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Supporting Information for:

Depletion Attractions Drive Bacterial Capture on Both Non-Fouling and Adhesive Surfaces, Enhancing Cell Orientation

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1. PEO Induces Depletion Aggregation of Log-Phase E. coli in Bulk Solution

Prior studies established the bulk solution depletion aggregation of stationary-phase *E. coli* cells that lack flagella,¹ the same strain in the current work. In the current work, the same types of studies were conducted with log-phase *E. coli* of the same strain to establish reversible depletion aggregation for log-phase cells in suspension. The current study also established that depletion-aggregated log-phase cells dispersed upon dilution. The reversibility of aggregation upon dilution confirms the depletion mechanism and distinguishes it from bridging or patch-wise aggregation that is not reversible upon dilution.

In the experiment of Figure S1A, log phase *E. coli* cells, at a concentration of $4x10^8$ cells/ml in a 0.5 wt% PEO solution (85,200 g/mol PEO in PBS), are initially aggregated and resuspend within seconds of 10-fold dilution into the same PBS. The phosphate buffered saline, PBS, is the same as that employed in the main paper. Of note, the PEO concentration, if represented as a volume fraction swept out each hydrated polymer coil, ϕ_p , would be $\phi_p = 0.45$. Also, if the cells were represented as a volume fraction, ϕ_{cell} , then based on their dimensions of 3.0 µm by 0.96 µm and treating cells as spherocylinders having a volume of 1.94 µm³, 4x10⁸ cells/ml is equivalent to $\phi_{cell} = 7.8 \times 10^{-4}$.

In the control study of Figure S1B log phase *E. coli* cells at a concentration of $4x10^8$ cells/ml initially aggregated in a 100 ppm solution of poly-l-lysine (PLL, having a nominal molecular weight of 15,000-30,000 g/mol from Sigma) in PBS. In contrast to the resuspension seen in Figure S1A, in Figure S1B cells remain aggregated upon 10-fold dilution in PBS. Here

aggregation remains because PLL is a polycation that adheres to cells and causes bridging aggregation or patch wise aggregation that can persist at low polycation concentrations. In the additional control of Figure S1C it is seen that log phase *E. coli* cells at a concentration of $4x10^8$ cells/ml do not aggregate in PBS, and they do not aggregate upon 10-fold dilution in the same PBS.



Figure S1. A) Log phase *E. coli* cells at a concentration of $4x10^8$ cells /ml aggregate in the presence of 0.5 wt% 85,200 g/mol PEO in PBS but resuspend upon 10-fold dilution in PBS. B) Log phase *E. coli* cells at a concentration of $4x10^8$ cells /ml aggregate in the presence of 100 ppm PLL in PBS but <u>do not</u> resuspend upon 10-fold dilution in PBS. C) Log phase *E. coli* cells at a concentration of $4x10^8$ cells /ml aggregate in the same PBS.

2. PEO Induces Depletion Aggregation of Log-Phase E. coli in the Flowing Suspension.

PEO causes depletion aggregation of log-phase *E. coli* cells in the flowing suspension and reservoir, a process that is visible within \sim 5 minutes of PEO introduction. Cells not yet aggregated on this short time scale adhere to the chamber surface by depletion aggregation, while aggregates tend to flow past, in Figure S2.

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Figure S2. Three video frames, taken 1 second apart, showing the travel of a depletion-induced aggregate (circled red) past a PLL-PEG coated surface containing singlets that have adhered via depletion forces (circled blue).

References

1. W. A. Niu, S. L. Rivera, M. S. Siegrist and M. M. Santore, Depletion Forces Drive Reversible Capture of Live Bacteria on Non-Adhesive Surfaces, *Soft Matter*, 2021, **17**, 8185-8194.