

Manuscript ID: LX17781

Manuscript title: Accelerated transport through sliding dynamics of rodlike particles in macromolecular networks

Author(s): Xuanyu Zhang, Xiaobin Dai, Md Ahsan Habib, Ziyang Xu, Lijuan Gao, Zhongqiu Tang, Xianyu Qi, Xiangjun Gong, Lingxiang Jiang, and Li-Tang Yan

First of all, we would like to thank the reviewers for their thoughtful comments on our original manuscript. Our detailed responses to them are as follow:

Responses to Referee A:

I think that the authors have adequately addressed the comments raised by the referees.

Reply: Thank you very much!

Responses to Referee B:

In the revised manuscript, the authors report new experimental results conducted at higher framerates and over longer periods of time. They also present experimental particle distributions to complement the simulations. Although these new experimental results are appreciated, ...I do not recommend publication without addressing these concerns.

Reply: Thank you very much for the expert comments!

1. The updated mean-squared displacements are over an order of magnitude smaller than in the previous version of the manuscript with a rod of length $2a_x$ moving approximately $0.1 \mu\text{m}^2$ over 100s in this revision but $\sim 50 \mu\text{m}^2$ over the same time in the previous experiments. As far as I can tell, the only differences in the new experiments are a higher magnification lens, a faster frame rate, and collecting additional frames. None of these experimental differences should affect the computed MSDs. The discrepancy between results indicates that there may be significantly more error and variability in these experiments than accounted for by the authors. Indeed, this discrepancy is larger than the difference in mean squared displacements between the nanorods with commensurate lengths and those without. As a result, any length commensuration effect may not be significant.

Reply: The comment is appreciated. In the last revision, based on the expert comments on the experiments, we carefully checked and remarkably improved the methods, protocol and devices for a higher resolution and much longer observation time, when we found that the experimental curves of MSDs in the original version did be abnormal because the diffusion exponents approximated to 2, behaving as the ballistic motion instead of 1 for the normal diffusion. After verifying the experiments very carefully, we found that this was due to the cumulative vibration and drift of the microscope stage used for imaging during a long-time measurement. Although it did not change the qualitative trend for the speeding-up dynamics at commensurate length, the values of D/D_0 were not accurate. Thus, in the last revision, besides a higher magnification lens, a faster frame rate, and collecting additional frames, we made a great effort to eliminate such an experimental error, through computing the overall drifting motion, which we will subtract away, adopting the reference frame of the particles' average position. This method can significantly reduce the creeping movement of the microscope and has been widely used elsewhere [1-3]. Indeed, with this correction, the diffusion exponents generated from the curves of MSDs converge to 1, as demonstrated in Fig. 1(b) in the main text, pointing to the normal diffusion. We are sorry that this case was not referred in the last response.

In the revision, we describe this aspect, which can be found from the 16th to 21st line on Page 3 in Supplemental Material.

2. Distributions of particle displacements (Fig 3).

2a. The tracking videos only go up to 600s, but the videos used for these distributions are taken up to 10,800s. Why do the authors not report the MSDs over this time scale? This would be particularly helpful for assessing the long term transport of rods with non-integer lengths.

Reply: Thanks for the careful comments. To ensure the experimental DPDFs with enough statistical significance, we extended the observation time up to 10800s, ensuring that at least 10 hopping events occur for the corresponding samples. Frankly, the measurement and data process within such a long period (10800s) is really challengeable; for example, the amount of the raw images is 108000, taking 216 GB for each length, and the computer memory for image processes, such as particle tracking, trajectory linking, ..., is almost energetically prohibited. Thus, the time range for MSDs is still set as 600s, *similar to* those used in some previous works where other parameters are also similar [4, 5]. It can be found that the MSD curves as well as the diffusion coefficients generated from them demonstrate the length dependence of the dynamics well [Fig.1(b) and (c)]. Furthermore, although extremely challengeable, we still calculate the MSDs for the two sample of DPDFs. As shown in Fig. R1, the MSDs within 600s for the corresponding samples match very well, and, in particular,

the diffusion exponent does converge to 1 for the long-term transport of the rod with non-integer length.

