

Peer Review File

Manuscript Title: The colloidal nature of complex fluids enhances bacterial motility

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

The paper focusses on the behaviour of E. coli bacteria in different colloidal suspensions. The colloids used vary within the hundreds of nanometers, with volume fractions for the suspensions ranging from the dilute to the concentrated regimes (up to ~20% solids). The authors report that the average bacterial swimming speed follows a non-monotonic behaviour that depends on both size and volume fraction of the colloids.

Bacteria display a constant wobbling as they swim, due to the misalignment of their flagellar bundle and the cell body axis. This wobbling angle appears to anticorrelate with the average swimming speed.

The central tenet of the manuscript is that the observed speed increase at small to intermediate solids fraction is the consequence of a decrease in bacterial wobbling due to hydrodynamic interactions between a cell and the surrounding colloids.

To support this, the authors develop two models, one phenomenological and the other more detailed which seem to reproduce the observed dependence of the normalised swimming speed vs. normalised wobbling angle and of the normalised maximum swimming speed (as ϕ is swept) vs. the normalised radius of the colloids. Previous results with polymers instead of colloids agree (broadly speaking) with the normalised curves when the polymers are considered as colloids of size equal to their hydrodynamic radius.

From the point of view of the community working on microbial motility, the manuscript is interesting in two ways. Firstly, it shows that there is a significant nonlinearity in the average swimming speed of the cells within a colloidal suspension. Secondly, it shows that the cells' behaviour -or at least some parts of it- is in fact similar to what is observed in dilute and semi-dilute polymeric suspensions if these are interpreted as appropriate colloidal suspensions. The question is whether the origin of the phenomenon is in fact the one that is proposed by the authors. This would widen significantly the appeal of the paper.

Unfortunately, although I agree with the authors that the theoretical curves in Figs. 2 and 3 look reasonable, there are several aspects of the theoretical model that I have reservations about (see below). For this reason I cannot recommend the paper for publication in Nature. I will be happy to see a new version if the editor decides to give the authors the chance for a revision.

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1) There are a series of papers on helical swimming and helical klinotaxis by Crenshaw which I think would be relevant for the paper, despite the fact that they do not focus on bacteria but on microalgae. I suggest the authors also look at more recent ones by A. De Simone (e.g. M. Rossi et al. PNAS 2017) and by K. Wan.

2) The model in the supplementary material introduces a parameter, ϵ , which is zero for a perfect hook. It is estimated here to be approximately 0.01. Following the derivation, I am not sure I understand the case of ϵ actually being equal to zero. In this case the angle β is equal to $\pi/2$, meaning that the angular velocity ω_{cm} is perpendicular to ω_t . Accordingly, the pitch of the helix is zero ($C_1=0$ in Eq.(34) of the SM). This means that the cell follows a circular trajectory. According to the interpretation of the authors in the supplementary material, this corresponds to a bacterium that in a putative experiment would be seen as immotile. This does not seem correct to me, and it puts a question mark on the authors' interpretation that the observed swimming speed of the bacteria is always the projection of their actual velocity along the axis of the helix (which is a key point in the model proposed).

3) It is not clear from the paper how is the pitch of the helix measured (apologies if I have missed it). I guess it is from the oscillations of the cell body? It would be good to have a few example curves of these oscillations, with more details on how the angles are measured, given the significant noise in bacterial trajectories and the fact that fitting the image of the cell body to an ellipse does not single out an orientation for -say- the major axis.

4) If I understand correctly, the authors propose that swimming close to a colloid alters the wobbling angle α of a bacterium due to the hydrodynamic interaction between the cell body and the colloid. This in turn has an effect on the angle θ and the projection of the swimming speed along the axis of the helix. As discussed in Sec.2.4.4 of the supplementary material, one of the issues of this mechanism is that the hydrodynamic interaction can either increase or decrease α depending on the orientation of the cell as it passes close to the colloid. To remedy this the authors argue that the colloids are depleted from the region internal to the helical trajectory. The result is that there is an imbalance between the cell-colloid interaction for colloids external and internal to the trajectory. I have to say that I am not at all convinced by this argument. The main issues that I see in this are:

i) each cell within the suspension follows a different path. Therefore, it seems to me that there is no chance for the imbalance in colloids' distribution to be established along a specific helix. I do not see why a cell would see a lower concentration of colloids in the internal region of its helical trajectory, for parts of the trajectory which it has not yet reached.

ii) if there really is an imbalance between interactions on the two sides of the " ω plane", then shouldn't the estimates for the experimental quantities depend on this imbalance? It seems to me that the current model assumes that there are only interactions with colloids "outside" of the helix.

iii) The experiments with the optical-tweezer-held colloid should not suffer from the issue of presence/absence of the imbalance. A cell that passes by will sometimes do so with the colloid

oriented in front of the “ ω plane” and sometimes in the back. As a result one should be able to see both types of alteration of the wobbling angle and therefore both an increase and a decrease in swimming speed. These should depend on the phase in the wobbling cycle of the cell as it passes by the colloid. I do not see any evidence for that. Rather, it seems that there is a general increase in the swimming speed close to the colloid (see Fig.4). As far as I see, the results reported for these experiments are qualitatively inconsistent with the model proposed.

iv) In suspension the colloids that a cell interacts with can both translate and rotate. This is initially taken into account (Eqs.(2) and (5) of the supplementary material) before dismissing the importance of colloidal rotation (“We assume the contribution to the torque from the angular velocities ω_{1z} and ω_{2z} is negligible [...]”). However, there is no support in the rest of the document of why the contribution from ω_{2z} should be negligible (or at least I cannot find it). Colloids in suspension can rotate as a cell passes by. I am not convinced that their rotation can be neglected, in particular for colloids that are smaller than the bacterium itself (as those in Fig1c).

5) I do not understand why the parameter K is compared to the bending stiffness of the flagellar hook, when the cell is propelled by a bundle of flagella.

6) On pg.11 of the supplementary material the authors refer to the centre of mass of the bacterial cell and of the flagellar bundle. I do not think that mass here plays any role whatsoever. Rather, I think that the authors should refer to the hydrodynamic centres of resistance of the bacterial body and of the flagellar bundle.

7) Sec.2.4.1 of the supplementary material: “[...] Interestingly, $\theta(\alpha)$... saturated to $\theta_{\max}$.”

From Eq.(31), β is a function of both α and ϵ . Therefore so is θ , which is just $\beta - \alpha$. For which value of ϵ is $\theta_{\max}$ calculated? For $\epsilon=0$ (perfect joint) $\beta=\pi/2$ so θ becomes $\theta=\pi/2-\alpha$. This is qualitatively different from what happens in Ext Fig2c.

Referee #2 (Remarks to the Author):

This paper addresses a question that has been puzzling for a long time: why bacteria can swim faster in complex fluids than in simple Newtonian liquids.

The authors show experimentally that the size of the colloids is the factor which determines bacterial speed. Larger colloids imply faster bacteria. Moreover, polymers behave in the same way as colloids, with their size is determined by their hydrodynamic radius.

They then give an explanation of their results by arguing that hydrodynamic interactions with the colloids exerts a torque on the bacteria which reduces wobbling, and then less wobbling gives a substantial speed up.

These are very interesting ideas but more work is needed to be entirely convincing that this is the right interpretation. Is it possible to repeat the experiments with a type of bacterium that does not wobble as a control? This should presumably give a much lower speed up.

In their list of why previous theories do not work the authors omit the two-fluid model introduced by Man & Lauga Phys. Rev. E 92, 023004 (2015) and used in ref [21] which showed (using simulations) a substantial speed up for a bacterium that doesn't obviously wobble.

The colloids are quite small compared to the bacteria. Is their recoil considered, and for how long is the bacterium near enough the colloid for the torque to act? For what range of γ can the approximations used to derive eq (1) be trusted?

I did not find the movie very convincing. The colloid is three times the size of the largest colloids used in the experiments so it is not a totally fair comparison. It is hard to identify the speed up by eye, although the data presented for bacterium orientation are more convincing.

So there is more work needed to make this paper Nature standard, although the results so far are very promising.

Referee #3 (Remarks to the Author):

This manuscript investigates the swimming behavior of *e. coli* in dilute colloidal suspensions. The goal is to provide new insights into an important problem in active matter, namely the observed enhancement of swimming speed by *E. coli* (a pusher-type swimmer) in dilute polymeric solutions. This is an issue that has been around for decades since the observations of Schneider & Doetsch in 1974 which shows a non-monotonic increase in *E. coli* swimming speed as a function of polymeric solution fluidity ($1/\text{viscosity}$) – note that this was not a systematic study – and subsequent explanations in Ref. 14 and Ref. 15 for the observed phenomena which we know now not to be adequate. More recently, experiments and simulation work have been performed to understand bacteria swimming behavior in polymeric solutions using fluids with tailored rheological properties (shear-thinning, elasticity) as well as continuum-based and molecular dynamic simulations – most studies show an increase in *E. coli* swimming speed in polymeric solutions. For relatively large molecules (Ref. 18 and Ref. 22, for example), experiments and continuum-based simulations ascribe the observed swimming speed to fluid elasticity and normal-stresses, while for smaller molecules shear-thinning and “polymer-depletion” (near swimmer) concept seem to dominate the dynamics (Ref. 16 and Ref. 21). The current manuscript seems to suggest that these works can be understood simply by re-scaling the swimming speed (and wobbling data) by particle or polymer size. The authors provide data that would indicate that swimmer/particle interaction is far more important than fluid rheological properties. In particular, boundary-induced torque (BIT) effects are promoted as the main source of swimming speed enhancement in both colloidal and polymeric solutions. Overall, the authors provide an intriguing idea/concept, namely that all the experimental data observed so far in polymeric solutions could be understood by just considering polymer/particle size relative to bacteria size. The model provided by the authors does seem to capture the correct trends

for their colloidal data and selective polymeric data. However, just because the colloidal and polymeric swimming speed vs particle size data trends are similar, it does not necessarily mean that the colloidal mechanism for speed enhancement is applicable to the polymeric data. I do not doubt the colloidal data or the proposed model (based on BIT), but I do doubt their applicability to polymeric solutions. The authors have not shown that the trends in bacterial rotational diffusivity, mean run time, and mean tumble time, for example, also show similar trends. Beyond that, how do the authors square their explanations with numerical simulations (Ref. 21, 22)? These numerical simulations show how polymers and their rheological properties affect bacterial swimming behavior. More specifically:

- The authors say (Page 2) “Previous studies of bacterial swimming in polymer solutions focused on the dependence of bacterial speeds on the concentration or the viscosity of polymer solutions, rather than size of polymeric molecules.” This is not an accurate statement. Both Martinez et al (Ref. 16) and Patteson et al (Ref. 18) study the effects of polymer molecular weight (MW), that is, molecule size. For example, Figure 4C and 5C in Ref. 18 show the effects of polymer size (i.e, MW) on *e coli* rotational diffusivity and speed in polymeric solutions (at same viscosity). Notably, Ref. 18 shows that *e. coli* swimming speed decreases with polymer size even when scaling for viscosity effects; rotational diffusivity remain relatively incentive to polymer size. Similarly, Figure 2 and Figure 4 in Ref. 16 show (beautiful) data on normalized swimming speed and body rotational for solutions of different MW – note that the larger size molecule behaves more in line with the data in Ref. 18. Clearly these authors seem to acknowledge and study the role of particle/polymer size on *E coli* swimming behavior. Furthermore, similar trends to are found to the one shown in this manuscript.
- How does bacteria running length/time scale with particle volume fraction? Is this in line with data shown in Ref. 18.
- The point above show how bacteria swimming speed can be controlled by both polymer type and molecular weight (size). It would be important for the authors to discuss those findings in more detail and add them to their analysis.
- Figure 4: I am a little concern about the findings in this figure. First, it is know known that *e coli* can be light sensitive (phototactic) – Perlova, et al J. Bacteriology, 2019. It is quite likely that the observed increased in speed is due to phototaxis.
- The authors should also discuss the work of Patteson et al, Soft Matter (2016), who found non-monotonic diffusion behavior of *e coli* with particle size. These are very dilute colloidal systems, but it seems that similar behavior is found.

