms. *E. coli* tested in such environments could rotate faster when compared to unstructured fluids with the same viscosity.

In this Perspective, we describe ABF movement in structured Newtonian fluids. In the following, the influence of viscosity on helical propulsion in unstructured Newtonian fluids is introduced. A model taken from the literature that considers the helical propulsion in structured Newtonian fluids is then presented along with experimental results.

Helical Propulsion in Unstructured Newtonian Fluids. The locomotion of helical swimmers in unstructured Newtonian fluids can be modeled by a 2×2 propulsion matrix¹⁷ that relates the linear and rotation velocity of a helix rotating about its major axis (given by u and ω , respectively) and translating along it with the force and torque (F and τ , respectively) it exerts on its environment along the same axis

$$\begin{bmatrix} F \\ \tau \end{bmatrix} = \begin{bmatrix} a & b \\ b & c \end{bmatrix} \begin{bmatrix} u \\ \omega \end{bmatrix} \quad (2)$$

The coefficients a , b , and c in the propulsion matrix are all linearly related to the viscosity:

$$a = \eta \cdot \bar{a} \quad (3)$$

$$b = \eta \cdot \bar{b} \quad (4)$$

$$c = \eta \cdot \bar{c} \quad (5)$$

where \bar{a} , \bar{b} , and \bar{c} contain only the geometric parameters, such as helicity angle, thickness of the filament, and radius of the helix.

The swim performance of magnetically actuated helices can be characterized by their effective pitch and step-out frequency. The effective pitch is the amount the microrobot moves forward per rotation around its helical axis. The effective pitch is always smaller than the geometric pitch of the helix due to slippage in the liquid. The step-out frequency is the maximum rotational speed that can be achieved and is a function of the microrobot's design and the maximum external (magnetic) torque available to rotate the microrobot.

The influence of viscosity on the effective pitch and step-out frequency can be modeled using eqs 2–5, assuming a free swimming helix with $F = 0$. The effective pitch is given by the slope of the frequency–velocity relationship:

$$p_{\text{eff}} = -\frac{\bar{b}}{\bar{a}} \quad (6)$$

The effective pitch is, therefore, independent of the viscosity of an unstructured Newtonian fluid. The step-out frequency is given by

$$\omega_{\text{max}} = \frac{\bar{a}}{\eta(\bar{a}\bar{c} - \bar{b}^2)\tau_{\text{max}}} \quad (7)$$

Summarizing all parameters \bar{a} , \bar{b} , and \bar{c} and τ_{max} into a constant C , eq 7 can be written as

$$\omega_{\text{max}} = \frac{C}{\eta} \quad (8)$$

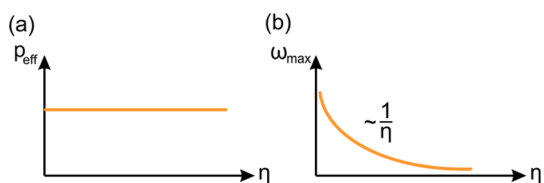


Figure 4. Schematic representation of the influence of viscosity on (a) the effective pitch and (b) the step-out frequency in unstructured, Newtonian fluids.

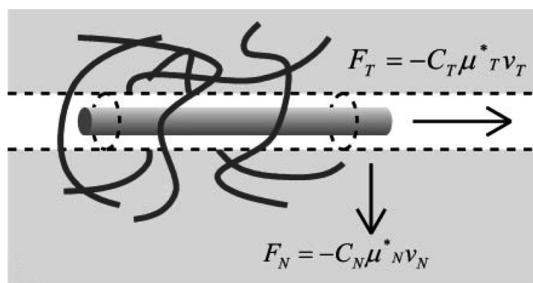


Figure 5. Model of the apparent viscosity by Magariyama and Kudo.¹⁸ The drag for the motion parallel to the filament axis is assumed to be related to the viscosity of the solvent, whereas the drag perpendicular to the filament axis is related to the viscosity of the solution. Reprinted with permission from ref 18. Copyright 2002 The Biophysical Society.