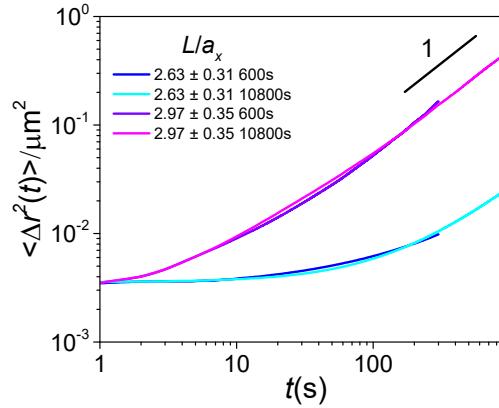


Fig. R1. Ensemble-averaged $\langle \Delta r^2(t) \rangle$ for different L/a_x with various measurement periods.

2b. The distributions at long times (i.e. $>750\text{s}$ for $L = 2.5$ and $>500\text{s}$ for $L = 3$) have a minimum at $z = 0$. This is wholly unphysical. If the particles are transporting according to Brownian motion, the distribution of displacements must be peaked at $z = 0$ to reflect the randomness of this motion.

Reply: The expert comment is appreciated. We fully agree with that the distribution of displacements must be peaked at $z = 0$ for the random motion. Indeed, our theoretical results in Fig.4(b) strictly follow this point. After considering the abnormal case in the simulation results very carefully, we found that the presence of minimum at $z = 0$ is attributed to the inappropriate correction for the drift beyond 600s. Please kindly let us explain it as follow:

As discussed in our reply to Comment 1, the cumulative vibration and drift should be eliminated for the neat dynamics. Such a correction is based on the overall drifting motion computed throughout the whole stage [1-3]. Although the MSDs were calculated based on the correction with the overall drifting motion computed within 600s, this overall drifting motion (within 600s) was misused as the correction for the calculation of the DPDFs with a much longer period over 10800s, resulting in the fact that all the DPDF curves beyond 600s were abnormal, as reflected by the minimum at $z = 0$ in each curve. We are deeply sorry for this case!

In the revision, we compute overall drift of the 10800s trajectories and thereby eliminate the cumulative vibration and drift exactly, resulting in the unbiased DPDFs of commensurate and non-commensurate rods. As shown in Fig. R2(a) and (b), the ensemble-averaged DPDFs reach its maximum at $z = 0$ for all times, presenting statistical characters of hopping and sliding dynamics respectively.

In the revision, Fig. R2(a) and (b) have been included, as Fig.3(c) and (d) respectively.

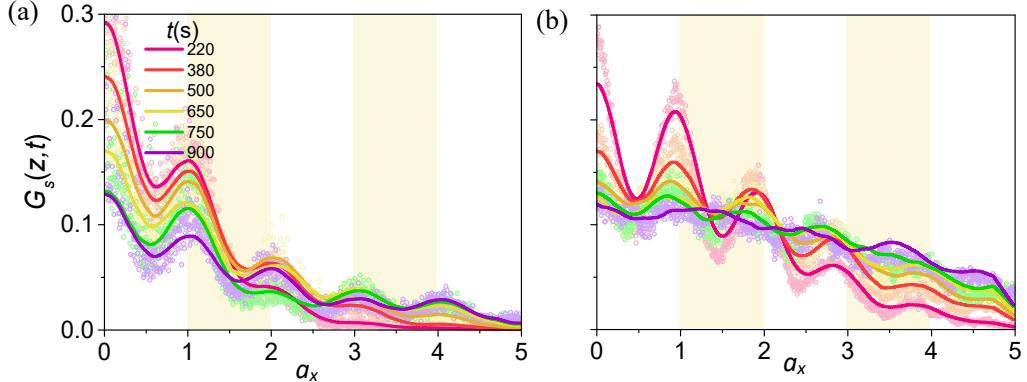


Fig. R2. Ensemble-averaged $G_s(z, t)$ of rods with (a) $L/a_x = 2.51 \pm 0.27$ and (b) $L/a_x = 2.97 \pm 0.35$ in experiments. Solid lines are smoothed results of circles.

2c. It is not clear whether the authors are presenting the displacements from the raw trajectories or from the wavelet-based methods. I suspect, however, that the distributions are calculated from the wavelet smoothed trajectories as the step over quantized values on the order of a_x in the simulations and $\sim 20\text{nm}$ (i.e. $1 a_x$) in experiments. The oscillations in the distributions may therefore just a result of the wavelength chosen to smooth the trajectories rather than being an independent calculation.