Author Rebuttals to Initial Comments:

We report below a detailed response to the reviewers' reports. The original reviewer comments are in *italic*, and our response appears in normal font. When addressing the reviewers' concerns, we also explicitly listed all the changes we made in the revised manuscript, which are color-coded red in the main text and supplementary materials.

Response to Reviewer 1:

We thank Reviewer 1 for carefully reading our manuscript and for the very insightful questions, which allow us to clarify the key points of our model and strengthen the conclusion of our paper.

From the point of view of the community working on microbial motility, the manuscript is interesting in two ways. Firstly, it shows that there is a significant nonlinearity in the average swimming speed of the cells within a colloidal suspension. Secondly, it shows that the cells' behaviour -or at least some parts of it- is in fact similar to what is observed in dilute and semi-dilute polymeric suspensions if these are interpreted as appropriate colloidal suspensions. The question is whether the origin of the phenomenon is in fact the one that is proposed by the authors. This would widen significantly the appeal of the paper.

We are very pleased that the reviewer find our work interesting to the microbial community. We would like to emphasize that our model is a logical consequence of our experimental findings, which were not contested by the reviewer. Instead of *some parts of cells'* swimming behaviors, our experiments have demonstrated that *all* the reported swimming features of flagellated bacteria in dilute polymer solutions are *quantitatively* similar to those in dilute colloidal suspensions. The comprehensive list of the seven different features of bacterial swimming that show the quantitative similarity is summarized below in our answer to Question 1 of Reviewer 3 as well as in Sec. 1 of the revised supplementary information (SI). Based on our experiments alone, it is beyond a reasonable doubt that common mechanism(s) dictate bacterial swimming in both dilute colloidal suspensions and dilute polymer solutions.

The natural question is then to ask what are the common property (or properties) shared by dilute colloidal suspensions and dilute polymer solutions. It has been long established in polymer science that the dynamics and rheology of dilute polymer solutions can be well described by those of dilute colloidal suspensions, where polymer coils are treated as colloids with a radius equal to the hydrodynamic radius of the coils (see e.g. the standard polymer textbooks "Polymer Chemistry" by Hiemenz and Lodge and "Polymer Physics" by Rubinstein and Colby). Once we have reached the key conclusion of our manuscript that the colloidal nature of complex fluids leads to enhanced motility of flagellated bacteria, there is not much room left for theoretical consideration except for the hydrodynamic interaction between bacteria and colloids, which eventually leads to our model. Below, we shall address the detailed questions of the reviewer on our model.

1) There are a series of papers on helical swimming and helical klinotaxis by Crenshaw which I think would be relevant for the paper, despite the fact that they do not focus on bacteria but on microalgae. I suggest the authors also look at more recent ones by A. De Simone (e.g. M. Rossi et al. PNAS 2017) and by K. Wan.

We thank the reviewer for providing us these interesting references. We have cited the review paper by Crenshaw as well as the recent papers by A. De Simone and K. Wan in the revised manuscript (Ref. 39-41 of the revised manuscript). As pointed out by the reviewer, bacteria and microalga are different in their

swimming kinematics. The origins of the helical trajectory in these two different categories of microorganisms are also qualitatively different. Driven by dynein motors, the flagella of microalga actively beat and adopt a series of 3D configurations in a beating cycle, which gives rise to the helical trajectory as demonstrated beautifully in Ref. 40 and 41. Lacking any internal motors, the flagella of bacteria can only rotate as a rigid helix, which is driven the flagellar motor embedded in the cell membrane at the root of the flagella. The helical trajectory of flagellated bacteria concerned in our study arises from the asymmetric off-axial flagellar geometry, where the major axis of cell body misaligns with the axis of flagella.

2) The model in the supplementary material introduces a parameter, ϵ , which is zero for a perfect hook. It is estimated here to be approximately 0.01. Following the derivation, I am not sure I understand the case of ϵ actually being equal to zero. In this case the angle β is equal to $\pi/2$, meaning that the angular velocity ω_{cm} is perpendicular to ω_t . Accordingly, the pitch of the helix is zero ($C_1=0$ in Eq.(34) of the SM). This means that the cell follows a circular trajectory. According to the interpretation of the authors in the supplementary material, this corresponds to a bacterium that in a putative experiment would be seen as immotile. This does not seem correct to me, and it puts a question mark on the authors' interpretation that the observed swimming speed of the bacteria is always the projection of their actual velocity along the axis of the helix (which is an key point in the model proposed).

We appreciate the insightful comment of the reference, which stimulated us to re-examine the hypothesis made in our model on the torque balance. We realized that the free parameter ϵ introduced in our original model is unnecessary. If we follow strictly the torque balance condition of the ideal universal joint proposed by Shimogonya *et al.* (Ref. 42)

$$\frac{B\omega_b \cos \alpha}{-d\omega_t} = 1,$$

we do not need to introduce any free parameter in the model. Mathematically, the revised model is equivalent to the original one, as the term $eV_b^z/(d\omega_t)$ plays the same role as the small parameter ϵ . Note that $eV_b^z/(d\omega_t) = e^2/[(A+b)d] \sim 10^{-2}$, quantitatively matching ϵ in the original model. Importantly, instead of a free parameter, eV_b^z is now the self-consistent outcome of the model itself. Since the velocity component along the direction of bacterial flagella V_b^z is non-zero (Eq. 29 of SI), $\epsilon \neq 0$ in the revised model. Our revised model is now free of fitting parameters, which quantitatively matches all the main findings of our experiments. The quantitative similarity between our original model and the revised model shows that our model predictions are indeed robust, insensitive to the fine detail of the balance equation. Please refer to our revised supplementary materials (Sec. 3) for the detailed derivation of the revised model.

Lastly, we should emphasize that the interpretation of the measured swimming speed of bacteria as the projection of their actual velocity along the axis of the helix is the geometric consequence of bacterial wobbling, where the 3D helical trajectory of bacteria is projected on the 2D imaging plane of optical microscopy (Refs. 24,32,38). In both our experiments and previous experiments, the swimming velocity of bacteria is measured based on the projected displacement of the center of bacterial body on the 2D imaging plane. Thus, the measured velocity over multiple helical periods must be the 2D projection of the actual 3D velocity of bacteria along the helical axis (see the more detailed discussion in Ref. 24).

3) It is not clear from the paper how is the pitch of the helix measured (apologies if I have missed it). I guess it is from the oscillations of the cell body? It would be good to have a few example curves of these oscillations, with more details on how the angles are measured, given the significant noise in bacterial

trajectories and the fact that fitting the image of the cell body to an ellipse does not single out an orientation for -say- the major axis.

Indeed, accurate measurements of the small pitch of helical trajectories are difficult. Instead of measuring the pitch ourselves with a potential biases to fit our own model, we directly obtain the data from the literature (Fig. 2a of Ref. 24). Note that experiments were done with another prototype flagellated bacteria, *B. subtilis*, which have a similar body plan, adopt the same locomotion mechanism and show the same swimming behavior as *E. coli* (see the discussion in Ref. 24 with example curves shown in Fig. 1c). One of the main contributions of our model is to demonstrate that the pitch of flagellated bacterial trajectory can be larger than 4 μm , the upper limit predicted by the existing wobbling model, which explains the puzzling experimental observations on the large pitches of the helical trajectories of *E. coli* and *B. subtilis*.

To clarify the confusion, we have now stated explicitly in the caption of Extended Data Figure 5b:

“Black squares are experimental data extracted from Fig. 2a of Ref. 24.”

4) If I understand correctly, the authors propose that swimming close to a colloid alters the wobbling angle α of a bacterium due to the hydrodynamic interaction between the cell body and the colloid. This in turn has an effect on the angle θ and the projection of the swimming speed along the axis of the helix. As discussed in Sec.2.4.4 of the supplementary material, one of the issues of this mechanism is that the hydrodynamic interaction can either increase or decrease α depending on the orientation of the cell as it passes close to the colloid. To remedy this the authors argue that the colloids are depleted from the region internal to the helical trajectory. The result is that there is an imbalance between the cell-colloid interaction for colloids external and internal to the trajectory. I have to say that I am not at all convinced by this argument. The main issues that I see in this are:

The breaking of the translational symmetry of the uniform distribution of colloids is one of the most interesting features arising naturally from our model, which gave us the strong confidence that the model indeed captures the key physics of the process. We do agree with the reviewer that the feature is not sufficiently explained in the original manuscript, which likely leads to the reservation of the reviewer. Below, we provide a more detailed explanation of this important feature.

i) each cell within the suspension follows a different path. Therefore, it seems to me that there is no chance for the imbalance in colloids' distribution to be established along a specific helix. I do not see why a cell would see a lower concentration of colloids in the internal region of its helical trajectory, for parts of the trajectory which it has not yet reached.

The fluid flow induced by the swimming of a bacterium, which is *long-range* in nature, strongly perturbs the uniform distribution of colloids near the bacterium. Particularly, the long-range hydrodynamic flow depletes colloids in the front of the bacterium within the internal region of bacterial helical trajectory before the bacterium reaches the position.

To understand the effect, let us consider the limits of small and large colloids first. (a) Small colloids. It has been well established that the far-field flow field of swimming *E. coli* can be described as a pusher-type dipolar flow, where the fluid flow moves away from the head and the tail of the bacterium and comes

in towards the side of the bacterium (see e.g. Fig. 1 in Drescher et al. *PNAS* **108**, 10940 (2011) and Kanazawa et al, *Nature* **579**, 364 (2020)). As the radius of the helical trajectory R_w is small on the order of the width of bacteria, the time-average flow of a wobbling bacterium can still be accurately approximated as the dipolar flow. For small colloids, the motion of the colloids simply follows the local flow field of the bacterium. Thus, colloids in front of the bacterium in the internal region of the helical trajectory are advected away by the long-range dipolar flow and depleted from the inner region. To verify this physical picture, we directly simulate the motion of 100-nm-radius colloids in the presence of a wobbling bacterium (see Zhang et al. *PNAS* **118**, e2100145118 (2021) for the details of the simulation method). As shown in the top row of Fig. S1, colloids that are initially within the internal region when the bacterium is far away are advected out of the internal region as the bacterium approaches the colloids.

