

Supplementary Information: Self-transport of swimming bacteria is impaired by porous microstructure

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1 Supplementary Methods

1.1 Calculation of effective mean free path

The effective mean free path, l_f , was shown to be the relevant length scale for accurately predicting the self-transport properties of swimming bacteria in the microfluidic model porous media examined here (see main text; Fig. 4). For both ordered and disordered lattices (approximately 201×201 pillars) having lattice spacing, c , and unit pillar diameter, d , the effective mean free path was calculated by randomly generating a total of 250,000 theoretical straight paths. For the ordered medium, paths were chosen with a random starting location on the surface of a centrally located pillar ($-\pi \leq \theta \leq \pi$). For the disordered medium, 250 different pillars from the central portion of the medium were used to seed 1,000 trajectories (each) with uniformly distributed starting positions on the pillar surface (accounting for cases with overlapping pillars). Two different probability distributions were chosen for generating the random initial angle relative to the local surface normal direction ($-\pi/2 \leq \beta \leq \pi/2$; Fig. 4c-d): (i) a uniform distribution with probability, $p(\beta) = 1/\pi$, and (ii) a distribution corresponding to a reflective boundary condition, $p(\beta) = \cos(\beta)/2$.

For each path, the distance, l_i , to the nearest intersection with an adjacent pillar surface was identified, and the effective mean free path was taken as the mean over all trajectories, $l_f = \langle l_i \rangle$. This process was repeated for 60 different lattices having a normalized lattice constant in the range $1.05 \leq c/d \leq 4$, which are commensurate with the model microfluidic porous media geometries examined here (Table 1; Fig. 4e, vertical black dotted lines). The effective mean free path normalized by the local pore size, l_f/d_p , reveals that swimming cells with relatively large persistence lengths can often travel several pores prior to interacting with a pillar (Fig. 4e), especially in media with relatively small pillar diameters and/or large porosities (i.e. large c/d).

1.2 Asymptotically equivalent mean squared displacement

Fits of the measured mean squared displacements (MSDs) are used to extract bacterial transport parameters including the persistence time, τ_p , and effective diffusion coefficient, D , in a range of porous media geometries (see main text, Fig. 3). We make use of a practical approximation of the MSD for a persistent random walk,¹ which has been shown to reliably extract the MSD cross-over time for single swimming cells.² For convenience, we first outline the traditional analytical results for $MSD(t)$ of a random walking cell^{3,4} with swimming velocity $\mathbf{v}(t) = V_s \mathbf{p}(t)$, where V_s is a constant swimming speed and $\mathbf{p}$ is a randomly evolving swimming direction with persistence time, τ_p . We then establish the approximate form,^{1,2} $MSD_A(t)$, to appropriately recover the persistence time from the measured MSD. In terms of the cell swimming velocity

correlation function, the MSD⁴ is written as

$$MSD(t'' - t') = \langle |\mathbf{r}(t'') - \mathbf{r}(t')|^2 \rangle = \int_{t'}^{t''} \int_{t'}^{t''} \langle \mathbf{v}(t_1) \cdot \mathbf{v}(t_2) \rangle dt_1 dt_2,$$

which is obtained assuming ergodicity. The velocity (and orientation) correlation function for random walk swimmers often decay exponentially³

$$C(t_1, t_2) = \langle \mathbf{v}(t_1) \cdot \mathbf{v}(t_2) \rangle = V_s^2 e^{-|t_2 - t_1|/\tau_p},$$

where the decay rate is $1/\tau_p$. We directly show that the orientation correlation functions for the swimming bacteria examined here also follow an exponential decay across all microfluidic porous media geometries examined (Fig. 2a). Inserting this exponential form into the MSD expression above yields^{3,4}

$$MSD(t) = V_s^2 \iint_{t'}^{t''} C(t_1, t_2) dt_1 dt_2 = 2V_s^2 \tau_p t \left(1 - \frac{\tau_p}{t} + \frac{\tau_p}{t} e^{-t/\tau_p} \right),$$

where $t = |t'' - t'|$.

An approximate form of the MSD has been used to accurately fit the MSD cross-over time, $MSD_A(t) = \lambda_1 t (1 - e^{-\lambda_2 t})$, which scales as $\sim t^2$ and $\sim t$ for short and long times, respectively^{1,2}. Below, we determine λ_1 and λ_2 such that MSD_A asymptotically matches MSD for both $t \rightarrow \infty$ and $t \rightarrow 0$. For long times in the diffusive regime, we find