Reply: Thanks for the comment. Actually, the distributions are calculated from the raw trajectories, instead of the wavelet-based methods. The wavelet-based method is used only to extract the local confinement diffusion and hopping events from the trajectories, as demonstrated in the insets of Fig.3. To explain the calculation protocol of the distributions in more detail, we present the DPDFs obtained directly from the experimental measurements shown as the half-transparent color lines, the ensemble-averaged DPDFs shown as the colored circles in each panel, and the smoothed lines for these circles in Figs.R3 and R4 corresponding to the hopping and sliding dynamics respectively. In the revision, these both figures have been included in the Supplemental Materials, as Figs.S7 and S8 respectively, to support the experimental results of DPDFs in Fig.3(c) and (d). In particular, the circles representing the ensemble-averaged DPDFs have also been included in Fig.3(c) and (d) for a more exact presentation.

(Please see the following page for Figs.R3 and R4, thanks!)

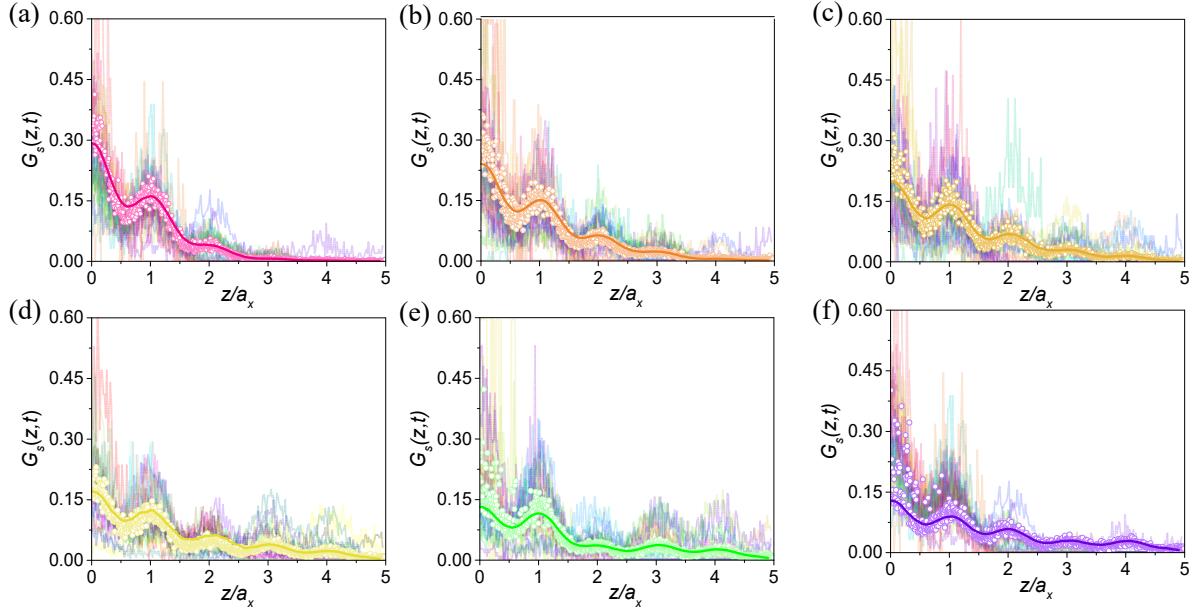


Fig. R3. The DPDFs obtained directly from the experimental measurements (half-transparent colored lines), the ensemble-averaged DPDFs (colored circles) and the smoothed lines for these circles (colored thick lines) of experimental $G_s(z, t)$ of rods with $L/a_x = 2.51 \pm 0.27$ at different times: (a) 220s (b) 380s (c) 500s (d) 650s (e) 750s and (f) 900s. The color of the half-transparent lines marks different particles.