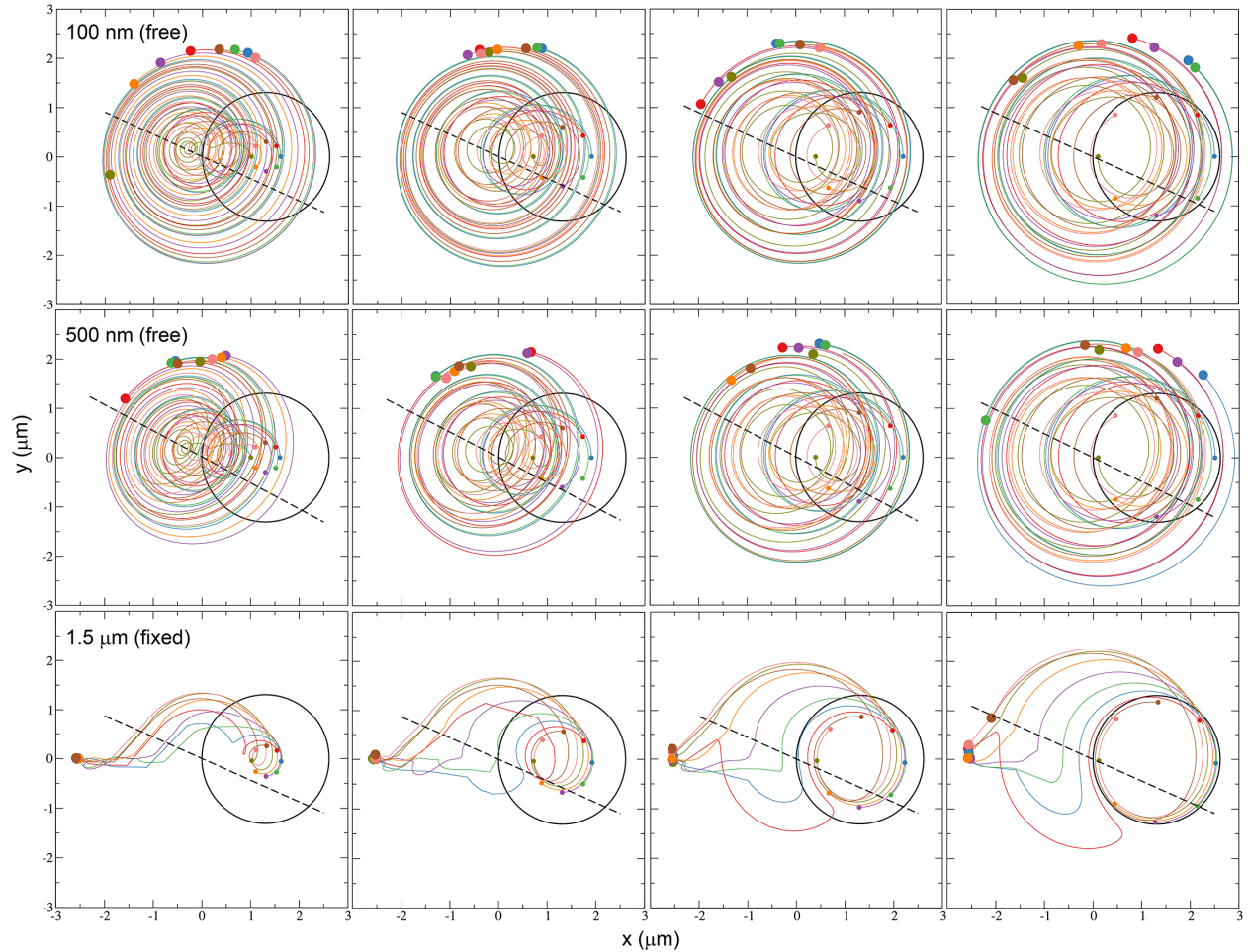


Fig. S1. Depletion of colloids from the cylindrical space enclosed by the helical trajectory of a wobbling bacteria. The radius of colloids is 100 nm (top row), 500 nm (middle row) and 1.5 μm (bottom row). While the 100 nm and 500 nm colloids are free, the large 1.5 μm colloids are pinned in the lab frame to match the condition of the optical tweezer experiments. The small color disks indicate the initial positions of colloids when the bacterium is far away from the colloids, where the distance between the bacterium and the colloids along the direction of the axis of the helical trajectory $-\omega_{cm}$ is $l_{bc} \equiv |(\mathbf{r}_b - \mathbf{r}_c) \cdot \omega_{cm}| / |\omega_{cm}| = 6.5 \mu\text{m}$. Here, $\mathbf{r}_b$ and $\mathbf{r}_c$ are the center of bacterial body and the center of the colloids, respectively. The large color disks indicate the positions of the corresponding colloids when $l_{bc} = 0$. The lines connecting the small and large disks represent the projection of the 3D trajectories of colloids on the plane normal to $-\omega_{cm}$ in the reference frame of the bacterium, where the bacterial body sits at the origin (0, 0) and orientates in the ω

plane indicated by the black dashed lines. The large black circles mark the boundary of the cylindrical space. The radius R_w and the pitch P of the helical trajectory of the bacterium are $1.3\ \mu\text{m}$ and $6.5\ \mu\text{m}$, respectively. The radius of bacterial body is $1\ \mu\text{m}$. All the colloids initially located inside the cylindrical space are depleted out of the space as the bacterium approaches the colloids, regardless particle sizes or whether particles are free or pinned. The results are obtained from numerical simulation, where the hydrodynamic interactions between one single colloid and one wobbling bacterium is simulated in each run.

(b) Large colloids. When colloids are larger than the size of bacteria, the colloids are almost immobile within the flow of a bacterium. As the bacterium approaches to colloids, under the influence of the long-range hydrodynamic interaction between the bacterium and colloids, the bacterium deviates from the colloids to avoid the direct collision onto the surface of colloids. In other words, large colloids push the bacterium away before the bacterium gets close to the colloids. In fact, given the small radius of the helical trajectory R_w , it is geometrically impossible for a large colloid to sit inside the internal region of the helical trajectory when the bacterium is close. If we switch the reference frame from the lab frame to the frame of the bacterium, the colloids in front of the bacterium move away from the bacterium, similar to the case of small particles in (a). We again run the direct simulation on the hydrodynamic interaction between $1.5\text{-}\mu\text{m}$ -radius immobile colloids pinned in the lab frame and a wobbling bacterium. The bottom row of Fig. S1 shows the motion of large immobile colloids in the reference frame of the bacterium. The colloids are always depleted from the internal region of bacteria helical trajectory as the bacterium approaches the colloids, regardless the initial position of the colloids.

For colloids of intermediate sizes between the two limits, both colloids and bacteria change their trajectories as they approach each other due to the long-range hydrodynamic interaction. Direct simulations again show clearly that the long-range hydrodynamic interaction between bacteria and colloids deplete the colloids in the front of bacteria within the internal region of bacterial helical trajectory (Fig. S1 middle row).

In short, the long-range hydrodynamic interaction perturbs the uniform distribution of free colloids around bacteria (but does not significantly change bacterial speed), whereas the short-range lubrication interaction induces the BIT on bacterial body and reduces the wobbling angle of bacteria. This separation of the hydrodynamic interaction into the far-field long-range contribution and the near-field short-range lubrication contribution is frequently used in the study of the dynamics of colloidal suspensions (Brady and Bossis, *Ann. Rev. Fluid Mech.* **20**, 111 (1988)) and in the study of bacterium-colloid interaction (see e.g. Kanazawa et al, *Nature* **579**, 364 (2020)).

To address the reservation of the reviewer, we have greatly extended our discussion on the symmetry breaking mechanism in Sec. 3.4.5 of the revised SI (highlighted in red) and added the simulation results as the new Extended Data Fig. 7. We encourage the reviewer to read the revised Sec. 3.4.5 for more systematic explanations there.

ii) if there really is an imbalance between interactions on the two sides of the “\omega plane”, then shouldn’t the estimates for the experimental quantities depend on this imbalance? It seems to me that the current model assumes that there are only interactions with colloids “outside” of the helix.

As shown in our simulations (Fig. S1), the long-range hydrodynamic interaction between bacteria and colloids is very effective in depleting colloids in the front of bacteria along their pathway. To construct a *minimum* model capturing the essential physics without introducing an extra unknown parameter that needs to be assessed via simulations, we simply assume the number of colloids in the helical cylinder is zero. This

simple assumption has yielded good quantitative agreement with our experiments. Adding another small “imbalance” parameter as a fitting parameter would certainly give even better quantitative description. Nevertheless, we believe such a step would reduce the predictive power and the appealing of our simple minimum model. The exploration of this possibility would be the topic of future theoretical studies.

iii) The experiments with the optical-tweezer-held colloid should not suffer from the issue of presence/absence of the imbalance. A cell that passes by will sometimes do so with the colloid oriented in front of the “ ω plane” and sometimes in the back. As a result one should be able to see both types of alteration of the wobbling angle and therefore both an increase and a decrease in swimming speed. These should depend on the phase in the wobbling cycle of the cell as it passes by the colloid. I do not see any evidence for that. Rather, it seems that there is a general increase in the swimming speed close to the colloid (see Fig. 4). As far as I see, the results reported for these experiments are qualitatively inconsistent with the model proposed.

As we have addressed in Question *i*) in the case of large colloids, the long-range hydrodynamic interaction between bacteria and an immobile colloid would deviate the trajectory of bacteria approaching the colloid. In the reference frame of bacteria, the colloid in front of bacteria moves away from bacteria. As a result, the colloid always falls outside the internal region of bacterial helical trajectory. When a bacterium swims by the colloid, if the opening between ω_b and ω_t (the angle $\pi - \alpha$) faces towards the colloid, the distance between the bacterium and the colloid would be at least a distance $2w$ away (Extended Data Fig. 6b), which is too large to exert a strong short-range lubrication force. Hence, the colloid would not significantly change the wobble angle and the swimming speed of bacterium. The distance between the bacterium and the colloid is small only when the opening between ω_b and ω_t faces away from the colloid, which invariantly decreases the wobble angle and increases the swimming speed. Thus, the presence of an immobile colloid either enhances the swimming speed of bacteria or does not change the swimming speed of bacteria significantly. To convincingly address the concern of the reviewer, we have run direct numerical simulation with pinned 1.5- μm -radius colloids as discussed above, which matches the condition of the optical tweezers experiments (the bottom row of Fig. S1). The results unambiguously verify the above physical picture. Particularly, at the shortest distance, the large immobile colloid always locates in the direction opposite to the opening of ω_b and ω_t outside the internal region of the bacterial helical trajectory, regardless the initial position of the colloid. (In the figure, the colloid always ends up on the left of the bacterial helical trajectory in the reference frame of the bacterium.) This configuration invariably reduces the wobble angle and enhances the bacterial swimming speed. Hence, the prediction of the model agrees well with our experiments of optical tweezers without any inconsistency.

As mentioned above, we have greatly expanded our discussion of the symmetry breaking mechanism in Sec. 3.4.5 of the revised SI, which provides a detailed convincing explanation of the tweezer experiments.

iv) In suspension the colloids that a cell interacts with can both translate and rotate. This is initially taken into account (Eqs. (2) and (5) of the supplementary material) before dismissing the importance of colloidal rotation (“We assume the contribution to the torque from the angular velocities ω_{1z} and ω_{2z} is negligible [...]”). However, there is no support in the rest of the document of why the contribution from ω_{2z} should be negligible (or at least I cannot find it). Colloids in suspension can rotate as a cell passes by. I am not convinced that their rotation can be neglected, in particular for colloids that are smaller than the bacterium itself (as those in Fig1c).

Without neglecting the rotation of colloids ω_2^z , the torque on the bacterial body can be calculated as (see the revised SI Sec. 2.1 for the derivation):

$$\tau_1^z = \frac{4\pi}{5} \mu r_b^2 V_b \frac{\gamma(4+\gamma)}{(1+\gamma)^2} \left[1 + \frac{4\gamma+1}{4(4+\gamma)} \right] \ln \frac{r_b(1+\gamma)}{2d} = \frac{\pi}{5} \mu r_b^2 V_b \frac{\gamma(17+8\gamma)}{(1+\gamma)^2} \ln \frac{r_b(1+\gamma)}{2d}.$$

In comparison with the original formula neglecting ω_2^z (Eq. 1 of the original text), we have an extra factor $\left[1 + \frac{4\gamma+1}{4(4+\gamma)} \right]$ highlighted in red. Note that $\gamma = R/r_b$ is the size ratio between colloids and bacteria. The approximation works better for smaller colloids, approaching $[1+1/16]$ when $\gamma \rightarrow 0$. For the largest colloids used in our experiments at $R = 500$ nm, the extra factor is $[1+1/6]$. So the torque induced by the rotation of colloids is at most 17% of the torque induced by the translation of colloids, which justifies our original approximation. Finally, within our model of bacterial wobbling, the angular velocity of bacterial body is always in the x - y plane. Thus, $\omega_1^z = 0$.