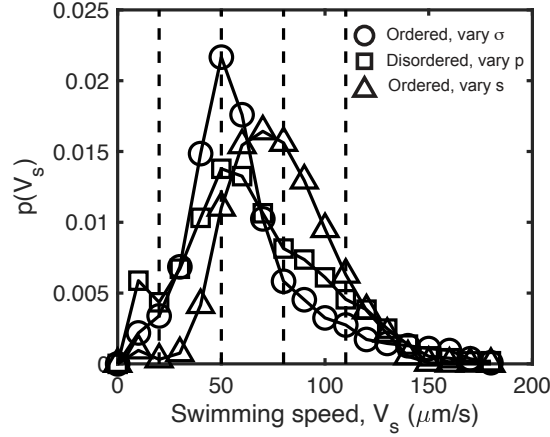
$$\frac{MSD_A(t)}{MSD(t)} = \frac{\lambda_1 t (1 - e^{-\lambda_2 t})}{2V_s^2 \tau_p t \left(1 - \frac{\tau_p}{t} + \frac{\tau_p}{t} e^{-t/\tau_p} \right)} \xrightarrow{t \rightarrow \infty} \frac{\lambda_1}{2V_s^2 \tau_p},$$

and thus, $\lambda_1 = 2V_s^2 \tau_p$. For short times in the ballistic regime and expanding in t , we have

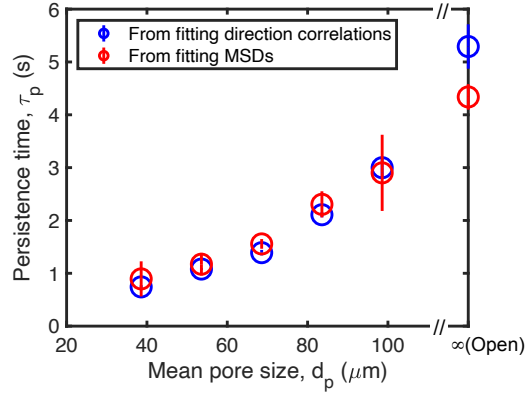
$$\begin{aligned} \frac{MSD_A(t)}{MSD(t)} &= \frac{2V_s^2 \tau_p t (1 - e^{-\lambda_2 t})}{2V_s^2 \tau_p t \left(1 - \frac{\tau_p}{t} + \frac{\tau_p}{t} e^{-t/\tau_p} \right)} \\ &= \frac{\lambda_2 t + O(t)}{1 + \frac{\tau_p}{t} \left(-1 + 1 - \frac{t}{\tau_p} + \frac{1}{2} \frac{t^2}{\tau_p^2} \right) + O(t)} \\ &= \frac{\lambda_2 t + O(t)}{\frac{t}{2\tau_p} + O(t)} \xrightarrow{t \rightarrow 0} \lambda_2 2\tau_p, \end{aligned}$$

and so, $\lambda_2 = 1/2\tau_p$. Finally, we obtain an approximate expression of the MSD, $MSD_A(t) = 2V_s^2 \tau_p t (1 - e^{-t/2\tau_p})$, or in terms of the effective diffusion coefficient, $MSD_A(t) = 4Dt (1 - e^{-t/2\tau_p})$. This expression is used to fit MSDs presented in the main text (Fig. 3; see also Methods) for the purpose of extracting the persistence time, τ_p , and effective diffusion coefficient, D . We verified the accuracy of this approach using our experimental data by comparing the persistence time measured directly from the swimming orientation correlation function (Fig. 2a) with the persistence time extracted from the MSD using the above approximation (Fig. 3), which show excellent agreement with one another (Fig. S2).

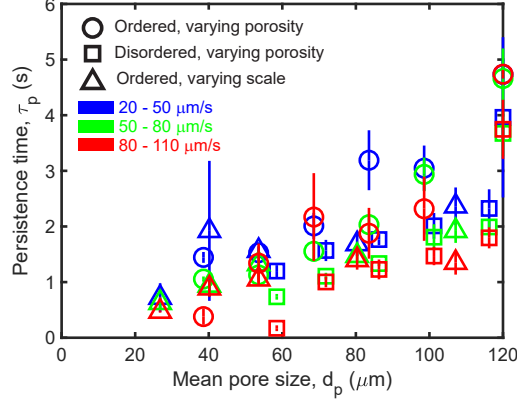
2 Supplementary Figures



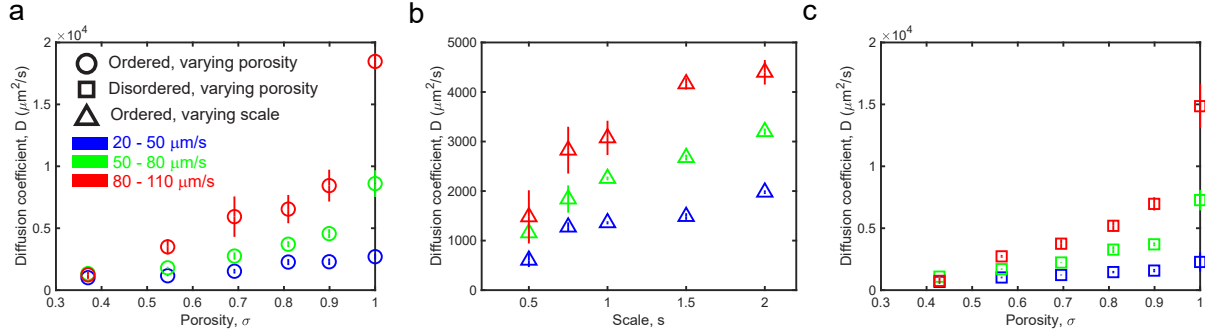
Supplementary Figure 1: Cells exhibit a consistent, broad range of swimming speeds. Probability distribution of individual bacteria swimming speeds (*Magnetococcus marinus*, MC-1), where V_s is averaged over individual cell trajectories. Data sets were acquired in the open sections of each porous media microchannel and correspond to: ordered with varying porosity (circles), disordered with varying porosity (squares), and ordered with varying scale (triangles). These three independent control experiments illustrate the consistency of the cell swimming speed distributions across experiments. Vertical dashed lines indicate cell swimming speed ranges for sub-population transport analyses in the main text: 20-50 $\mu\text{m/s}$, 50-80 $\mu\text{m/s}$, and 80-110 $\mu\text{m/s}$.