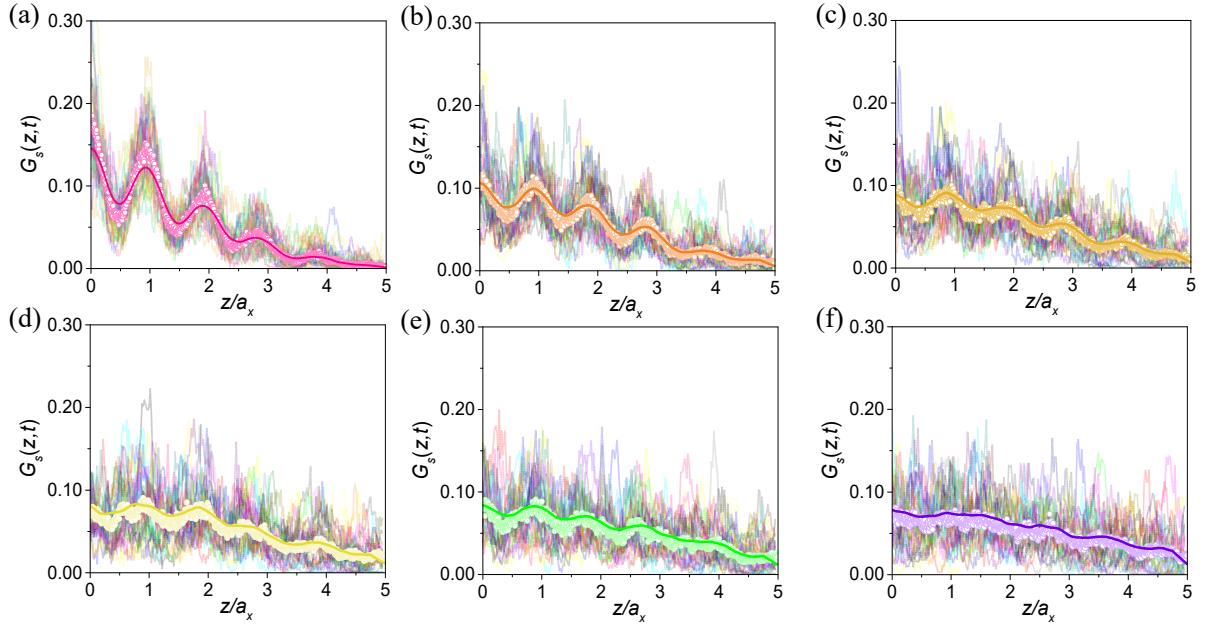


Fig. R4. The DPDFs obtained directly from the experimental measurements (half-transparent colored lines), the ensemble-averaged DPDFs (colored circles) and the smoothed lines for these circles (colored thick lines) of experimental $G_s(z, t)$ of rods with $L/a_x = 2.97 \pm 0.35$ at different times: (a) 220s (b) 380s (c) 500s (d) 650s (e) 750s and (f) 900s. The color of the half-transparent lines marks different particles.

2d. The authors report the tracking resolution to be 20 nm which is comparable to a_x . If this is truly the resolution, then the oscillations over this same value in the distribution cannot be statistically resolved. In other words, the binning of displacements is smaller than the resolution at which displacements can be determined. Therefore, the minima between integer multiples of a_x may exist because the algorithm cannot resolve motion over those distances

Reply: Thanks for the comment. The algorithm does not guarantee tracking resolution. As a matter of fact, in this work we use the Crocker-Grier algorithm in particle tracking experiments, by which the particle's position can be localized with *subpixel* accuracy through taking the average position of not only the brightest pixel but also its neighboring pixels, weighted by brightness [6]. As schemed in Fig. R5, using a 2D Gaussian fitting, it is not difficult to reduce the standard deviation of the position measurement to better than 1/10 pixel even with moderate signal noise [6]. Thus, by this algorithm, the binning of displacements can be smaller than the resolution, and the oscillation and randomness of the particle can be statistically resolved.

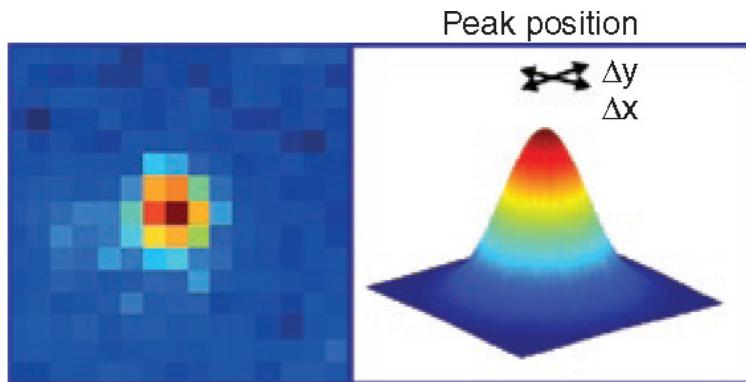


Fig. R5. When a single particle is present in the microscopy, the x-, y-coordinates of the particle at a given time point are derived from the central position of its diffraction limited intensity profile by, for example, fitting it to a 2D-Gaussian function (right). Hence, a positional accuracy far below the optical resolution is obtainable. Adapted from Ref. [7].