To address the concern of the reviewer, we adopted the accurate formula with the effect of ω_2^z above in the revised manuscript. As expected, the new formula yields the quantitatively same result with a slightly larger bending modulus of $K = 7.15 \times 10^{-19}$ N·m. The comparison of the two formulas is shown below.

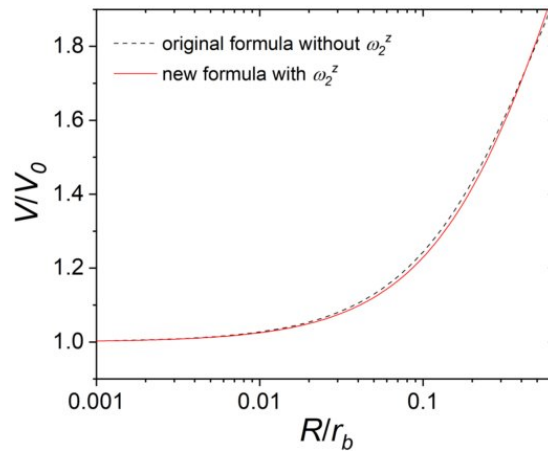


Fig. S2. The comparison of the formula without the effect of the rotation of colloids (the dashed line) and that with the effect of the rotation of colloids (the red line).

A minor revision: we have used a different coordinate system in the torque calculation and in the wobbling model in the original manuscript. z in the torque calculation should be x in the Extended Data Figure 4b. To avoid the confusion, we have redefined the coordinate system in the torque calculation in the revised supplementary materials, so the two systems are now consistent.

We have added the detailed derivation of the formula with the effect of colloidal rotation ω_2^z (or ω_2^x in the new coordinate) in the revised SI Sec. 2.1.

5) I do not understand why the parameter K is compared to the bending stiffness of the flagellar hook, when the cell is propelled by a bundle of flagella.

The bending stiffness of the hook is about two to three orders of magnitude smaller than that of the flagellar filament (Son, Guasto & Stocker, Nat. Phys. 2013; Jabbarzadeh & Fu, PRE 2018). A single flagellum (including the flagellar filament and the hook) behaves like springs in series. Under the external torque, the

component with the larger compliance would suffer the most bending. The total bending stiffness of the flagellum, K_t , can be estimated as

$$\frac{1}{K_t} = \frac{1}{K} + \frac{1}{K_f},$$

where K and K_f are the bending stiffness of the hook and the flagellar filament, respectively. Since $K \ll K_f$, $K_t \approx K$. As a flagellar bundle is typically consistent of three or four individual flagellum, each with its own hook, we may further approximate the configuration of a flagellar bundle as springs in parallel. Hence, the bending stiffness of the flagellar bundle would be $K_b = nK_t \approx nK$, where $n = 3$ or 4 is the number of flagella in the bundle. As our model provides only an order of magnitude estimate of the bending stiffness of the flagellar bundle, the order one factor n would not affect the conclusion of our calculation.

We have added the above discussion in the supplementary information (Sec. 2.2).

6) On pg.11 of the supplementary material the authors refer to the centre of mass of the bacterial cell and of the flagellar bundle. I do not think that mass here plays any role whatsoever. Rather, I think that the authors should refer to the hydrodynamic centres of resistance of the bacterial body and of the flagellar bundle.

We have changed the center of mass to the hydrodynamic center of resistance in the revision.

7) Sec.2.4.1 of the supplementary material: “[...] Interestingly, $\theta(\alpha)$... saturated to θ_{max} .” From Eq.(31), β is a function of both α and ϵ . Therefore so is θ , which is just β/α . For which value of ϵ is θ_{max} calculated? For $\epsilon=0$ (perfect joint) $\beta=\pi/2$ so θ becomes $\theta=\pi/2-\alpha$. This is qualitatively different from what happens in Ext Fig2c.

As we have eliminated the free parameter ϵ in the revised model, the question becomes irrelevant. Please see our answer to Question 2 above.

Response to Reviewer 2:

We are very pleased to see the positive comment of the reviewer on our work. We also thank the reviewer for the very useful and constructive suggestions, which help us greatly improve the quality of our manuscript. Below, we will address the comments of the reviewer in detail:

Q1: These are very interesting ideas but more work is needed to be entirely convincing that this is the right interpretation. Is it possible to repeat the experiments with a type of bacterium that does not wobble as a control? This should presumably give a much lower speed up.

To the best of our knowledge, all flagellated bacteria wobble. We have also consulted Prof. Howard Berg from Harvard, a known expert in bacterial strains, who commented that all *E. coli* strains wobble. Flagellated bacteria wobble because of the misalignment of the axis of flagellar bundle with the symmetric axis of bacterial body. As the chance that the flagellar bundle of a bacterium perfectly aligns with the symmetric axis at the pole of its body is slim, the bacterium is almost certain to show some degree of wobbling. To overcome the limitation, we reanalyzed our experiments with *E. coli* in our revision, which provides much more convincing evidence addressing the question of the reviewer.

Our new analysis is based on the following observation. In a single batch of *E. coli* in pure buffer without colloids, there is a natural distribution of the degree of wobbling among different bacteria. While some *E. coli* show strong wobbling, others show only weak wobbling. The average of the distribution gives the average wobble angle in buffer, $\theta_0 = 45^\circ$, which has been shown in Fig. 3 at $\phi = 0$ of our manuscript. Based on the hypothesis of the reviewer, those bacteria that wobble weakly should have higher swimming speeds even in buffer. Thus, the degree of motility enhancement of the weakly-wobbling bacteria in colloidal suspensions should be much weaker. In a sense, we can treat bacteria that wobble weakly as “the new type of bacteria” asked by the reviewer. To test the hypothesis, instead of the *average* swimming speed versus the *average* wobble angle of bacteria in colloidal suspensions of different concentrations shown in Fig. 3, we analyzed the swimming speed of *individual* bacteria as a function of their wobble angle in buffer and in a colloidal suspension of a fixed concentration, respectively (Fig. S3a below). We chose the colloids of radius $R = 500$ nm and concentration $\phi = 4\%$, where the enhancement of the average swimming speed is strongest. We found that the two curves overlap well. Particularly, at the small wobble angle of $\sim 20^\circ$, the speed enhancement of bacteria in colloids is less than 15%, which is much lower than the enhancement of the average swimming speed of bacteria in colloids over 80% (Fig. 2). Thus, the new measurements confirm the hypothesis of the reviewer and unambiguously demonstrate that the decrease of wobble angle is the leading factor for the motility enhancement. Figure S3b further showed that the distribution of the wobble angle of bacteria in buffer and in the colloidal suspension. The distribution obviously shifts towards small wobble angles in the suspension. Hence, the average swimming speed of bacteria increases in colloidal suspensions, because there are more weakly-wobbling bacteria in the suspensions. With a fixed wobble angle, the swimming speed of a bacterium is nearly constant, independent whether it swims in buffer or in colloidal suspensions.

Thanks to the insightful suggestion of the reviewer, the new analysis provided strong evidence to support the causal relation between the decrease of wobble angle and the increase of bacterial swimming speed. A further evidence on the causal relation is further provided by the temporal correlation between the decrease of the wobble angle and the increase of the swimming speed when bacteria swim next to a colloid (see our response to Q4 of the reviewer below). We have added the new plot as the Extended Data Figure 3 of the revised manuscript. The following sentence is added in the main text:

“This anticorrelation is observed not only between the average swimming speed and the average wobble angle of bacteria in suspensions of different ϕ (Fig. 3), but also between the swimming speed and the wobble angle of individual bacteria at each given ϕ (Extended Data Fig. 3).”

In addition, we have added the following comment in the last paragraph of Sec. 3.1 of the revised supplementary information:

“Such a strong anticorrelation emerges not only at the average level but also at the level of individual bacteria, where an individual bacterium that has a larger wobble angle also exhibits a slower swimming speed, independent of whether the bacterium is in pure buffer or in suspensions (Extended Data Fig. 3a). In addition, a single bacterium shows simultaneously the increase of swimming speed and the decrease of the wobble angle when the bacterium swims next to a colloid (Supplementary Video). **These observations together form the most stringent test on the causal relation between bacterial swimming and wobbling.**”

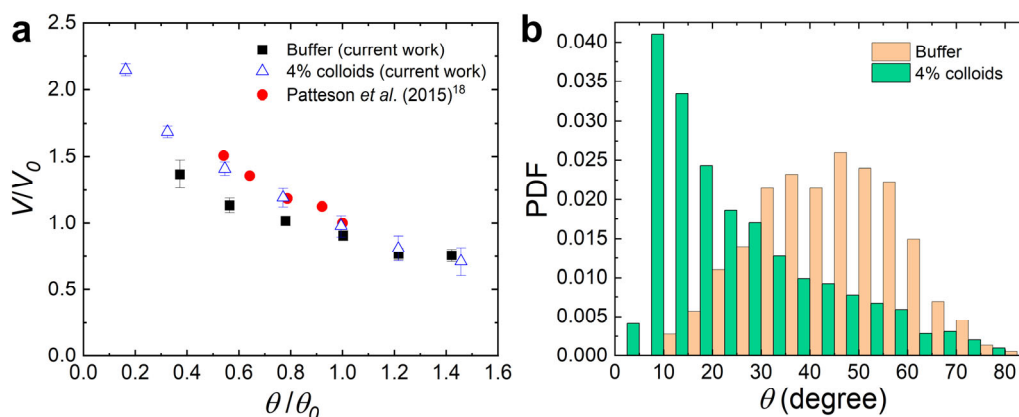


Fig. S3. The causal relation between bacterial wobbling and motility enhancement. **a**, The swimming speed of bacteria as a function of the wobble angle of bacteria in pure buffer (solid squares) and in a suspension of colloids of radius $R = 500$ nm and volume fraction $\phi = 4\%$ (blue triangles). $V_0 = 13 \mu\text{m/s}$ and $\theta_0 = 45^\circ$ are the average swimming speed and the average wobble angle of bacteria in buffer, respectively. As a comparison, the average swimming speed versus the average wobble of bacteria in polymer solutions of different concentrations from Ref. 18 is also shown (red disks). **b**, The probability distribution function (PDF) of the wobble angle of bacteria in buffer and in the colloidal suspension.

Q2: In their list of why previous theories do not work the authors omit the two-fluid model introduced by Man & Lauga Phys. Rev. E 92, 023004 (2015) and used in ref [21] which showed (using simulations) a substantial speed up for a bacterium that doesn't obviously wobble.

We thank the reviewer for bringing this important reference to our attention. We have added the reference in the revised manuscript (new Ref. 23).

