Bacterial Fluctuations in double emulsions

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Project description. Original non-equilibrium phenomena arise in active matter in the presence of confinement. In this project, we aim to investigate the swimming of *E. coli* bacteria confined by double emulsions, which have gained significant popularity in food, cosmetic, therapeutic and many other industries. Our results will provide key experimental evidence to understand confinement effect of active matter.

Assessments: methodology, key implementations and results. **We first demonstrate a well controlled experimental system: double emulsions generated by a capillary microfluidic device.** By varying the flow rates of each phase, we are able to produce double emulsions with continuously controllable sizes, allowing us to explore the parameter space with fine resolution. The device, as well as the double emulsions produced, are shown in Figure 1. **We also developed robust characterization techniques: droplet tracking and droplet particle image velocimetry (DPIV).** Droplet tracking combines a generic circle finding method and a specific template matching method to ensure the automated data processing. DPIV is a modified version of PIV to address the droplet motion driven by bacteria inside the droplet, using a "moving mask". The ideas and results of these techniques are illustrated in Figure 2. **In collaboration with theorists, we analyzed our data with the guidance of a theoretical model under the framework of "collisional noise".** The model highlights two characteristic time scales of the inner droplet motion in our experimental system. The first one is the ballistic-diffusive transition (t_1) and the second one is the confinement-induced "saturation" (t_2). We measured these time scales for various geometries and bacterial concentrations, as shown in Figure 3. The t_1 data show sharp contrast to the bulk measurements (Wu and Libchaber 2000), suggesting that the confinement is taking effect. The t2 data show a tendency to follow our prediction based on a simple "spring" argument (red line in Fig. 3B). However, the significant scattering of the data again indicates confinement effects that are not correctly accounted for. Further investigations are required to better understand these data.

Plan and outlook. (I) **Double emulsion is certainly an interesting and important geometry to study active matter. However, our preliminary data suggest that there are intertwined factors in the system, which scatter the data.** For example, when we measure the ballistic-diffusive transition time t1, we already notice 3 factors that may take effect. In addition to the confinement from the outer droplet, which may force bacteria to change directions and thus reduce their persistence, there are also the "buoyant force" experienced by the inner droplet, as well as the additional "shell" confinement posed by the inner droplet. Rather than studying double emulsion directly, we can untwist the factors by studying smaller and neutrally buoyant particle and then approach more complicated systems. (II) Collective motion of *E. coli* bacteria adds another complication to understanding the experiment. We have observed vigorous collective motion in water droplets, even as tiny as several bacterial length in diameter. It is remarkable that bacteria manage to self-organize into collectively moving groups in such crowded and confined environments. By studying the flow and structural configurations in dense bacterial droplets, we can elucidate the dynamics behind the collective motion under confinement. Moreover, we can gain insight into the survival strategies of microorganism by characterizing these collective motions. (III) Dense bacterial droplets show bizarre phenomena, which keep challenging our understanding of active matter and confinement, and eventually help us understand better. For example, we have recently observed a "resurrect" phenomenon, where a "frozen" droplet is reactivated by an active droplet and goes back to collective motion state (illustrated in Figure 4). Further investigations into such phenomena may provide better understanding of the collective motions dynamics under confinement.