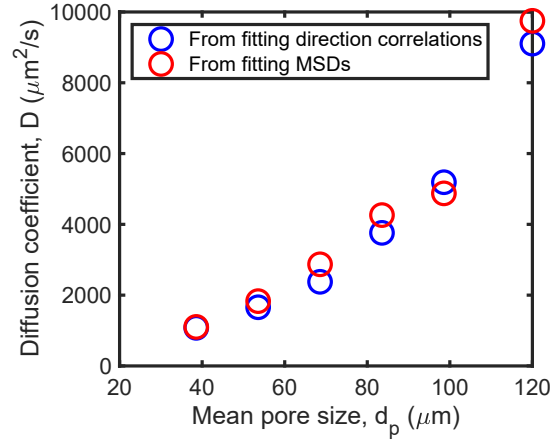
Supplementary Figure 2: Persistence swimming time of cells decreases with decreasing mean pore size. Sample data illustrating the reduction of persistence time, τ_p , with decreasing mean pore size, d_p . Persistence times are computed using two different methods (see Methods): (i) exponential fitting of the swimming direction correlation function (Fig. 2a), and (ii) fitting the mean squared displacements (MSDs) with an analytical model for a persistent random walk (Fig. 3). The strong agreement between the two methods confirms the robustness of our analyses. Error bars represent the standard error of the persistence time computed by dividing the experimental cell trajectories random subsets ($N = 3$).



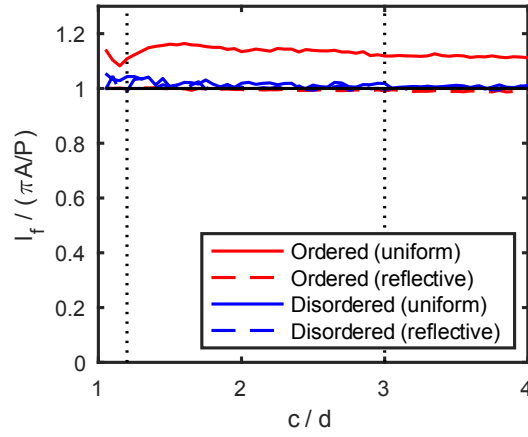
Supplementary Figure 3: Persistence swimming time of the cells decreases with mean pore size for all geometries and cell swimming speed ranges. The persistence times presented here are extracted by fitting the MSDs with an analytical model (Fig. 3; see also Methods). Error bars represent the standard error of the persistence time computed by dividing the experimental cell trajectories into random subsets ($N = 3$).



Supplementary Figure 4: Effective cell diffusion coefficient decreases with decreasing porosity and scale. Effective cell diffusion coefficients measured from mean squared displacement (MSD) fits (Fig. 3) for swimming bacteria in (a) ordered lattice with varying porosity, (b) ordered lattice with varying scale, and (c) disordered lattice with varying porosity. In (a-c), all three swimming speed ranges are shown corresponding to blue, green, and red markers (see also Supplementary Fig. 1). Error bars represent the standard error computed by dividing the experimental cell trajectories into random subsets ($N = 3$).



Supplementary Figure 5: Effective cell diffusion coefficient decreases with decreasing mean pore size. Sample data illustrating the reduction of diffusion coefficient, D , with decreasing mean pore size, d_p . Diffusion coefficients are computed using two different methods (see Methods): (i) exponential fitting of the swimming direction correlation function (Fig. 2a) to obtain persistence time, τ_p , and $D = V_s^2 \tau_p / 2$, and (ii) fitting the mean squared displacements (MSDs) with an analytical model for a persistent random walk (Fig. 3). The strong agreement between the two methods confirms the robustness of our analyses (see also Supplementary Fig. 2).



Supplementary Figure 6: Comparison of computed effective mean free path to the Santalo equation. The computed effective mean free path, l_f , is shown normalized by the Santalo equation, $\pi A/P$, which is a function of the measured wetted area, A , and perimeter, P , of the porous media. The normalized effective mean free path is in very good agreement with the theoretical Santalo equation, despite the latter being an exact relation on for periodic media with reflective boundary conditions. This empirical relationship holds across a wide range of lattice size to pillar diameter ratios, c/d , measured in experiments, and for different disorders and scattering distributions.

Supplementary References

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- ⁴ Bechinger, C., Leonardo, R. D., Reichhardt, C. & Volpe, G. Active Particles in Complex and Crowded Environments. *Rev. Mod. Phys.* **88**, 045006 (2016).