Supporting Information

Rapid Detection of *Salmonella enterica* via Directional Emission from Carbohydrate-Functionalized Dynamic Double Emulsions

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1. Materials & Methods

1.1 Chemicals

All following chemicals and solvents were purchased from Sigma-Aldrich, Alfa Aesar, Acros, VWR, or SynQuest, and used as received unless otherwise noted: Sodium dodecylsulfate (SDS, ≥99%), Zonyl FS-300 (Zonyl, 40% solids), Sudan Red 7B (95%), perylene (≥ 99%), poly(styrene)-block-poly(acrylic acid) (PS:PAA: 30,000:2,000, PDI ≤ 1.1), 3-aminophenylboronic acid, *N,N'*-dicyclohexylcarbodiimide (DCC), *N*-bromosuccinimide (NBS), 1,2-dibutoxybenzene, triisopropyl borate, *n*-butyllithium (2.5M in hexanes), D-(+)-Mannose, D-(+)-Glucose, Mannan (from Saccharomyces cerevisiae), phosphate-buffered saline (PBS) solution: Sigma Aldrich; D-Fructose: Alfa Aesar; dichloromethane (DCM), tetrahydrofuran, methanol, toluene: VWR; 2-trifluoromethyl-3-ethoxyperfluorohexane (HFE-7500): SynQuest; and heptacosafluorotributylamine (FC-43): Acros Organics. Antibodies**:** Anti-human IgG (Fc specific)-FITC antibody: Sigma-Aldrich; Anti-Salmonella typhimurium antibody: Abcam. The boronic acid surfactants 3,4-dibutoxyphenyl-boronic acid ($B(OH)_2$ **1**) and polystyrene-block-poly(acrylic acid-co-acrylamidophenylboronic acid) ($B(OH)_2$ **2**) were synthesized following literature procedures[1,2]. In brief, the polymeric surfactant $B(OH)_2$ **2** was prepared via post-polymerization functionalization of polystyrene-block-poly(acrylic acid) in a DCC coupling reaction using 5.0 eq. of 3-aminophenylboronic acid. The characterization of both boronic acid surfactants was consistent with the previous reports. F-PDI was synthesized following a literature procedure[3]. In brief, F-PDI was prepared via Heck reaction between octabrominated PDI and 1H,1H,2H-perfluoro-1-decene. The characterization of F-PDI was consistent with the previous report. DI water was used for the preparation of the continuous phases.

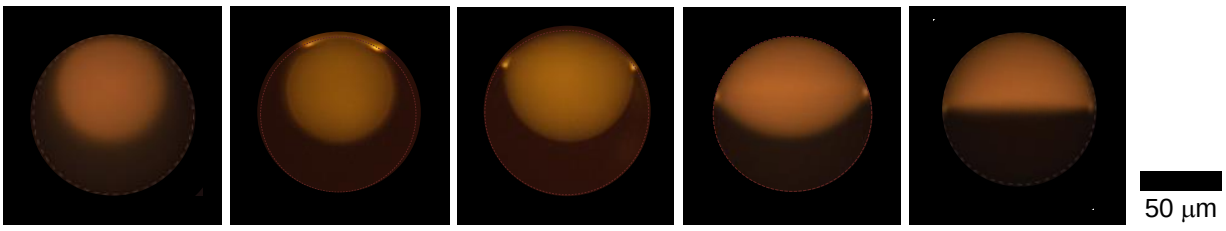
1.2 Instruments

NMR spectra were recorded using a Bruker Avance 400 MHz NMR spectrometer. ATR-FTIR spectra were obtained using a Thermo Scientific Nicolet 6700 FTIR with a Ge crystal for ATR. Polymer molecular weights were determined at room temperature on a HP series 1100 GPC system in THF at 1.0 mL/min (0.5 mg/mL sample concentrations), approximate molecular weights were estimated using a polystyrene calibration standard. Absorption spectra were obtained using an Agilent Cary 4000 UV/Vis spectrophotometer. Photoluminescence and excitation spectra were acquired on a HORIBA Jobin Yvon Fluorolog-3 spectrofluorometer (model FL-321) equipped with a 450 W Xenonlamp as the excitation source and a F-3000 Fiber Optic Mount that allows for fluorescence imaging outside of the sample compartment. The F-3000 couples to the T-box; light is focused from

