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Supplemental Information

Interplay of motility and polymer-driven depletion forces in the initial stages of bacterial aggregation

Michael K. Porter,[a] Asher Preska Steinberg[a] and Rustem F. Ismagilov[a,b*]

[a] Division of Chemistry & Chemical Engineering
[b] Division of Biology & Biological Engineering
California Institute of Technology, 1200 E. California Blvd. Pasadena, CA 91125
* rustem.admin@caltech.edu

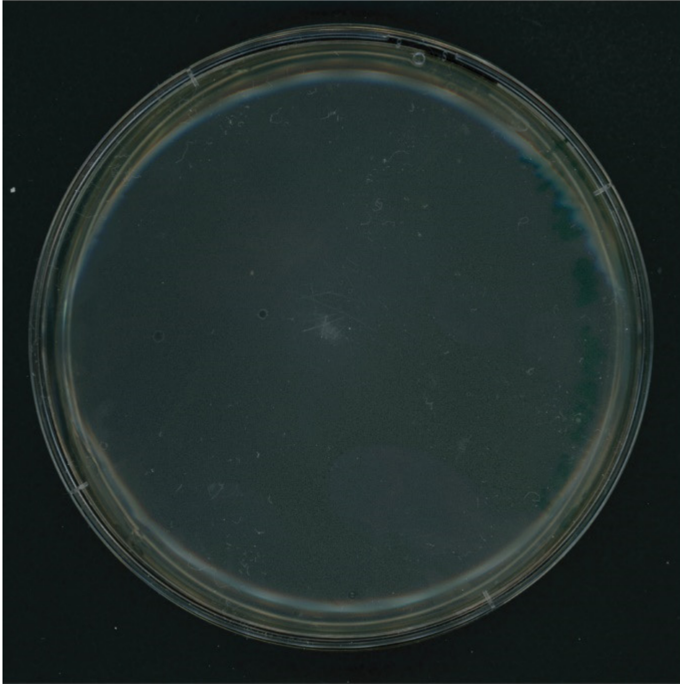
Supplemental Experimental section

Figures S1-S2

Contributions of non-corresponding authors

Supplemental Experimental section

Viability of *E. coli* K12 after treatment with sodium azide was tested by plating cells onto lysogeny broth (LB) with 1.5% agar. Cells were prepared as described in the Experimental section, and were left to incubate at room temperature for 1 h before plating. Duplicate agar plates were incubated at 35 °C overnight and then colonies were counted. None of the cells in the azide-treated group survived, whereas the cells washed in motility buffer maintained viability.



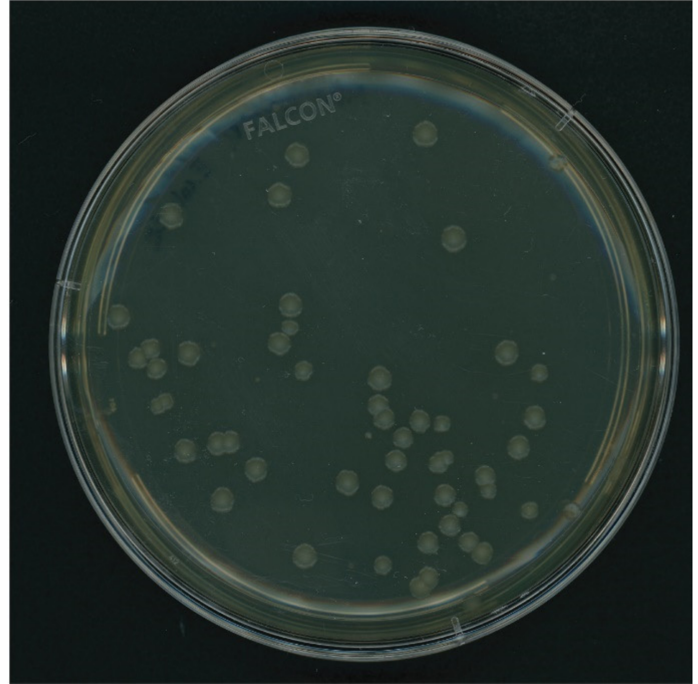


Fig S1. Confirming the deactivation of *Escherichia coli* via plating. *Escherichia coli* washed (a) with sodium azide in motility buffer and (b) motility buffer (no sodium azide) which were then plated on LB and 1.5% agar.