Ref. 21 studied bacterial swimming in polymer solutions, where they simulated polymer molecules as coarsen-grained colloidal chains in background Newtonian fluids. Their study indeed showed a significant increase in bacterial swimming speed up to 70% in polymer solutions and $\sim 40\%$ in colloidal suspensions. Nevertheless, neither the rheology of the fluids nor the geometry of bacteria in the simulation matches experiments, which casts doubt on the numerical accuracy of the results. For example, Figure 2a of Ref. 21

shows that the viscosity of colloidal suspensions at a volume fraction of 20% is only about 20% higher than that of the solvent. In comparison, well-established measurements on the rheology of colloidal suspensions have shown that the viscosity of hard-sphere colloidal suspensions at 20% volume fraction is more than 2.5 times (i.e. 250%) higher than that of the solvent (see e.g. Fig. 1 of Wagner and Brady, *Phys. Today* 2009). Moreover, the width of bacterial body adopted in the simulation is only 4 times larger than the thickness of the flagellar filament and is the same as the diameter of the flagellar helix. In comparison, the body width of *E. coli* is ~ 25 to 50 times thicker than the flagellar filament and is ~ 2 to 3 times larger than the diameter of the flagellar helix. Hence, although the study did provide intriguing ideas, it is very likely that the quantitative value of the speed enhancement is the outcome of the unphysical parameters used in the simulation.

The ultimate test of any simulations or theories must be a quantitative comparison with experiments (e.g. the benchmark experiments by Patteson *et al.* in Ref. 18) using *physically relevant* parameters. We have implemented this rigorous test in this study. All the parameters used in our model are consistent with physical parameters from direct measurements.

Q3: The colloids are quite small compared to the bacteria. Is their recoil considered, and for how long is the bacterium near enough the colloid for the torque to act? For what range of γ can the approximations used to derive eq (1) be trusted?

Indeed, small colloids are driven by the dipolar flow induced by swimming bacteria. Under such a flow, colloids in the front of bacteria are depleted and pushed away from bacteria, whereas colloids on the side of bacteria are pulled towards bacteria, which promotes the close encounter between bacteria and colloids in a configuration favorable for the wobbling reduction. Please see our response to Question 4(i) of Reviewer 1 for the detailed description of the bacterial flow and the recoil of small colloids.

Based on the simple dimensional analysis, the time scale for the interaction between a colloid and a bacterium should be $t_b = D/V$, where D is the diameter of colloids and V is the swimming speed of bacteria. A dimensionless time is needed in order to determine whether t_b is long or not. Since bacteria interact with many colloids to maintain the steady-state wobble angle, t_b should be compared with the mean free flight time of bacteria t_f , during which bacteria swim freely without interacting with any colloids. Without the boundary-induced torque, the wobble angle of bacteria relaxes back to its equilibrium value during the free flight. t_f can be easily estimated geometrically as $t_f = \frac{2D}{3V} \left(\frac{D}{2r_b + D} \right)^2 \frac{1}{\phi}$. Here, $2r_b$ is the width of bacteria and ϕ is the volume fraction of colloids. Hence, the dimensionless interaction time is given as

$$\frac{t_b}{t_f} = \frac{3}{2} \phi \left(\frac{2r_b}{D} + 1 \right)^2.$$

At a fixed ϕ , the relative interaction time actually becomes longer for smaller colloids. Although the absolute interaction time decreases for small colloids, the frequency of close encounters between bacteria and colloids increases. Even for large colloids of the order of the size of bacteria, $t_b/t_f \sim O(1)$ when $\phi = \phi_{max} \approx 5\%$.

The lubrication approximation is valid when the gap distance d between bacteria and colloids is smaller than the size of bacteria and colloids. As the shortest distance between bacteria and solid surfaces is about $d \sim 50$ nm based on previous experiments (Ref. 36). The lubrication approximation would break down in principle when the size of colloids is less than ~ 50 nm, which leads to $\gamma \sim 0.05$. Nevertheless, as many

applications of the lubrication theory have shown, the theory can actually provide a fairly good approximation even in the case where d is comparable or even larger than the size of the system.

To address the question, we have added the discussion of the recoil of colloids and the interaction time in the revised Supplementary Information (Sec. 3.4.5). In addition, we have also explicitly stated the valid range of the lubrication approximation in SI Sec. 2.1, when the approximation is applied.

Q4: I did not find the movie very convincing. The colloid is three times the size of the largest colloids used in the experiments so it is not a totally fair comparison. It is hard to identify the speed up by eye, although the data presented for bacterium orientation are more convincing.

First, it is difficult to pin colloids of small sizes in optical tweezers without influencing the swimming of bacteria nearby. There are a couple of advantages of using large colloids. Thanks to the weak diffusion of large colloids, we only need to use a weak laser power to pin the colloids. Moreover, the large perimeter of the colloids also prevents bacteria from getting too close to the trap center, where the influence of the trap is strong. Second, we could not conduct bulk experiments with 1.5- μm -radius colloids due to the problem of sedimentation. The sedimentation speed of colloids is proportional to the square of particle sizes. Nevertheless, we would like to emphasize that our model applies equally well for 1.5- μm colloids, which predicts quantitatively similar speed enhancement and wobbling reduction near the colloids. Such a prediction has been unambiguously verified by both our experiments and our new simulations in the revision. Particularly, our experimental measurements on the anti-correlation between wobble angle and bacterial velocity using 1.5- μm colloids are quantitatively similar to that of small colloids (green squares in Fig. 3b). Our new simulations with pinned 1.5- μm colloids demonstrate the same particle behavior of the large colloids as that of small colloids under the bacterial flow (see the bottom row of Fig. S1 and our answer to Q4(i) and (iii) of Reviewer 1). Thus, the essential physics does not change with the 1.5- μm colloids.

To address the concern of the reviewer directly, we have plotted the swimming speed of the bacterium along with its orientation when the bacterium swims by the colloid (see the revised Supplementary Video). The speed enhancement can be clearly identified from the measurement, which is synchronized with the reduction of the wobble angle. The result again supports the causal relation between the decrease of wobbling and the speed-up of bacterial swimming.

Response to Reviewer 3:

We thank Reviewer 3 for their positive comments on our work. We also appreciate the very constructive suggestions/comments of the reviewer, which help us to strengthen further the conclusion of our manuscript on the common origin of bacterial motility enhancement in dilute polymer solutions and colloidal suspensions.

Overall, the authors provide an intriguing idea/concept, namely that all the experimental data observed so far in polymeric solutions could be understood by just considering polymer/particle size relative to bacteria size. The model provided by the authors does seem to capture the correct trends for their colloidal data and selective polymeric data. However, just because the colloidal and polymeric swimming speed vs particle size data trends are similar, it does not necessarily mean that the colloidal mechanism for speed enhancement is applicable to the polymeric data. I do not doubt the colloidal data or the proposed model (based on BIT), but I do doubt their applicability to polymeric solutions. The authors have not shown that the trends in bacterial rotational diffusivity, mean run time, and mean tumble time, for example, also show similar trends.

We are very pleased that the reviewer find the idea/concept of our manuscript interesting and approve the validity of our theoretical model of bacterial swimming in colloidal suspensions.

As shown in Fig. 1a and b of our manuscript, the trajectories of bacteria become smoother and less tortuous in dilute colloidal suspensions, similar to the trajectories of bacteria in dilute polymer solutions (see Fig. 1 of Ref. 18). This observation indicated that bacterial rotational diffusivity decreases and bacterial run time increases in colloidal suspensions, the trends qualitatively agreeing with those in polymer solutions. Following the suggestion of the reviewer, we have now quantitatively analyzed the rotational diffusivity, the mean run time and mean tumble time of bacteria in colloidal suspensions with increasing concentrations (Fig. S4). As expected from Fig. 1a and b, all these quantities show *quantitatively* similar trends as those shown in the benchmark experiments of Patteson et al. on dilute polymer solutions (Ref. 18).

Specifically, the rotational diffusivity of bacteria D_R decreases from 2.8 rad²/s to 0.4 rad²/s with increasing colloidal concentrations and is nearly independent of the size of colloids in the dilute limit (Fig. S4a). In comparison, D_R decreases from ~ 3 rad²/s to 0.7 rad²/s in dilute polymer solutions and is also incentive to polymer molecular weights. The mean run time τ_r increases from 0.8 s to 2.3 s (Fig. S4b) and the mean tumble time τ_t increases from 0.14 s to 0.28 s with increasing colloidal concentrations (Fig. S4c). In comparison, τ_r increases from 0.9 s to 3.5 s and τ_t increases from 0.16 s to 0.37 s with polymer concentrations.

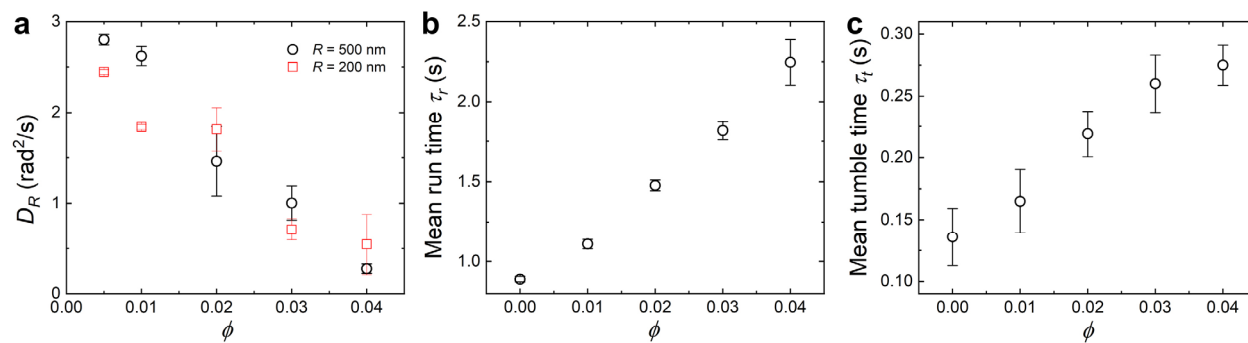


Fig. S4. The rotational diffusivity (a), the mean run time (b) and mean tumble time (c) of bacteria in colloidal suspensions of increasing concentrations. Black circles are for suspensions of colloids of radius

$R = 500$ nm, whereas red squares are for colloids of $R = 200$ nm. The results are quantitatively similar to those reported in Figs. 3D, 4A, 4B and 4C of Ref. 18.

Thus, instead of one similar trend pointed out by the reviewer (Point (ii) below), our experiments have shown that *all* the **seven** reported swimming features of flagellated bacteria in dilute colloidal suspensions are *quantitatively* similar to those in dilute polymer solutions. These seven features are summarized below:

- (i) The non-monotonic trend of the swimming speed of bacteria with the increasing concentration of colloids (or equivalently the increasing concentration of polymer) (Fig. 1c);
- (ii) The correlation between the swimming speed of bacteria and the size of colloids (or equivalently the size of polymer coils) (Fig. 2);
- (iii) The nearly linear anticorrelation between the wobble angle of bacteria and the swimming speed of bacteria (Fig. 3b);
- (iv) The decrease of the rotational diffusivity of bacteria with the increasing concentration of colloids (or equivalently the increasing concentration of polymer) (Extended Data Fig. 2a);
- (v) The rotational diffusivity of bacteria is nearly independent of the size of colloids (or equivalently the size of polymer coils) (Extended Data Fig. 2a);
- (vi) The increase of the run time of bacteria with the concentration of colloids (or equivalently the concentration of polymer) (Extended Data Fig. 2b);
- (vii) The increase of the tumble time of bacteria with the concentration of colloids (or equivalently the concentration of polymer) (Extended Data Fig. 2c).

From the experimental evidence on this comprehensive list of bacterial swimming behaviors, it is beyond a reasonable doubt that some common mechanism(s) dictate the swimming of bacteria in both dilute colloidal suspensions and dilute polymer solutions.