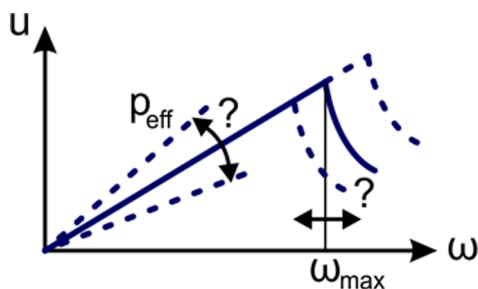


Figure 6. Potential influence on changes of the effective pitch and step-out frequency when testing artificial bacterial flagella in heterogeneous fluids.

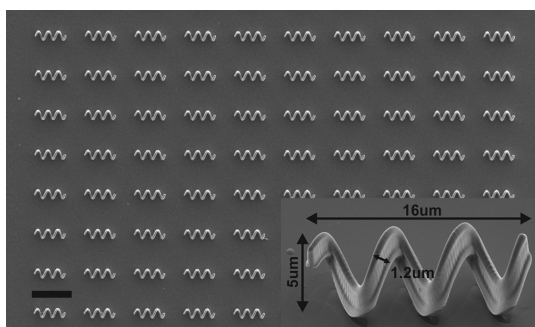


Figure 7. Scanning electron micrograph image of an array of fabricated artificial bacterial flagella. Scale bar corresponds to 20 μm . Inset shows an individual ABF and its dimensions. Reprinted with permission from ref 21. Copyright 2012 IEEE.

which makes the inverse proportionality of the step-out frequency with the viscosity apparent. Figure 4 shows schematics representing p_{eff} and ω_{max} as functions of the viscosity η in the case of an unstructured Newtonian fluid.

Helical Propulsion in Structured Newtonian Fluids. Microorganisms have been reported to move faster in structured than unstructured fluids. Berg and Turner argued that the organisms can slip through the mesh of fibers inside the fluid and

even “push” themselves forward, which reduces the slip, that is, increases the effective pitch, of the helical motion.⁸ Magariyama and Kudo proposed a descriptive model based on resistive force theory (RFT) to explain the increased speed of helical microorganisms in structured fluids.¹⁸ They introduced two apparent viscosities acting on the helical filament (see Figure 5). The drag acting in the direction normal to the filament is assumed to be a function of the viscosity of the solution. The drag acting in the direction parallel to the filament is assumed to be a function of the viscosity of the solvent only. This viscosity difference leads to a larger drag ratio between the motion parallel and perpendicular to the filament, which increases the effective pitch.

The model assumes that the thickness of the filament is smaller than the space between the fibers in the fluid. A single flagellum of an *E. coli* bacterium is only tens of nanometers, and even when several flagella bundle together, the combined flagella diameter is still only on the order of 100 nm. It is expected that a helix with a diameter larger than the gap between the structures in the fluid does not swim faster, as it simply “sees” the viscosity of the solution and not of the solvent. Artificial bacterial flagella have a thicker filament than *E. coli* bacteria, and it is therefore interesting to see if the change in MC concentration influences the swimming behavior. In particular, we examined if the effective pitch and step-out frequency are influenced by the structures in the fluids (see Figure 6).

Experimental Results in Methyl Cellulose. Arrays of ABFs with a diameter and length of 5 and 16 μm (see Figure 7), respectively, were fabricated by three-dimensional laser lithography. The polymer helices were coated by a 100 nm nickel layer and a 5 nm titanium layer. This fabrication method was introduced by Tottori *et al.* and detailed instructions can be found in their paper.¹⁹

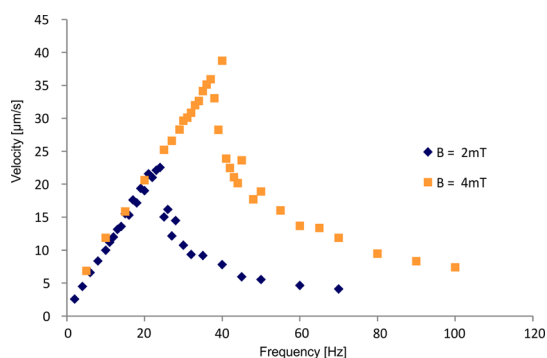


Figure 8. Frequency–velocity plot of typical swim experiments in 0.1% methyl cellulose.