3. I strongly disagree with the authors' argument that rotational dynamics do not play a role in the transport of nanoparticles through polymer networks, at least not for the experiments. In the response letter, the authors present theoretical arguments that state the rotational time scale can be estimated by $t=t_r0 \exp(-(\Delta E_{\text{rot}})/kT)$ where they estimate $\Delta E=7.1$ kT for a 1.5 a_x long rod with a diameter of 1 a_x and $t_r0=1/D_r=(8\pi\eta r_h^3)/kT$ with r_h being the hydrodynamic radius of the particle. Assuming a particle with $r_h=100$ nm (a dramatic overestimate of the rods used in this work) and a solvent viscosity of 0.89 mPa s for water,

the rotational diffusivity would 200 s^{-1} with a rotational time scale of 0.005s . Even accounting for the energetic barrier presented by the polymer, $t \approx 7\text{s}$. This means that the particles should be rotating every 7 seconds in these experiments (or faster for much shorter particles) and that rotational dynamics must be accounted for. If the simulations do not replicate this rotational motion, then they do not accurately reflect the physics of the experiments.

Reply: The expert comment is highly appreciated. Clarifying the role of rotational dynamics in the transport of nanoparticles through polymer networks does be important. However, we note that there is a great difference in the viscosity used in the calculation here and our previous response, that is, $0.89 \text{ mPa}\cdot\text{s}$ vs 1.1cP ($1.1\text{Pa}\cdot\text{s}$), which should have remarkable effect on the calculation results of the rotational time scale. After considering this case very carefully, we find that both of these values are inappropriate, because (1) $0.89 \text{ mPa}\cdot\text{s}$ is the viscosity of pure solvent (water), instead of the solution with polymers, which should possess a higher viscosity; (2) the value of $1.1\text{Pa}\cdot\text{s}$ was taken from Ref. [8] where the system is porcine gastric mucin, instead of PEGDA used in this work. As this value is very essential, in this revision we measure the viscosity of our experimental systems, i.e., nanorod in the polymer solution, by performing additional particle tracking experiments of a nanorod in 4% PEGDA 20000 solution. In particular, a typical nanorod with diameter $d = 20\text{nm}$ and length $L = 40\text{nm}$ is used in the measurement.

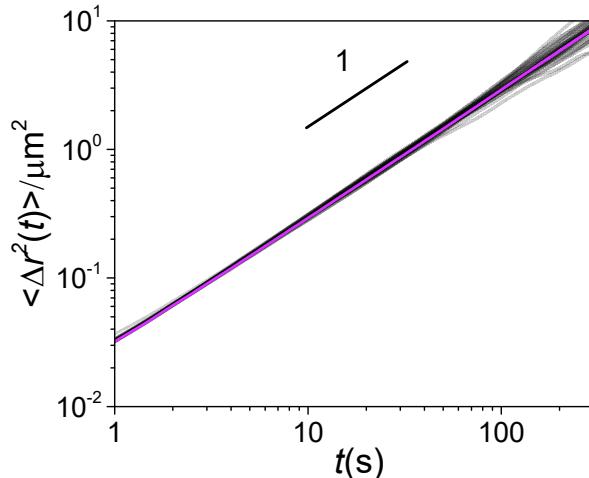


Fig. R6. Time-averaged $\langle \Delta r^2(t) \rangle$ (gray lines), ensemble-averaged $\langle \Delta r^2(t) \rangle$ for 10800s (magenta line) plotted against time on the log–log scale, where $d/a_x = 1.0$ and $L/a_x = 2.0$.