The natural question is then to ask what are the common property (or properties) shared by dilute colloidal suspensions and dilute polymer solutions. It has been long established in polymer science that the dynamics and rheology of dilute polymer solutions can be well described by those of dilute colloidal suspensions, where polymer coils are treated as colloids with a radius equal to the hydrodynamic radius of the coils (see e.g. the standard polymer textbooks “Polymer Chemistry” by Hiemenz and Lodge and “Polymer Physics” by Rubinstein and Colby). Once we have reached the key conclusion of our manuscript that the colloidal nature of complex fluids leads to enhanced motility of flagellated bacteria based on our experiments, there is not much room left for theoretical consideration except for the hydrodynamic interaction between bacteria and colloids, which eventually leads to our model. This theoretical consideration is also verified by our experiments with optical tweezers.

The reviewer has commented that “*I do not doubt the colloidal data or the proposed model (based on BIT)*”. If the physics of the colloidal model is correct as approved by the reviewer, we are not aware of any possible mechanisms that can obliterate the colloidal nature of polymer coils in dilute solutions well established in polymer science and annihilate its effect on bacterial swimming. It is indeed rather surprising that such an important property of dilute polymer solutions has not been considered heretofore.

To emphasize the quantitative similarities, we added the following comment in the main text of the revised manuscript:

“Together, we observe the *quantitative* similarities of *seven* different bacterial swimming features in dilute colloidal suspensions and polymer solutions, which are summarized in Sec. 1 of Supplementary Information.”

We then include the comprehensive list above in Sec 1. of the revised Supplementary Information.

Beyond that, how do the authors square their explanations with numerical simulations (Ref. 21, 22)? These numerical simulations show how polymers and their rheological properties affect bacterial swimming behavior.

Neither the rheology of colloidal suspensions/polymer solutions nor the geometry of bacteria used in the simulation of Ref. 21 matches well-established experimental data, which casts doubt on the numerical accuracy of the prediction of the simulation. Please see our answer to Question 2 of Reviewer 2 above for the detailed explanation.

Ref. 22 by Binagia *et al.* studied the locomotion of squirmers in a viscoelastic fluid, where the fluid is treated as a continuum medium following the Giesekus constitutive model. First, squirmers have a different geometry and adopt a very different swimming kinematics, compared with flagellated bacteria such as *E. coli*. More importantly, the speed enhancement observed in the simulations is only about 10 - 15%, which is significantly lower than the speed enhancement up to 80% observed experimentally in dilute colloidal suspensions and more than 50% observed in dilute polymer solutions. The results indicate viscoelasticity is not sufficient to explain the experimental observations.

We would like to emphasize that we are not arguing that other polymer properties such as viscoelasticity do not affect bacterial swimming. Rather, we aim to show, via our systematic experiments and quantitative modeling, that the colloidal nature of polymer solutions is the leading factor influencing bacterial swimming and bacterial motility enhancement. Such an important factor has not been considered before.

• *The authors say (Page 2) “Previous studies of bacterial swimming in polymer solutions focused on the dependence of bacterial speeds on the concentration or the viscosity of polymer solutions, rather than size of polymeric molecules.” This is not an accurate statement. Both Martinez et al (Ref. 16) and Patteson et al (Ref. 18) study the effects of polymer molecular weight (MW), that is, molecule size. For example, Figure 4C and 5C in Ref. 18 show the effects of polymer size (i.e, MW) on e coli rotational diffusivity and speed in polymeric solutions (at same viscosity). Notably, Ref. 18 shows that e. coli swimming speed decreases with polymer size even when scaling for viscosity effects; rotational diffusivity remain relatively incentive to polymer size. Similarly, Figure 2 and Figure 4 in Ref. 16 show (beautiful) data on normalized swimming speed and body rotational for solutions of different MW – note that the larger size molecule behaves more in line with the data in Ref. 18. Clearly these authors seem to acknowledge and study the role of particle/polymer size on E coli swimming behavior. Furthermore, similar trends to are found to the one shown in this manuscript.*

We apologize for any inaccurate account of the literature that may lead to the misunderstanding of our original manuscript. Nevertheless, we do like to point out the molecular weight of polymer is a general quantity, which determines not only the size of polymer molecules but also many other properties of polymer systems. Indeed, almost all the physical properties of polymer depend on its molecular weight (e.g. viscosity, G' , G'' , diffusivity, relaxation times, glass transition temperature, etc.). Thus, stating molecular weights alone cannot distinguish the effect of particle sizes from the effects of other polymer properties, which are not limited only to viscosity. For example, two polymer molecules of different molecular weights

have different Rouse relaxation times. With the information of molecular weights alone, it is unclear whether it is the Rouse relaxation time or the size of polymer coils that affects bacterial swimming. We have carefully read the two references. Neither of the two references has stated that it is the size of polymer molecules, instead of other polymer properties associated with molecular weights, that determines bacterial motility enhancement. Rather, Ref. 16 attributed the motility enhancement to metabolizable organic impurities in polymer solutions, whereas Ref. 18 attributed the enhancement to the elasticity of polymer chains. To the best of our knowledge, our study is the first to explicitly isolate the role of the hydrodynamic radius of polymer on bacterial motility enhancement. Please also see our response to the question after the next below.

To address the concern and avoid any possible misinterpretation, we have modified our manuscript and explicitly stated the results of previous experiments:

“Although the size dependence can be inferred from recent experiments on polymers of different molecular weights^{16,18} and has been discussed in a recent theory¹⁷, our study provides the explicit evidence on the particle-size dependence of motility enhancement.”

• *How does bacteria running length/time scale with particle volume fraction? Is this in line with data shown in Ref. 18.*

The running time, the tumble time and the rotational diffusivity of bacteria in dilute colloidal suspensions show quantitatively similar trends as those in dilute polymer solutions shown in the benchmark experiments of Ref. 18. Please see our answer to the first question above for details.

We have added the following sentence and a new figure (Extended Data Fig. 2) in the revised manuscript:

“Analyses of the rotational diffusivity, the mean run time and mean tumble time of bacteria confirm their quantitatively similar trends (Extended Data Fig. 2).”

• *The point above show how bacteria swimming speed can be controlled by both polymer type and molecular weight (size). It would be important for the authors to discuss those findings in more detail and add them to their analysis.*

We fully agree with the reviewer that polymer type also plays an important role in bacterial swimming. As we have argued above, molecular weight is not equivalent to polymer size. Different types of polymers with the same molecular weight (or the same number of repeat units) can have completely different hydrodynamic radii and, therefore, have drastically different effects on the swimming of bacteria. In addition to the molecular weight, the size of a polymer coil also depends on the molecular structure and the solvent quality. For example, branched polymers such as Ficoll have relatively small sizes even at high molecular weights, which then have a weak effect on bacterial motility enhancement. On the other hand, linear chains such as methylcellulose (MC) and polyvinylpyrrolidone (PVP) have much larger size at the same molecular weight and therefore have a much stronger effect on the speed enhancement. Our study provides a unified picture to understand bacterial swimming in polymer systems of different types and molecular weights, which agrees well with existing experiments. Such a unified picture that quantitatively explains all the main features of bacterial swimming in dilute polymer solutions and colloidal suspensions has not been achieved previously.

We have added the above discussion in our revised manuscript (see **Methods: Hydrodynamic radius and concentration of polymer solutions**).

• *Figure 4: I am a little concern about the findings in this figure. First, it is known that e coli can be light sensitive (phototactic) – Perlova, et al J. Bacteriology, 2019. It is quite likely that the observed increased in speed is due to phototaxis.*

The paper by Perlova *et al.* shows the phototaxis of bacteria under *blue* light. In contrast, our optical tweezer uses a 1064 nm infrared laser, which is operated at a much lower photon energy. Indeed, the photon energy of the laser is even lower than that of the microscope illumination light. If the IR light induced the phototaxis of *E. coli*, the illumination light of conventional microcopy would also induce phototaxis, which has not been reported before. To address the concern of the reviewer directly, we applied the infrared laser to our bacterial suspensions without pinning colloids. We did not observe any phototaxis or speed enhancement for bacteria swimming 1.5 μm away from the trap center in experiments.

• *The authors should also discuss the work of Patteson et al, Soft Matter (2016), who found non-monotonic diffusion behavior of e coli with particle size. These are very dilute colloidal systems, but it seems that similar behavior is found.*

We thank the reviewer for providing the new reference. The interesting work of Patteson and co-workers studied the diffusion of *passive* colloidal tracer, instead of the diffusion of *E. coli*, in bacterial suspensions. They showed that the diffusion of passive tracers exhibits a non-monotonic trend with the size of the tracers. The paper does not discuss the swimming behavior of bacteria, which is the focus of our current manuscript. In a sense, our current study is complementary to the extensive research in recent years on the enhanced diffusion of passive colloidal tracers in active bath of microswimmers. Instead of probing the influence of bacterial swimming on the motion of passive colloids, we showed here that the presence of low-concentration colloids can in turn profoundly modify the swimming behaviors of bacteria.

Given the limit space of the main text, we have added the following comment and the new reference in the revised supplementary information (the end of Sec. 3.5):

“Our current study is also complementary to the extensive research in recent years on the enhanced diffusion of colloidal tracers in active bath of microswimmers¹⁸⁻²². Instead of probing the influence of bacterial swimming on the motion of colloids, we show here that the presence of low-concentration colloids can in turn profoundly modify the swimming behaviors of bacteria.”

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

I commend the authors for the extra work they have undertaken and which has improved greatly the original manuscript. Although I still have reservations on the answer to some of my questions, the new data presented in Ext.Data Fig.3 lends a very strong support to the connection between the change in wobbling angle and the increase in average speed.

Overall, I therefore decided that I am happy to recommend the paper for publication.

Referee #2 (Remarks to the Author):

Re-reading the manuscript and the replies of the authors, I remain of the opinion that this work is best published elsewhere. The ideas are nice but, even more than in the first version of the manuscript, they appear to be over-sold.

I am not convinced by the authors' replies to my first round of comments:

Q1: I do not understand Fig S3a. The authors state that this is the swimming speed of individual bacteria plotted against their wobble angle. I would have expected a large number of points, showing trends maybe, but not a smooth curve -- i.e. a point for each individual bacterium. Fig S3b certainly shows nicely that the wobble angle tends to be smaller in the colloidal suspension. Why the sharp drop off at $\theta=0$? Does this data fit the theory?

Q2: I agree that the model used in ref 21 uses unphysical parameters (although it does seem to fall on the curve in Fig 2 of this manuscript). However, I was asking about the theory in ref 23 – I was hoping for a measured discussion about whether this could be a possible interpretation of the results presented here?

Q3: On p3 of the SI the authors say that “The resistance matrix has been calculated previously in the limit when $(r_b, R) \gg d$ using the lubrication theory”. They then answer my question by saying that lubrication theory holds up to $(r_b, R) \sim d$. I would have thought that the first condition is correct. Indeed, there are a lot of approximations in the theoretical development, which are not summarised clearly, and there is no attempt to consider the robustness of the results to parameter choice.

Referee #3 (Remarks to the Author):

I thank the authors for providing additional data and for improving the model. The new version of the manuscript shows the colloidal effects more explicitly, and the connection to polymeric fluids data is convincing in my opinion. Thus, I would like to recommend the manuscript for publication. I believe it is an important contribution to the field to microbial motility in complex fluids.

Author Rebuttals to First Revision:

As both Reviewer #1 and #3 support the publication of our manuscript without further questions, we report below only a detailed response to the report of Reviewer #2. The original reviewer comments are in *italic*, and our response is in normal font. When addressing the reviewer's questions, we also explicitly state the changes we made in the revision.