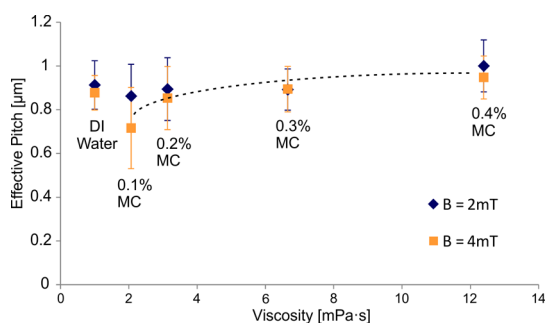


Figure 9. Effective pitch of artificial bacterial flagella in water and methyl cellulose. The average values from swim tests with 6–8 ABFs are shown, with bars indicating ± 1 standard deviation. The dashed line is drawn to show the trend but has not been fitted.

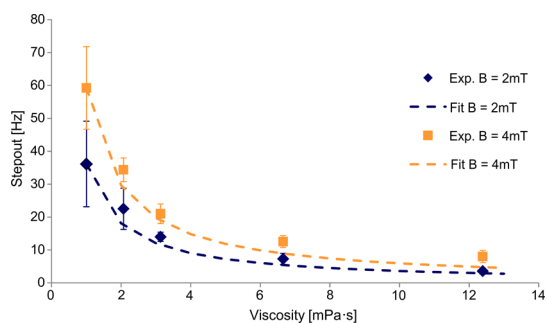


Figure 10. Step-out frequency of artificial bacterial flagella at different methyl cellulose solutions. Experiments performed at 2 and 4 mT. Shown are the average values from swim tests with 6–8 ABFs with bars indicating ± 1 standard deviation. Dashed line shows theoretical curve $f_{\max} = C/\eta$ for unstructured fluids. The close resemblance of the experimental data and the curve indicates that the step-out frequency in MC behaves similarly to the step-out frequency in an unstructured fluid.

The ABFs were tested in four MC concentrations (0.1, 0.2, 0.3, and 0.4%) and in water for reference. Figure 8 shows a swim result in 0.1% MC at two different magnetic field strengths. The frequency–velocity plot looks analogous to experiments conducted in water; that is, there is a linear frequency–velocity relationship at low frequencies and a velocity drop off after the step-out

frequency. The swim experiments were repeated with multiple swimmers and the effective pitch and step-out frequency measured.

Figure 9 shows the effective pitch measured for multiple samples in water and MC. Each data point represents the average of swim tests with 6–8 different microrobot prototypes. A slight increase in the effective pitch is seen from 0.1 to 0.4%

(indicated with a dashed line in Figure 9) but only to approximately the value found in water without MC fibers. The increase in the effective pitch is, however, minimal. The increase in MC concentration and effective pitch is correlated at a significance value of 0.05 and 0.01 for $B = 2$ and 4 mT, respectively (by means of Pearson correlation test). The amount of increase of the effective pitch, however, cannot be shown to be significant as the spread of the results is large.

Figure 10 shows the step-out frequencies of ABFs in water and MC. Two dashed lines representing the theoretical decrease of the step-out frequency based on eq 8, that is, $f_{\max} = C/\eta$ derived for unstructured fluids, with $C_{2\text{mT}} = 36.125$ mPa and $C_{4\text{mT}} = 59.25$ mPa, have been drawn. The decrease in step-out in MC follows the theoretical curve for unstructured fluids fairly well, indicating that the effects of the structured fluid on the drag torque are not significantly different from the effects of unstructured fluids. It is noteworthy that the variability of the step-out values for different swimmers becomes smaller for higher viscosities, suggesting that it is easier to get repeatable performance from different robots as the fluid becomes more viscous.