As shown in Fig. R6, the MSD of nanorod in the polymer solution exhibits the normal diffusion, with diffusion exponent 1 as expected at all time scale. Using a linear regression in

log space, we can easily fit this ensemble MSD to a power law, $\langle \Delta r^2 \rangle = At$, where $A = 0.0295\mu\text{m}^2/\text{s}$. For 2-dimensional diffusion, the diffusion coefficient of the nanorod can be calculated as

$$D = \frac{A}{4} = 0.0737\mu\text{m}^2$$

The hydrodynamic radius of the nanorod can be estimated as $r_h = (3Lr^2/4)^{1/3} = 22.9\text{nm}$ [9]. Using Stokes-Einstein relation, $D = k_B T / (6\pi\eta r_h)$, one can easily calculate the real viscosity of the nanorod, $\eta = 1.32\text{Pa}\cdot\text{s}$.

Then the rotational time scale, $t_{r0} = 1/D_r = (8\pi\eta r_h^3)/k_B T = 0.0997\text{s}$. As for the rod with $d/a_x = 1.0$ and $L/a_x = 2.0$, the energy barrier it experiences when rotating reaches $10.3 k_B T$. Accounting for the energetic barrier presented by the confinement of the polymer network, the waiting time for a rotating event, t_{hop} , can be calculated as

$$t_{hop} = t_{r0} \exp\left(\frac{\Delta E_{rot}}{k_B T}\right) = 2.96 \times 10^3 \text{s}$$

which is much larger than the observation time in experiment (600s). Actually, almost all the rod lengths used in our experiments are equal or even larger than $L = 40\text{nm}$, indicating that the rotational dynamics does play a trivial role in the transport of nanorods in these systems. The only one exception is the rod with $L=30\text{nm}$ ($L/a_x = 1.5$) and the calculation of t_r for this system is about 200s. Although this value is a little smaller, the rotational event take place in a low probability if considering the observation time of 600s, which, we believe, has a little effect on the longitudinal motion of this rod in the macromolecular network and cannot modify the physics of the whole experimental systems. We note this case in the revision, which can be found from the 16th to 20th line on Page 15 in Section VI in Supplemental Material.

In the revision, we have appended the above calculation and description, which can be found from the last line on Page 6 to the top 11th lines on Page 7 for the calculation of viscosity as well as the estimation of waiting time for a rotating event in Section VI in Supplemental Material.

Additionally, the authors should be readily able to identify the presence of rotational dynamics (or off axis dynamics) by looking at the persistence length of particle trajectories. If the particles move only along their major axis, then the trajectories should form straight lines across the sample rather than randomly diffusing in 2D (or 3D taking into account motion in and out of the focal plane).

Reply: We really appreciated such an expert suggestion. Fig.R7 presents the representative trajectories for rods with different lengths corresponding to the hopping and sliding dynamics. One can identify that the particles do move only along their major axis, and the trajectories form straight lines across the sample rather than randomly diffusing in 2D.

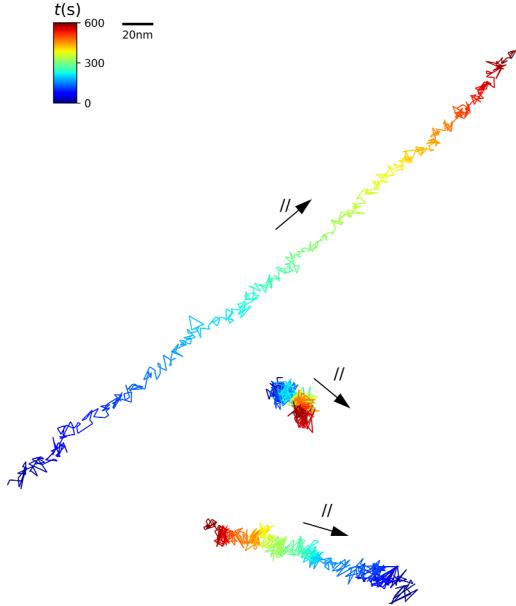


Fig. R7. Trajectories of rods with different L/α : 2.02, 2.63 and 2.97 from top to the bottom, corresponding to the sliding, hopping and sliding dynamics respectively. The color and scale bars on the left top indicate the values of the temporal and spatial scales of the trajectories.

4. The authors report experimental MSDs and displacement distributions along the z -axis (along the major axis of the nanoparticles). Because these nanoparticles are well below the optical diffraction limit, it is unclear how the authors resolve the major axis of the nanoparticle. Indeed, it is never described how the authors separate off axis/rotational motion from translational measurements. Resolving dynamics in different axial directions is highly non-trivial and must be discussed by authors.

