Electronic Supplementary Material (ESI) for Soft Matter.
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Supporting Information

Trapping and Assembly of Living Colloids at Water/Water Interfaces

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Experimental Setup. PDMS wells were created with ~500 μm deep molds. The solution was placed in the well and closed with a coverslip. The device was turned over to image on an inverted confocal laser scanning microscope, as depicted in Fig. S1.

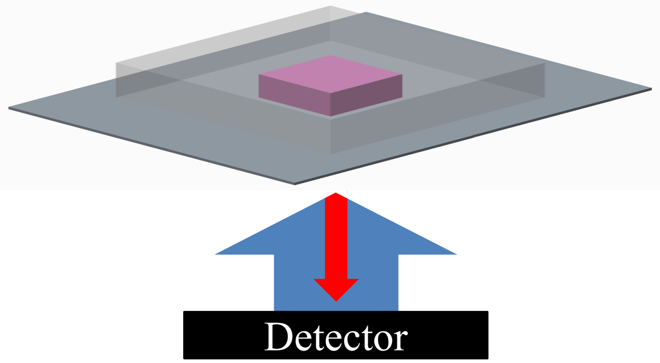

Fig. S1. Schematic of experimental setup. Casein-xanthan suspension was enclosed in a PDMS cell on top of a coverslip. Total sample thickness was 500 μm. Imaged on inverted confocal laser scanning microscope (CLSM).

Motility study. We monitored both WT and GFP *E. coli* strains during various periods throughout the phase separation at 20 μm above the coverslip for short periods of time. These movies (S1-S3) reiterate the free motion that the WT *E. coli* has immediately after mixing versus 24 hours after mixing. Once trapped at the interface, they do not swim. This lack of motility is either because they are trapped at the interface and cannot move or they have turned off their motility gene. No additional verification was done investigating the gene expression through time. The lack of motion of the GFP *E. coli* in Movie S3 is shown as verification that we have used a non-motile strain.

MovieS1.avi	Movie S1. WT *E. coli* within sample well are monitored 20 µm from cover slip immediately after mixing. Casein is stained red and the *E. coli* are stained green with SYTO 9. The bacteria can be observed swimming across the viewing window multiple times. Scale bar 50 µm.
MovieS2.avi	Movie S2. WT *E. coli* within sample well are monitored 20 µm from cover slip 24 h after mixing. Casein is stained red and the *E. coli* are stained green with SYTO 9. The bacteria are no longer observed swimming across the viewing window. Scale bar 50 µm.
MovieS3.avi	Movie S3. Non-motile GFP *E. coli* within sample well are monitored 20 µm from cover slip immediately after mixing. Casein is stained red and the *E. coli* are green. The bacteria are not moving, nor are they at the interface yet. Scale bar 50 µm.

CLSM scans of WT E.coli, GFP E. coli, and inert colloids. Representative Z-scans of the slices shown in Fig. 1 – 3 are given below in Movie S4-S8. In each movie, as in the main paper, red indicates the casein region, stained with Rhodamine B, green indicates live, WT *E. coli*. All scale bars are 50 µm.

MovieS4.avi	Movie S4. Z-scan from top to coverslip of live, WT *E. coli*, t = 0
MovieS5.avi	Movie S5. Z-scan from top to coverslip of live, WT *E. coli*, t = 16 h
MovieS6.avi	Movie S6. Z-scan from top to coverslip of live, WT *E. coli*, t = 24 h
MovieS7.avi	Movie S7. Z-scan from top to coverslip of live, WT *E. coli*, t = 40 h
MovieS8.avi	Movie S8. Z-scan from top to coverslip of live, WT *E. coli*, t = 7 d

CLSM scans of Pseudomonas strains. Two *Pseudomonas* strains were added to the system to see the behavior. *Pseudomonas aeruginosa* (PA01) studies were carried out exactly as the *E. coli* studies grown in a hydrated, light-protected environment at room temperature (Fig. S1). The images presented in Fig. S2 are mixtures of 2 wt% casein in PBS (no xanthan). Including xanthan drives the bacteria to the interface,

as shown in the main paper, and the PA01 decomposes the casein region within 24 hours. It is easier to see the breakdown of the casein region without the xanthan, as seen in Fig. S2B, in which the red fluoresced region appears patchy with bacteria around these regions. PA01 was stained green with SYTO 9 stain. PA01 is a positive casein clearer so it follows that the casein region gets cleared more rapidly with this bacterium compared to *E. coli*. As such, after only 40 h (Fig. S2C), the PA01 sample resembles the 7 d *E. coli* sample (Fig. 1E) with its lack of red fluorescence and apparent flattening. The species *P. sp. 62* is a marine bacteria that grows ideally around 27 °C and a representative figure is presented in Fig. S3. The bacteria attach at the casein-xanthan interface after 24 h but interestingly do not consume the matrix as rapidly as the PA01.