the excitation spectrometer onto the fiber-optic bundle, and then directed to the sample. Fluorescence emission from the sample is directed back through the bundle and into the front-face collection port in the sample compartment. Emulsion droplets were deposited into an Invitrogen Attofluor Cell Chamber from Thermo-Fisher Scientific prior to the collection of the emission spectra. For the preparation of monodispersed complex emulsion droplets the following equipment was purchased from Dolomite Microfluidics: two Mitos P-Pumps, Basic, and the Remote partnered with an external Remote Chamber 30 mL, Telos® High Throughput Droplet System, Telos 2 Reagent Chip (100µm), 1.6mm O.D. x 0.25mm I.D. FEP tubing, End Fittings and Ferrules, Linear Connector Funnel with FEP Tubing, 1/16" x 0.8mm, 10 meters, and T-Connector.

1.3 Imaging and Microscopy

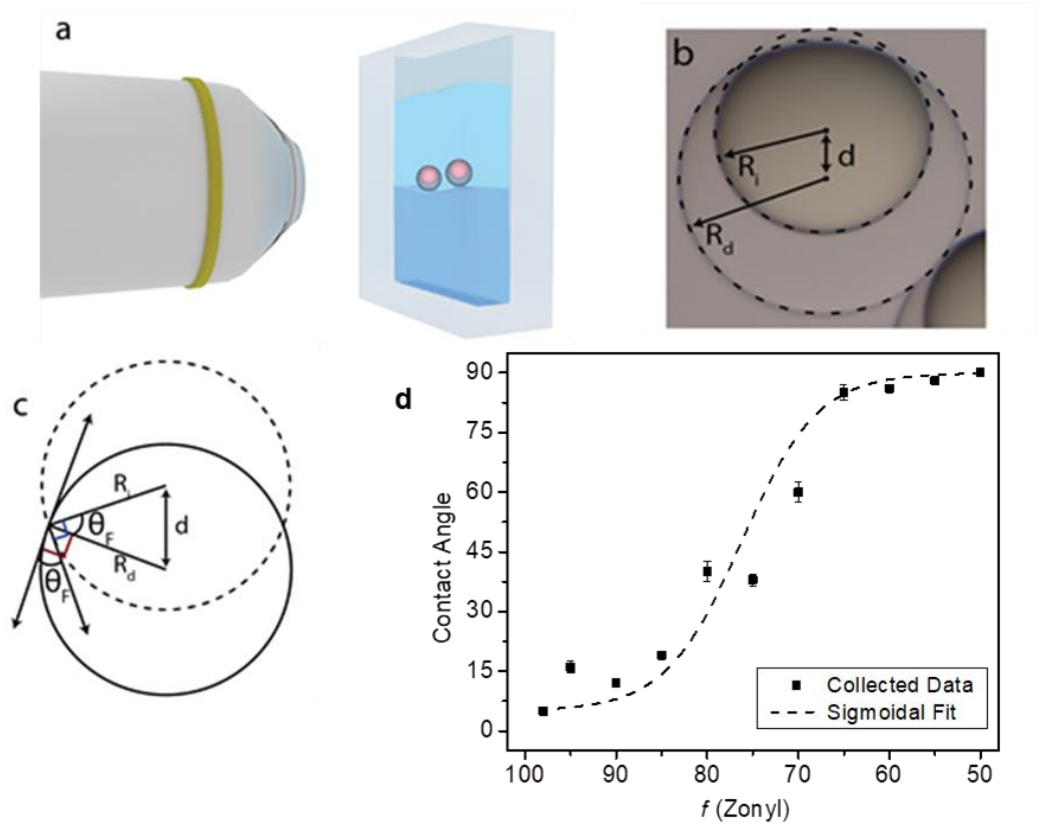
Lateral confocal cross-sections of the droplets were imaged using a Nikon 1AR ultrafast spectral scanning confocal microscope. Fluorescence and bright-field images were taken with a Zeiss Axiovert 200 inverted microscope equipped with a ZeissAxioCam HRc camera. Side-views of the droplets were taken using a custom built horizontal microscope comprised Olympus 20x objective (NA = 0.5), a Thorlabs tube lens (effective focal length = 200 mm), and an Allied Vision Prosilica GT camera. A white screen was placed behind the sample, and the sample was illuminated from the side using a Fiber-Lite MI-152 lamp for bright field images. Fluorescent images were taken using an LED coupled into a filter cube containing 450 nm shortpass filter, 425 nm dichroic, and 550 nm longpass filter between the objective and tube lens. For these experiments, the droplets were placed onto an index-matched hydrogel substrate enclosed between two coverslips. This index matched hydrogel allows the droplets to be imaged perpendicularly to their gravitational axis. The same setup was used for recording fluorescent side-view images of dyed complex emulsions.