DISCUSSION AND OUTLOOK

We tested ABFs in water and four concentrations of MC solutions, which are structured Newtonian fluids. We chose MC because previous publications reported the increased swimming speed and rotational rates achieved by microorganisms inside this environment. We found that ABFs could successfully locomote in MC solutions with viscosities of over 10 mPa·s, which is over 10 times the viscosity of water (≈ 1 mPa·s). This can be considered to be a promising preliminary result for using ABFs in bodily fluids such as blood or the cerebrospinal fluid with viscosities of ≈ 4 and ≈ 1 mPa·s, respectively.^{12,13} Further tests are important to investigate

their capability in fluids other than MC.

Artificial bacterial flagella are influenced by the structures inside the fluid even though the macroscopic fluid properties of the methyl cellulose solutions are considered Newtonian.

The ABFs showed similar behavior to the microorganisms, as the effective pitch increased with growing MC concentration. Initially, however, adding MC to the water decreased the effective pitch. The step-out frequency decreases more rapidly with increasing viscosity than the effective pitch increases with MC concentration, which is why larger MC concentrations lead to lower maximum velocities.

The locomotion model of Magariyama and Kudo assumes a helical filament size that is smaller than the gap size between the MC fibers.¹⁸ Despite the ABFs being fabricated at the resolution limits of the direct laser writing tool, they have a filament thickness that is larger than the *E. coli*'s bundled tails by approximately a factor of 10. This is assumed to be a strong contributor to the small observed increase of the effective pitch (and the large observed change in step-out frequency) of the ABF in MC.

In summary, the ABFs are influenced by the structures inside the fluid even though the macroscopic fluid properties of the MC solutions are considered Newtonian. The structures have a stabilizing influence on the swimmers, decreasing the variability in the results and showing a slight increase in the effective pitch with increasing fiber concentration. A vast number of contributing factors remain to be

examined in future investigations into nano- and microscopic propulsion in nonideal fluids. In addition to studying the influence of the relative size between the helical propeller and the structures inside the fluid, such as is presented by Schamel *et al.* in this issue of *ACS Nano*,¹⁰ the following points should be considered in future research efforts.

Other Types of Heterogeneous Fluid Models: Leshansky proposed a different type of heterogeneous fluid model by assuming sparsely distributed spherical particles inside the fluid.²⁰ He showed that the effective pitch of helical swimmers is increased by the presence of the particles inside the fluid. This model does not make any assumptions on the thickness of the helical filament and may, therefore, account for swim behavior of ABFs in heterogeneous fluids. For example, helical propulsion in blood, which contains RBCs, may be well represented by Leshansky's locomotion model.

Other Types of Fluids: We selected MC as a swim environment for the ABFs because it allowed the direct comparison to the swim behavior of *E. coli* tested by Berg and Turner.⁸ They showed that *E. coli* rotate faster in MC than in other types of fluids containing particles with the same macroscopic viscosity. Their experiments demonstrated that microscopic propulsion is not only a function of the macroscopic viscosity of the fluid but also a function of the types of microscopic fibers and particles inside the fluid. Knowing this, it is of absolute importance to test microrobots in representative environments when evaluating their potential use for navigating in bodily fluids. Different bodily fluids, such as blood, urine, cerebrospinal fluid, or vitreous humor, contain different fibers and proteins. Hence, the successful propulsion in one such fluid cannot guarantee the success in another fluid (even if the viscosity is the same or smaller).

Surface Functionalization: The swim performance of microrobots is not

It is of absolute importance to test microrobots in representative environments when evaluating their potential use for navigating in bodily fluids.

only a function of the particles inside and the viscosity of the fluids. It is assumed that the interactions between the swimmer's surface and the surrounding fluid play an important role. For example, it is desirable for the ABF surface to have low affinity for the fibers inside the fluid to avoid attraction and attachment (this would cause slow-down or immobilization of the robot; see Figure 1). Another interesting avenue to examine is the difference in swim performance for microrobots with hydrophobic and hydrophilic surfaces.

Conflict of Interest: The authors declare no competing financial interest.

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