Response to Reviewer #2:

We thank Reviewer #2 for carefully re-reading our manuscript and for further clarifying their questions. These questions help us further clarify the technical points of our work and improve the quality of the manuscript.

Re-reading the manuscript and the replies of the authors, I remain of the opinion that this work is best published elsewhere. The ideas are nice but, even more than in the first version of the manuscript, they appear to be over-sold.

As we have emphasized in our response to the first run of the reviewers' report and in our revised manuscript, the main conclusion of our manuscript, i.e., the colloidal nature of complex fluids leads to enhanced motility of flagellated bacteria, is firmly supported by our experiments without recourse to any theoretical modeling. In particular, our experiments have demonstrated that *all* the reported swimming behaviors of flagellated bacteria in dilute polymer solutions are *quantitatively* similar to those in dilute colloidal suspensions (see the comprehensive list of the seven different behaviors in Sec. 1 of the supplementary information (SI)). Based on our experiments, it is beyond a reasonable doubt that the colloidal nature of complex fluids leads to the motility enhancement of flagellated bacteria, a factor that has not been considered heretofore. We are very glad that the reviewer does not question either the validity of our experiments or the importance of our findings. Rather, the specific questions raised by the reviewer below all focus on the technical details of our model or the specific way we presented our data. Below, we shall address these questions and summarize the approximations adopted in our model. However, we would like to emphasize again that the conclusion of our manuscript is valid, independent of these technical points. This view is clearly shared by both Reviewer #1 and #3, who explicitly support the publication of our work after the first revision.

Q1: I do not understand Fig S3a. The authors state that this is the swimming speed of individual bacteria plotted against their wobble angle. I would have expected a large number of points, showing trends maybe, but not a smooth curve -- i.e. a point for each individual bacterium. Fig S3b certainly shows nicely that the wobble angle tends to be smaller in the colloidal suspension. Why the sharp drop off at $\theta=0$? Does this data fit the theory?

(i) For Fig. S3a, the data points represent the averaged results of many individual bacteria binned over a small range of wobble angles, an averaging procedure frequently used to illustrate the trends of bacterial swimming behaviors. Due to intrinsic cell-to-cell variations in body shape, metabolism, and the distribution and number of flagella, the swimming behaviors of bacteria naturally display large fluctuations. Thus, to extract any meaningful physical trends, one has to either average over many bacteria binned over a small

range of the property under study (e.g. Fig. 4E and F in Ref. 36) or fit scattered points with regression lines (e.g. Fig. 4 in Ref. 18 and Fig. 3 in Ref. 32). This general rule certainly applies for showing the swimming speeds of bacteria in colloidal suspensions of different volume fractions in our study, as well as in polymer solutions of different concentrations in all previous studies (Figs. 2 and 3). In Fig. S3a, we adopt the first approach and use the averaged results to illustrate the trends. Fitting with polynomial regression lines would lead to the same conclusion.

To address the concern of the reviewer, we have plotted all the raw data points from individual bacteria in 4% colloidal suspensions in the revised Fig. S3a as the background, together with the original averaged results in the foreground to illustrate the physical trends. For clarity, we do not show the results of individual bacteria in buffer, which show a similar degree of scattering. We replicate the revised Fig. S3 below for the convenience of the reviewer (Fig. R1).

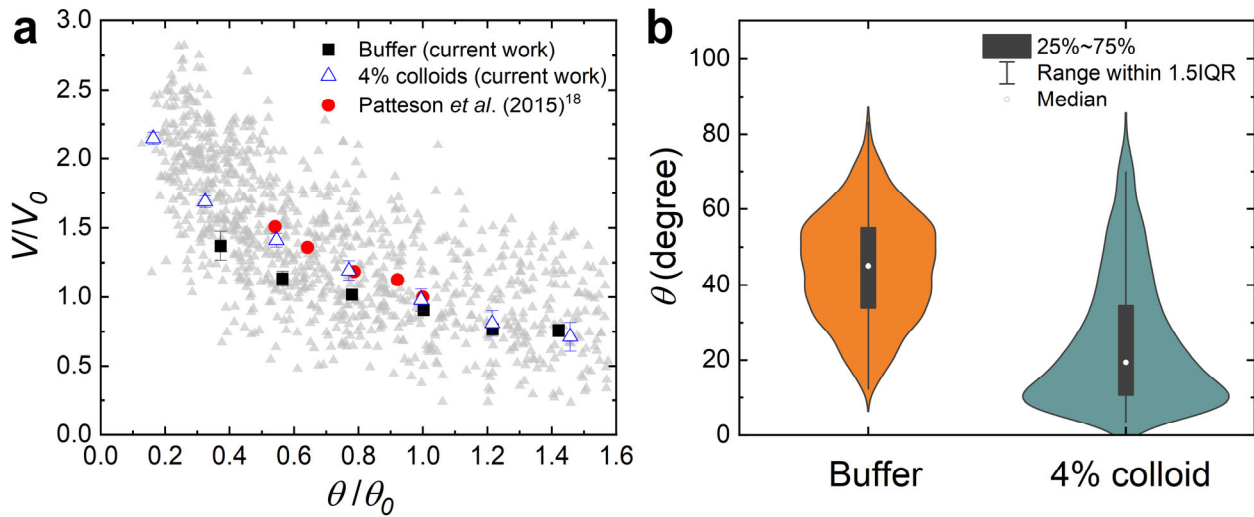


Fig. R1 | Replication of the revised Extended Data Fig. 3. Note that we represent the probability distributions of the wobble angle of bacteria using a violin plot in **b**. In addition to the distributions showing vertically on the side, the plot also shows the median and interquartile range (IQR) of the distributions, which conveys more complete statistical information of the data. The detailed caption of the figure can be found in the revised manuscript.

(ii) For Fig. S3b. Thanks for the question of the reviewer, which stimulated us to consider the origin of the sharp drop of the probability distribution of wobble angles at zero. Indeed, our model gives a nice explanation of this interesting result, which provides further support to the validity of our model.

With increasing volume fractions of colloidal suspensions, the position of the peak of the probability distribution of the wobble angle shifts towards smaller angles. However, the probability of finding bacteria without wobbling remains small, independent of whether there are colloids or not. Hence, as correctly pointed out by the reviewer, the probability distribution drops sharply near zero wobble angle at sufficiently high colloidal volume fractions. As we have explained in our response to the first run of the reviewer's report, the chance that the flagellar bundle of a bacterium perfectly aligns with the symmetric axis of the body of the bacterium is slim. Thus, the probability that bacteria show no wobbling is small in pure buffer without colloids. Our experiments and model have shown that colloids reduce bacterial wobbling. Thus, the key question that needs to be addressed here is why colloids cannot reduce wobble angle down to zero

and increase the number of bacteria without wobbling. Our model provides the answer of this nontrivial question, which shall be discussed next.

As discussed in Sec. 3.4.5 in SI, a symmetric breaking mechanism exists in our model, where colloids are depleted from the inner cylindrical region of the helical trajectory of wobbling bacteria. A bacterium interacts only with colloids outside of the cylindrical region close to its body (the near side), which invariably reduce its wobble angle (Fig. R2a below). In particular, the bacterium does not interact with a colloid on the far side of the cylindrical region due to the short-range nature of the lubrication interaction and the relatively large distance between the bacterium and the colloid. However, as the wobble angle approaches zero, bacteria swim in much straighter lines. The radius of the cylindrical region shrinks to zero correspondingly (Fig. R2b). As a result, bacteria can now interact with colloids on both the near side and the far side of the cylinder via the short-ranged lubrication interaction. Thus, a colloid is equally likely to enhance or reduce the wobble angle of a bacterium in this limit. The average effect of colloids on reducing wobbling vanishes as the wobble angle approaches zero. Hence, increasing colloidal concentrations can shift the peak of the distribution to smaller angles, but cannot increase the number of bacteria with zero wobble angle, which therefore leads to the sharp drop of the distribution at high enough volume fractions. Quantitative analysis of the distribution needs the biological information on the cell-to-cell variations in body and flagellar shape, metabolism, etc, which is beyond the scope of our physical model.

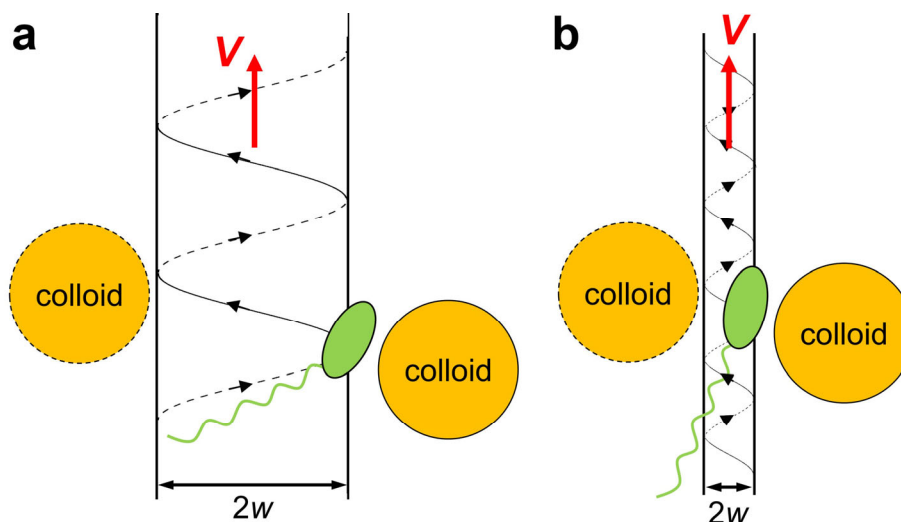


Fig. R2 | Schematics explaining the sharp drop of the probability distribution at zero wobble angle. **a**, When the wobble angle is large, a bacterium can only interact with the near-side colloid (the solid colloid) via the short-ranged lubrication interaction, which always reduces the wobble angle of the bacterium (see Sec. 3.4.5 in SI). The colloid on the far side of the cylindrical region (the dashed colloid) is too far away to interact with the bacterium via the lubrication interaction. **b**, When the wobble angle approaches zero, the cylindrical region shrinks to zero correspondingly. The bacterium can now interact with both the near-side colloid that reduces wobbling and the far-side colloid that enhances wobbling. Hence, colloids do not affect the wobble angle of the bacterium on average in this limit.

To address the question of the reviewer, we have added the above discussion as a new prediction of our model in Sec 3.4.5 of the revised SI (highlighted in red). In addition, to present the more complete statistical information of the probability distributions of bacterial wobble angles including their median and

interquartile range, we use a violin plot in the revised Fig. S3b (see Fig. R1b above), instead of the histograms in the original figure.

Q2: I agree that the model used in ref 21 uses unphysical parameters (although it does seem to fall on the curve in Fig 2 of this manuscript). However, I was asking about the theory in ref 23 – I was hoping for a measured discussion about whether this could this be a possible interpretation of the results presented here?

We apologize for the misunderstanding of the original question of the reviewer. Following the suggestion, we now discuss the theory in Ref. 23 in the context of our experiments. Before delving into the technical details, we would like to thank the first author of Ref. 23, Yi Man, for the fruitful in-person discussion. Our response below has been formed through the stimulating discussion with Dr. Man, who supports our conclusion below.