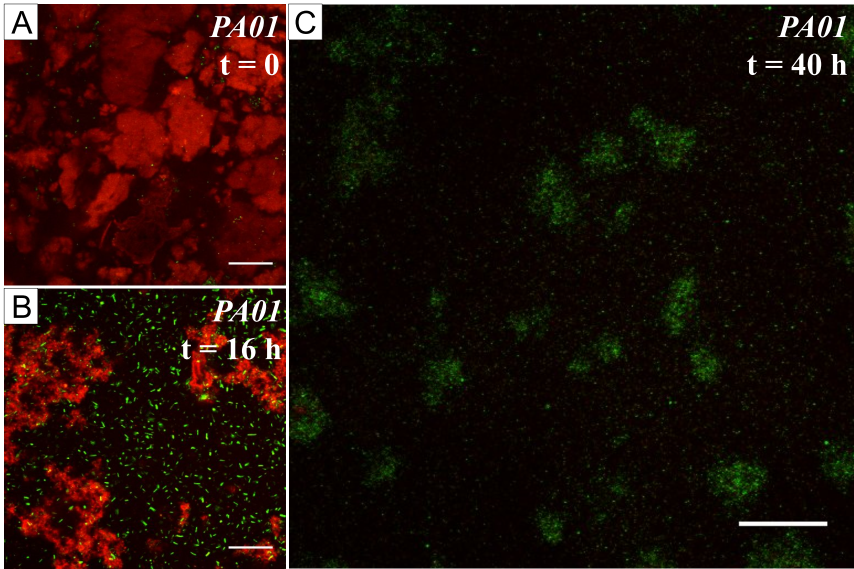

Fig. S2. Time progression of *P. aeruginosa* (PA01) imaged in CLSM 20 μm above coverslip. Casein is stained with Rhodamine B and PA01 is stained with SYTO 9. (A) Immediately after mixing (B) 16 hours after mixing (C) 40 hours after mixing. All scale bars are 50 μm.

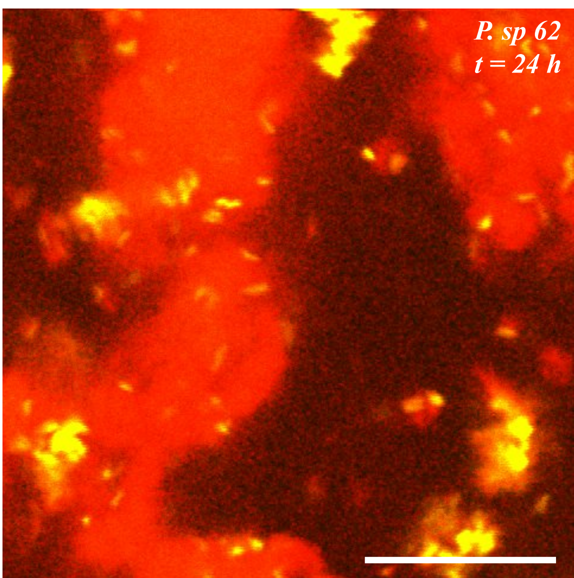

Fig. S3. Image of *Pseudomonas sp. 62* in 2 wt% casein, 0.08 wt% xanthan solution 20 μm above coverslip. Scale bar 20 μm.

Drop elution studies. Simple drop elution experiments were performed to qualitatively characterize the interfacial tension between the casein and xanthan phases as shown in the main text in Fig. 4A and 4B. Full drop movies shown in Movie S9 and S10. Non-Newtonian characteristics are especially evident in Fig. S4, in which the drop has a mostly uniform shape but of which part is torn.

MovieS9.avi	Movie S9. 10 wt% casein solution eluted into 0.1 wt% xanthan solution. Corresponds to stills from Fig. 4A.
MovieS10.avi	Movie S10. 10 wt% casein solution eluted into DI water. Corresponds to stills from Fig. 4B.

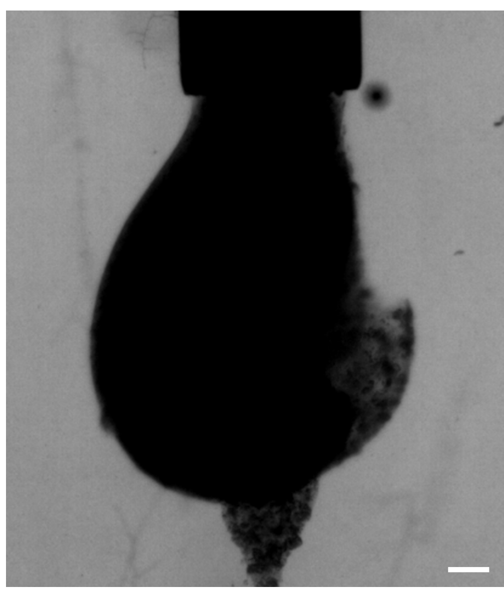

Fig. S4. Frame from pendant drop experiment in which 10 wt% casein was eluted into 0.1 wt% xanthan. Scale bar is 2000 μm.