Reply: Thanks for the comment. Indeed, the consideration of the dynamics in different axial directions is non-trivial. As discussed in our reply to Comment 3, the particles do move only along their major axis and the rotational dynamics play a trivial role in the transport of nanoparticles in most of experimental systems. To further verify that, we also evaluate the MSDs in different axial directions, that is, parallel and perpendicular to the major axis. As shown in Fig. R8, the MSD along the major axis is much faster than that along the direction perpendicular to the major axis. More importantly, the transverse displacements are found to

be highly subdiffusive, indicating strong transverse localization. The residual slight increase of MSD with time ($\text{MSD} \sim t^{0.2}$ empirically) likely reflects limited motions such as the thermal fluctuation of the polymer mesh; in contrast, the parallel displacements are close to linear in elapsed time ($\text{MSD} \sim t^1$ empirically). This corroborates the confined off-axis dynamics, consistent with the results of some previous works [10, 11].

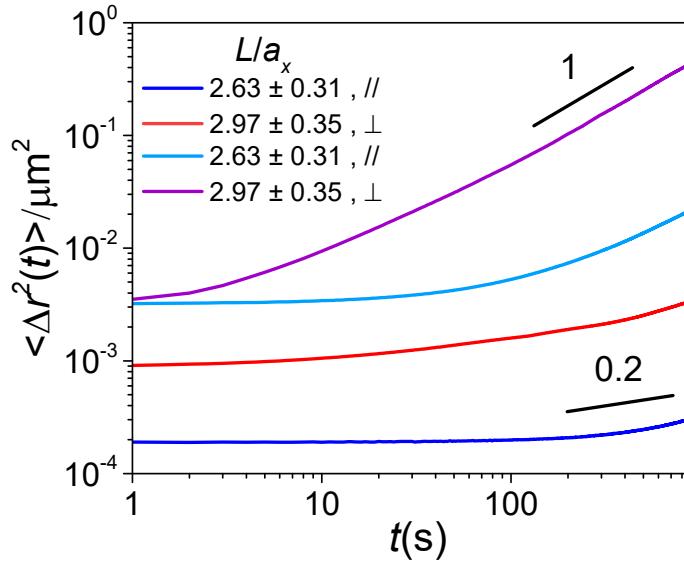


Fig. R8. MSDs in different axial directions for rods with different lengths. (\parallel) parallel to the major axis; (\perp) perpendicular to the major axis.

Responses to Referee C:

This work details the speed-up dynamics experienced by anisotropic thick rods where the length of the rod is some integer multiple of the mesh size, using a combination of theory, simulations, and experiments. Compared to the original submission from 2021, the results have been strengthened by the inclusion of new experiments to support the hypothesis, and removal of lengthy speculation around the relevance of these results for motile bacteria penetrating macromolecular networks.

I was pleased to observe that the authors followed up on a suggestion I had made previously and conducted controlled experiments using artificial nanorods to verify the theoretical predictions. This is a much more convincing approach than arbitrarily extracting parameters on the sizes of various mucus-dwelling motile bacteria from the literature.

The authors appear to have already responded thoroughly to the comments from the latest round of revisions, providing essential details about the experimental conditions and various measurement approaches. The measurements appear to agree with the simulations both in terms of msd curves, as well as the displacement probability distributions. Thus, I'm now broadly supportive of publication on the following conditions.

Reply: Thank you very much for the expert comments and kind suggestions!

1. Given the nature of the experimental data and simulations and their importance in deriving the conclusions from the paper, and for the sake of reproducibility and transparency I highly recommend making as much of the raw data (e.g. particle trajectories) and simulation codes available as electronic Supplementary Material. Specific example movies of the hopping vs sliding regimes could also be supplied for ease of reference.

Reply: Thanks for the comment. The necessary data outside of the main text, including the trajectories, DPDFs et al. have been included in Supplemental Materials. Other relevant data have also been deposited into a public data repository, which can be found in https://github.com/dxbdx/a/dateset_of_accelerated_transport.

2. Some clarification/careful motivation of the choice of experimental parameters (e.g. $d/a_x = 1$) compared to the simulation results (which start at $d/a_x = 1.2$) are necessary. Indeed the results of figure 4 would suggest that at d/a_x close to unity, the free energy U_b should also be less than $k_B T$? So why not just perform experiments with nanorods of thicker diameter in the first place?