The theory in Ref. 23 cannot be applied to explain our experiments, as the system considered in Ref. 23 is qualitatively different from flagellated bacteria studied in our experiments in terms of both their swimming gaits and body geometries:

- Different swimming gaits. Ref. 23 considers the swimming of an infinitely long filament without a body that executes *planar waving*, a system that is more appropriate for spermatozoa. In contrast, an *E. coli* bacterium in our study consists of a large body and a rigid helical flagellar bundle of finite length. The helical flagellar bundle of bacteria rotates rigidly, instead of beating in a planar wave. A recent review by Eric Lauga, one of the authors of Ref. 23, considers bacteria with rotating helical flagella and spermatozoa with planar waving filaments as two qualitatively different categories in terms of their swimming behaviors and underlying theoretical frameworks (Velho Rodrigues, Lisicki, Lauga, *PLOS One* **16**, e0252291 (2021)). Thus, the prediction of the theory in Ref. 23 cannot be used to explain our experiments on flagellated bacteria.
- Different geometries. Ref. 23 considers a filament *without* a body. For a spermatozoon whose filament has an undulation amplitude much larger than the characteristic size of its body, this might be a reasonable approximation. However, the width of the body of an *E. coli* bacterium (0.5 μm) is wider than the diameter of its flagellar helix (0.4 μm). The force and torque balances between the body and the flagellar bundle play the essential role for the locomotion of *E. coli* and neglecting the body can lead to qualitatively different predictions.

Even within the framework of Ref. 23, the theory in Ref. 23 cannot explain the key findings of our experiments. Quantitatively, the theory predicts only a mild speed enhancement of a few percent for colloids of radius $R = 500$ nm and volume fraction $\phi = 4\%$, which cannot explain the significant mobility enhancement ($\sim 80\%$) observed in our experiments. Specifically, Eq. (56) of the reference shows that the thickness of the depletion layer h follows $h = 2R(1 - \phi/\phi_m)$, where ϕ_m is the maximum volume fraction of colloidal suspensions. Here, $\phi_m = 64\%$ for the random close packing of mono-disperse spheres. Applying the equation to our system with $R = 500$ nm and $\phi = 4\%$, we have $h = 0.94$ μm . Furthermore, the wavenumber of *E. coli* flagella is $k = 2\pi/\lambda = 2.86$ μm^{-1} , where $\lambda = 2.2$ μm is the pitch length of flagellar helix. Thus, $kh = 2.68$. Based on this value, from the prediction of the theory in Fig. 8a of Ref. 23, even at the viscosity ratio of $\beta = 0.5$, the speed enhancement is only a few percent, which is much smaller than 80% enhancement observed in our experiments at $R = 500$ nm and $\phi = 4\%$. At the viscosity ratio of $\beta = 0.9$ that is more relevant to our experiments, the speed enhancement would be even smaller, almost negligible. Note

that the thickness of bacterial flagella is $\rho = 20 \sim 30$ nm, which gives $k\rho = 0.06 \sim 0.09$, matching the parameter used in Fig. 8a.

Qualitatively, the prediction of the theory on the particle-size dependence of the speed enhancement is opposite to the trend observed in our experiments. Since our experiments have shown that the volume fraction of colloids ϕ at which the speed enhancement is strongest is approximately constant independent of the size of colloids (Extended Data Fig. 8), Eq. (56) of Ref. 23, $h = 2R(1 - \phi/\phi_m)$, predicts that the thickness of the depletion layer h is smaller for smaller colloids. The theory in Ref. 23 further predicts that the speed enhancement is stronger when the thickness of the depletion layer h is smaller. Taken together, the theory predicts that the speed enhancement increases with the decrease of the size of colloids. This prediction is opposite to what we observed in our experiments, where the speed enhancement increases with the size of colloids (Fig. 2).

It should be emphasized that, although the theory of Ref. 23 cannot be applied to our experiments on flagellated bacteria, the study is certainly an important theoretical development for understanding the motion of filaments with planar waving in complex fluids.

A minor point: The data point from Ref. 21 was the only numerical result in the original Fig. 2 of our manuscript. To avoid the confusion and any potentially misleading information, we have removed the data point of Ref. 21 from Fig. 2. In the revised Fig. 2, we now compare only experimental results of the swimming speed of *E. coli*. Nevertheless, we still keep Ref. 21 in our revised manuscript as an important and inspiring work on bacterial swimming in polymer solutions.

Q3: On p3 of the SI the authors say that “The resistance matrix has been calculated previously in the limit when $(r_b, R) \gg d$ using the lubrication theory”. They then answer my question by saying that lubrication theory holds up to $(r_b, R) \sim d$. I would have thought that the first condition is correct. Indeed, there are a lot of approximations in the theoretical development, which are not summarised clearly, and there is no attempt to consider the robustness of the results to parameter choice.

(i) Regarding the lubrication approximation: As the characteristic size of bacteria $r_b \approx 1$ μm is fixed, which is always significantly larger than the small gap between bacteria and colloids at $d \approx 50$ nm, we only need to compare the radius of colloids R with d for the valid range of the lubrication approximation.

From the practical point of view, our interest is naturally in the regime when R is large and comparable to the size of bacteria, where the motility enhancement is most obvious and the lubrication approximation is accurate. Although the lubrication approximation is less accurate when R is small and comparable to d , the motility enhancement of bacteria is weak and barely above one at small R (Fig. 2), which is not the focus of our study.

From the theoretical point of view, we agree with the reviewer that the lubrication approximation is strictly valid only in the limit when the slope of the gap $d/R \rightarrow 0$. However, it is commonly known in fluid mechanics calculations that “the lubrication approximation often works well far beyond the regime in which it is formally valid” and can provide quantitative predictions even when the local slope is not small (see e.g. the recent publications: Krechetnikov, *Phys. Fluids* **22**, 092102 (2010); Espin and Kumar, *Phys. Rev. Fluids* **2**, 014004 (2017); Hooshanginejad et al. *J. Fluid Mech.* **901**, A19 (2020)). The quote above is directly from the second reference. Specifically related to the application of the lubrication approximation in colloidal

interactions, the early work of Goldman and co-workers has shown that when the gap thickness between a colloid and a flat wall is equal to the radius of the colloid, the torque on the colloid from the lubrication approximation approaches zero (Goldman *et al.*, *Chem. Eng. Sci.* **22**, 637 (1967)). More accurate calculation beyond the approximation shows a negligible torque of 0.0048. As a comparison, the torque is nearly two orders of magnitude higher at ~ 0.25 when $d/R = 0.1$ for 500 nm colloids. Here, the torque is non-dimensionalized by $8\pi\eta R^2 U$, where η is the viscosity of the solvent and U is the speed of the colloid. The lubrication approximation has also been used at large gaps in Stokesian dynamics simulations, which are the most widely used numerical method in studying the dynamics of colloidal suspensions with hydrodynamic interactions. The simulations have been well tested and yielded many quantitative predictions on colloidal dynamics (see the review by Brady and Bossis, *Annu. Rev. Fluid Mech.* **20**, 111 (1988) and papers that cited this review).

(ii) Regarding approximations in our model: We took five approximations (including the lubrication approximation above) in order to solve the mathematical problem analytically. It is hard, if not impossible, to solve the full problem rigorously without taking any approximations. Nevertheless, the approximations we adopted in our model are either easily justified or standard, which have been frequently used in previous theoretical studies of bacterial swimming. Following the suggestion of the reviewer, we summarize explicitly all the approximations in our model and justify why these approximations are valid below. We would like to emphasize again that these technical details of our model do not affect the conclusion of our manuscript, which is firmly established based on our experimental findings.

- The lubrication approximation
We have discussed the validity of the approximation above.
- A constant magnitude of bacterial tangential velocity
We have shown in our wobbling model that, under a constant rotary motor torque, the variation of the rotational speed of bacterial flagella is less than 7% over the entire range of wobble angles. As the bacterial tangential velocity is linearly proportional to the rotational speed of flagella, the variation of bacterial tangential velocity is also less than 7%.
- Approximating bacterial body as a sphere
Bacterial body has a small aspect ratio less than 3. A previous study has shown that the difference in the drag coefficients of bacterial body of different orientations is less than 8%, which leads to at most 8% motility enhancement (Ref. 20), far less than the 80% motility enhancement observed in our experiments. In fact, the drag coefficient of even an infinite long cylinder moving along its radius is only two times larger than the drag coefficient of the cylinder moving along its symmetric axis at low Re . Such a small difference reflects the nature of low- Re Stokes flow, where the hydrodynamic effect induced the shape of an object is strongly mitigated due to the viscous damping (see “*A Physical Introduction to Suspension Dynamics*” by Guazzelli and Morris). Thus, it is well justified to treat bacterial body as a sphere, an approach that has been adopted by many previous studies (see e.g., Ref. 35 and 36).
- Position of flagella with respect to the center of bacterial body
We put the flagellar bundle directly underneath the center of bacterial body. Thus, the position of the flagellar tip with respect to the center of the body is simply $|z_{t,0}| = l + a$, where l is the length of the flagellar bundle and a is the radius of the bacterial body. The largest possible deviation of the actual $|z_t|$ from this approximation is reached when the flagellar bundle is formed at the side of the bacterial body and perpendicular to the line connecting the center of the body and the proximal end of the flagella. In this extreme configuration,

$$|z_t| = \sqrt{l^2 + a^2} = l\sqrt{1 + (a/l)^2} \approx l\left(1 + \frac{1}{2}\frac{a^2}{l^2}\right),$$

which leads to the maximum error $(|z_t| - |z_{t0}|)/|z_{t0}|$ still less than 14%. Here, we have used the Taylor expansion and the fact that l is much larger than a in the last step. Note that this extreme configuration has never been reported for bacteria in the run phase during swimming in experiments (Ref. 32). Common swimming configurations would give even much smaller errors.

- No hydrodynamic coupling between bacterial body and flagellar bundle

We formulate the resistance matrices of the body and the flagella separately without considering the hydrodynamic coupling between the two in our analytical model, which is again a standard practice for calculating the locomotion of bacteria (see e.g. Refs. 15 and 16, as well as the recent review by Velho Rodrigues et al., *PLOS One* **16**, e0252291 (2021) and the classic paper by Purcell, *PNAS* **94**, 11307 (1997)). Our numerical simulation including the hydrodynamic coupling (Fig. S7) also qualitatively confirms the prediction of our analytical model.

All the approximations above have been clearly stated and justified in SI when we discussed the details of our model. As the reviewers' report and our response would be published along with the manuscript if it were accepted by *Nature*, the summary above would also be available to any readers who are interested in seeing all the approximations in one place.

(iii) Regarding the choice of parameters: Our model does not contain any free fitting parameters. The coefficients of the resistance matrices are the only parameters used in our model. These parameters are numerically calculated using the method of Stokeslets based on the geometry of *E. coli*, which has been well documented in the literature and confirmed in our own measurements. Certainly, the geometry of bacteria varies due to cell-to-cell variations within a biologically relevant range. We have tested different bacterial geometries by changing the size of bacterial body and the shape of flagellar bundle. Specifically, we vary the characteristic radius of bacterial body from 0.7 μm to 1.0 μm , the pitch length of flagellar helix from 2 μm to 2.2 μm , the radius of flagellar helix from 0.2 μm to 0.3 μm and the thickness of flagella from 20 nm to 50 nm. In addition, we have also changed the number of pitches of flagellar bundle from 2 to 3 and the number of flagella in the bundle from 1 to 3. Our results are robust. The key predictions of our model including the size-dependence motility enhancement (Fig. 2) and the anti-correlation between wobble angle and swimming speed (Fig. 3) are qualitatively the same within the range.

To address the concern, we have added the following sentence in Sec. 3.3 of the revised SI:

“We have tested different bacterial geometries by changing the size of bacterial body and the shape of flagellar bundle within a biologically relevant range. The key predictions of our model including the size-dependence motility enhancement (Fig. 2) and the anti-correlation between wobble angle and swimming speed (Fig. 3) are robust and do not change qualitatively within the range.”