Extended Data Figure 1. Fluorescent side-view micrographs of dyed complex emulsions in five different morphologies. The higher light intensity at the three-phase contact line represents the TIR light that is directed in different out-coupling angles.

2. Contact Angle Determination

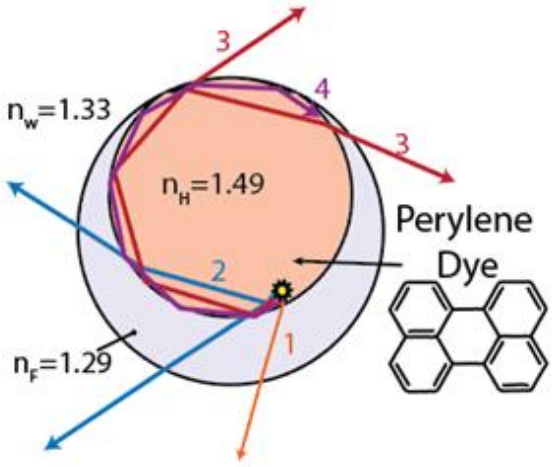
Contact angles between the fluorocarbon, hydrocarbon, and water phases were determined from side view images taken using a horizontal imaging setup as shown in Extended Data Figure 2. An index matched hydrogel substrate was used to hold the droplets in place. In all cases, the droplets appeared overall to be spherical ($\theta_F + \theta_H = 180$ degrees). Therefore θ_F was used as the sole parameter for describing the morphology of the droplet. In order to determine the contact angle, ImageJ was used to fit circles manually to the droplet internal interface as well as the overall outer interface. When the interface between the two liquids is imaged through the outer phase of the droplet, the image of the interface is magnified (or in the case of a lower refractive index outer phase de-magnified). In order to correct for the magnification, the paraxial approximation was used, resulting in a correction factor of $h_{real} = \frac{n_{medium}}{n_{fluoro}} h_{image}$. This correction was applied to the curvature R_i and distance d. From the two circles that define the inner and outer interfaces of the droplets, the contact angle can be determined using the law of cosines: $\cos(\theta_F) = (R_i^2 + R_d^2 - d^2)/2R_iR_d$.



Extended Data Figure 2. Contact angle determination. (a) Horizontal imaging setup for recording side-view images of the complex emulsions. An index matched hydrogel substrate was used to hold the droplets in place; (b) Side-view image analysis and (c) Contact angle calculation; (d) Plot of the experimentally determined contact angles for droplets prepared in different surfactant ratios and sigmoidal fit used for assigning experimentally determined emission intensities (recorded as a function of *f* (Zonyl)) to specific contact angles (see Figure 2).