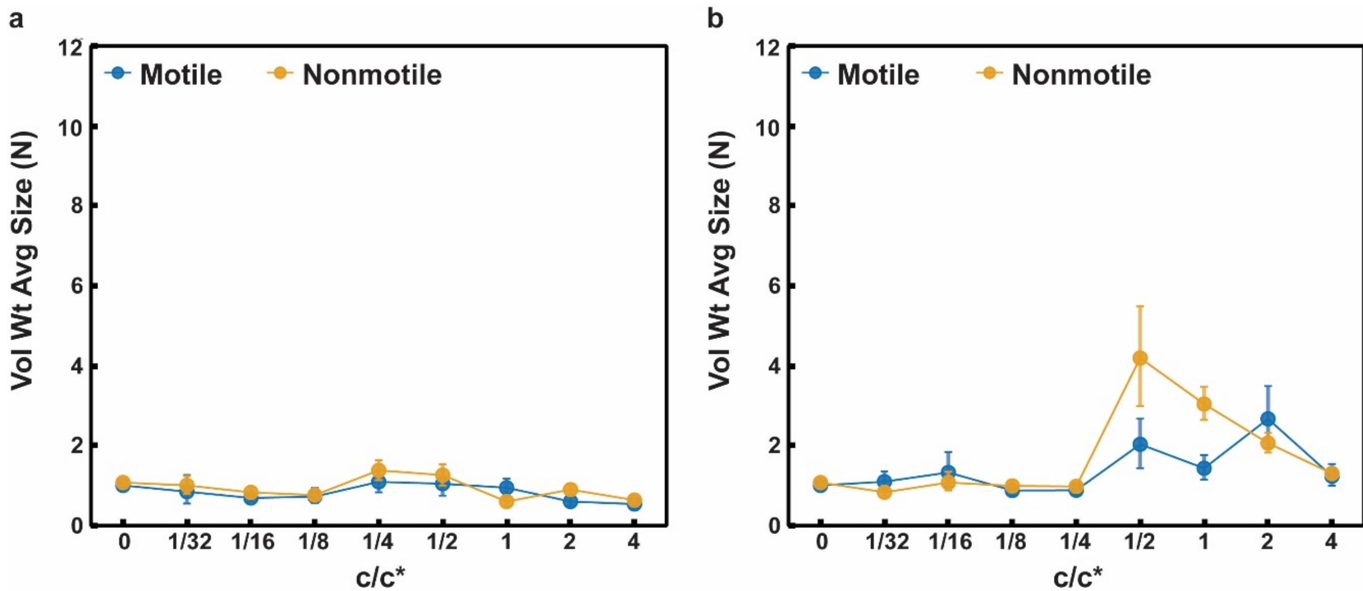


Fig S2. A comparison of the aggregation of motile and nonmotile *E. coli* K12 in a range of concentrations of 10 kDa and 100 kDa PEG. Volume-weighted average aggregate sizes (Vol Wt Avg Size) of nonmotile and motile *E. coli* K12 for serial dilutions of (a) 10 kDa PEG and (b) 100 kDa PEG. Aggregate sizes were measured 10 min after cells were mixed with PEG using a cell concentration of $10^9\ cells/mL$. Volume-weighted average sizes in terms of bacteria per aggregate (N) are plotted against polymer mass concentration (c) normalized by overlap concentration (c^*). Overlap concentration was estimated to be 85 mg/mL for 10 kDa PEG and 8.5 mg/mL for 100 kDa PEG. Vertical error bars are 95% empirical bootstrap confidence intervals using the bootstrapping protocol described in the "Imaging Analysis" section of the Methods in Ref.[1] Data for the 10 kDa PEG concentration were compiled from one biological replicate and data for the 100 kDa PEG concentration were compiled from two biological replicates (where each replicate is a separate bacterial culture). For each concentration of PEG, each replicate was obtained from one z-stack that was comprised of at least 120 slices.

Aggregation of motile and nonmotile *E. coli* was measured in a serial dilution of 10 kDa and 100 kDa PEG around their respective overlap concentrations. In 10 kDa PEG, neither motile nor nonmotile bacteria aggregated at the concentrations tested (c/c* = 1/32 to 4). A similar observation was made using PEG-coated particles instead of bacteria in a similar MW PEG solution in our previous publication (Preska Steinberg *et al*. 2019).

In the 100 kDa PEG solutions, nonmotile *E. coli* aggregated in a manner qualitatively consistent with polymer-driven depletion aggregation, as reported with PEG-coated particles tested with the same MW PEG in the same concentration range (c/c* = 1/32 to 4). The nonmotile bacteria aggregation measured in the 100 kDa PEG is similar but smaller in magnitude compared to the aggregation measured in the 1 MDa PEG, indicative of the smaller depletion potential exerted from the smaller MW PEG. Minimal aggregation was measured for the motile *E. coli* in 100 kDa PEG, suggesting that at this MW and concentration range, the swim force is strong enough to overcome the depletion potential.

Contributions of non-corresponding authors

M.P.

1. Designed experiments, interpreted results, and analyzed data
2. Optimized aggregation measuring experiment for bacteria
3. Performed experiments to generate all data for figures 2, 3, 4, S1, and S2
4. Drew schematic in figure 1 and obtained images for figure 1
5. Performed numerical calculations for figure 3 using code adapted from A.P.S.
6. Contributed to writing abstract, introduction, results/discussion, and conclusion sections of the manuscript
7. Contributed to writing of supplemental information

A.P.S.

1. Contributed to experimental design, interpretation of results, and analysis of data.
2. Contributed to adaptation of image analysis pipeline for use in this system.
3. Co-developed numerical methods and contributed to adaptation of theory for use in figure 3.
4. Contributed to writing abstract, introduction, results/discussion, and conclusion sections of the manuscript.
5. Contributed to writing of supplemental information.