Reply: The comment is appreciated. Actually, in the simulations and theoretical analysis, the thicker rods (which start at $d/a_x = 1.2$) are considered in order to limit their off-axis dynamics and demonstrate the nature of the rod-length dependent entropic free energy barrier. However, the free energy barrier is almost impossible to be determined for the experimental systems. Thus, considering the possible contribution of the weak interaction between the macromolecular network and the rod, a slightly smaller diameter of rods is adopted in the experiments, which facilitates the comparison between experimental and theoretical results because they have almost equal level of free energy barrier. Indeed, one can find from, for example, Fig.1(c) that such a process does result in a good agreement between experimental and theoretical results.

3. Finally, as the ‘ultimate’ proof - ideally one should be able to observe/record the differently sized nanorods undergoing the distinct regimes of behavior at the same time as the mesh structure (I suspect PEGDA will be autofluorescent). This may or may not be possible given the optical resolution of their imaging system, but perhaps the authors would like to try?

Reply: Thanks for the comment. It is interesting to observe/record the differently sized nanorods undergoing the distinct regimes of behavior at the same time. However, considering the dark field techniques used for the analysis of the trajectories, it is extremely challengeable to realize because it can't be able to determine the shape of each rod in a system due to the extremely small length scale. Thus, only one type of nanorods, whose size are first determined and the size distribution is very narrow, are used in an experimental system, so that the physics of the dynamics can be determined based on such a neat system.

Minor points

1. r_c was undefined? (For all systems, the network mesh size is fixed at $a_x = 3.35r_c$)

Reply: Thanks for the comment. r_c is the length unit in the coarse-grained simulations. In the revision, it has been defined, which can be found in the 26th line of the right column on Page 2 in the main text (marked in red).

2. in the Supplementary Materials - spurious comment on 'that our model captures the entropic nature arisen by the larger diameters of bacterium' (no bacteria in current version)

Reply: Thanks for your careful revision. We are sorry for this typo error. This sentence has been deleted in the revision of Supplementary Materials.

3. typo page 2: 'to detect the particiles' presumably 'particles'

Reply: Thanks for the comment. “particiles” has been revised into “particles” in the revision, which can be found in the 22nd line of the left column on Page 2 in the main text (marked in red).

With these statements, we sincerely hope that the paper is now suitable for publication.

Thanks!

Li-Tang Yan

Reference

- [1] B. S. Schuster, L. M. Ensign, D. B. Allan, J. S. Suk, and J. Hanes, *Adv. Drug Deliv. Rev.* **91**, 70 (2015).
- [2] K. M. Spillane, J. Ortega-Arroyo, G. De Wit, C. Eggeling, H. Ewers, M. I. Wallace, and P. Kukura, *Nano Lett.* **14**, 5390 (2014).
- [3] M. Lindén, V. Ćurić, E. Amselem, et al. *Nat. Commun.* **8**, 15115 (2017).
- [4] M. Gardel, M. Valentine, and D. Weitz, *Microrheology*, in *Microscale Diagnostic Techniques*, edited by K. S. Breuer (Springer Berlin Heidelberg, Berlin, Heidelberg, 2005)
- [5] I. Y. Wong, et.al., *Phys. Rev. Lett.* **92**, 178101 (2004).
- [6] J. C. Crocker, and D. G. Grier, *J. Colloid Interface Sci.* **179**, 298 (1996).
- [7] N. Ruthardt, D. C. Lamb, and C. Bräuchle. *Mol. Ther.* **19**, 1199 (2011).
- [8] J. Celli, B. Gregor, B. Turner, N. H. Afdhal, R. Bansil, and S. Erramilli, *Biomacromolecules* **6**, 1329 (2005).
- [9] M. Yu, J. Wang, Y. Yang, C. Zhu, Q. Su, S. Guo, J. Sun, Y. Gan, X. Shi, and H. Gao, *Nano Lett.* **16**, 7176 (2016).
- [10] B. Tsang, Z. E. Dell, L. Jiang, K. S. Schweizer, and S. Granick. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 3322 (2017).
- [11] N. Fakhri, A. D. Wessel, C. Willms, M. Pasquali, D. R. Klopfenstein, F. C. MacKintosh, and C. F. Schmidt. *Science* **344**, 1031 (2014).