3. Ray-Tracing Calculations

Raytracer was implemented in MATLAB (version R15b). Each ray contains data for its location, direction, amplitude, and polarization, as well as the trajectory that the ray has followed up until that point. All of the interfaces of the droplet were treated as spherical. Intersections between the spherical interfaces and the rays were determined analytically and the direction vector $\vec{d}_{\mathrm{t}}$ of refracted rays was determined using a vector version of Snell's law: $\vec{d}_{\mathrm{t}} = \frac{n_1}{n_2}\vec{d}_{\mathrm{i}} + \left(\frac{n_1}{n_2}\cos(\theta_{\mathrm{i}}) - \sqrt{1 - \left(\frac{n_1}{n_2}\right)^2 [1 - \cos^2(\theta_{\mathrm{i}})]}\right)\vec{n}$; where $\vec{d}_{\mathrm{i}}$ is the direction of the incident ray, $\vec{n}$ is the surface normal, θ_{i} is the angle that the incoming ray makes with the surface normal, and n_1 and n_2 are the refractive indices before and after the interface. Transmission and reflection coefficients were determined using the Fresnel equations. The density of starting rays was 20 million rays per unit volume. The units were set so that the radius of the droplet was 1. Matlab's rand() function was used to initialize starting coordinates (x, y, z) within the volume of the hydrocarbon phase. As well as to initialize the starting direction (ϕ, θ) of each ray. In order to correctly homogeneously distribute starting ray-directions, the polar coordinate was initialized by: $\theta_i = \cos^{-1}(2p_i - 1)$ where p_i is uniformly distributed between 0 and 1. Rays that intersected droplet interfaces more than 1000 times were eliminated (these rays are trapped within the droplet), as well as rays whose amplitude was less than 1% of the starting intensity of the ray. Once the rays exited the droplet, their intensity was summed and binned according to their emmision direction. The bin size used was 1 degree for each ϕ and θ. Because the rays were binned by their solid angle, the intensity of each bin was corrected by a factor of $\frac{1}{\sin\theta}$ in order to account for smaller bin sizes near the poles.



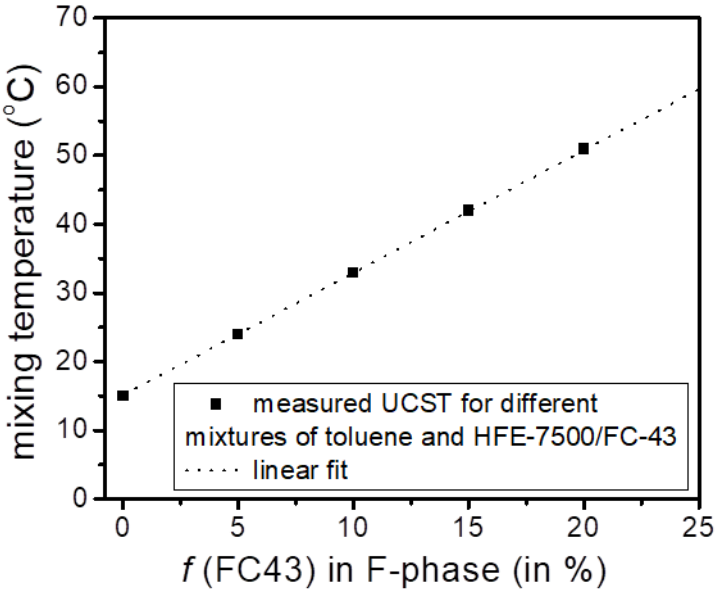
Extended Data Figure 3. Schematic ray diagram explaining the directional light emission from within the hydrocarbon phase of complex emulsions: Rays 1 and 2 represent light rays that intersect the hydrocarbon/fluorocarbon interface at an angle less than the critical angle for total internal reflection, $\theta_c = \sin^{-1}(n_{FC}/n_{HC}) = 60°$; a fraction of that light is reflected while the rest transmits through the interface. This transmitted light, which is present in all droplet morphologies was simply treated as background emission. Other rays, such as Rays 3 and 4, intersect the internal interface at an angle larger than the critical angle θ_c. These rays are guided along the interface by total internal reflection until they encounter the hydrocarbon/water interface, where they can escape the droplet (Ray 3) or continue to be totally internally reflected (Ray 4), depending on the droplet's geometry and the refractive index contrast.

4. Fabrication of complex emulsions

4.1 General Fabrication Procedure

Emulsification was conducted at temperatures above the critical temperature (32 °C) of the dispersed phase solvents, and the emulsion was subsequently cooled to below the critical temperature to induce phase separation inside the droplets, as described previously[9]. Emulsions were fabricated using either bulk emulsification or a microfluidics device, which generates polydisperse or monodisperse droplets, respectively. Both procedures allow the formation of droplets with highly uniform morphology and composition. A vortex mixer was used for bulk emulsification. Emulsion droplets with well-controlled sizes were fabricated using a Dolomite Microfluidic Setup inside a laminar flow hood using a Telos 2 Reagent Chip (100µm). Two Mitos P pressure pumps, one for the mixed droplet phases and one for the continuous phase, were used for controlling the flow rate. After heating the droplet phase above the upper critical solution temperature, the fluids were driven by pressurizing the two individual droplet and continuous chambers with N_2 providing a pulseless, stable flow to the flow focusing chip (pressures: droplet phase: 300 mbar; continuous phase: 320 mbar). The droplet phase was split into two crinkled adjacent flow resistors which provide additional flow stability and mixing. Hydrocarbon and fluorocarbon oils with a large refractive index contrast, toluene (RI=1.49) and a 9:1 mixture of 2-trifluoromethyl-3-ethoxyperfluorohexane (HFE-7500) and heptacosafluorotributylamine (FC-43) (RI=1.29) were used in this study. The ratio of FC-43 in the fluorocarbon phase allows for adjusting the upper consolute temperature of the mixture to the desired value (see Extended Data Fig. 4).

Extended Data Figure 4. Side-view images of complex bi-phase emulsion droplets in five different morphologies. The hydrocarbon phase is dyed red (Sudan Red 7B) to enhance contrast between the droplet phases. At the far left is a droplet with the hydrocarbon completely encased by the fluorocarbon phase (i.e. FC/HC/W) and on the far right is a droplet with the fluorocarbon phase completely encased by the hydrocarbon phase (HC/FC/W). The orientation of the phases represents their gravity-aligned structures that place the denser fluorocarbon phase at the bottom. The droplets were prepared by a simple phase-separation method within a continuous phase containing different ratios of hydrocarbon (SDS) and fluorocarbon (Zonyl FS-300) surfactants.



Extended Data Figure 5. Experimental determination of the influence of the fraction of FC-43 in the F-phase on the upper critical solution temperature of a toluene-HFE-7500/FC43 mixture.

4.2 Fabrication of Dyed and $B(OH)_2$-containing Complex Emulsions

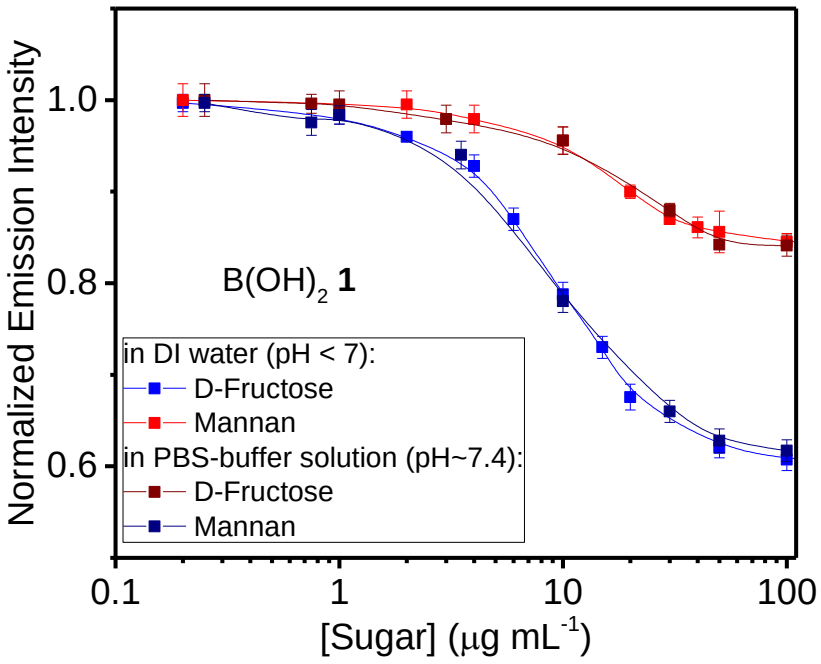
Toluene dyed with perylene (1.5 mM) was used as the hydrocarbon phase for the preparation of dyed complex emulsions. For the preparation of $B(OH)_2$-functionalized emulsion droplets, the $B(OH)_2$ surfactants 3,4-dibutoxyphenyl-boronic acid ($B(OH)_2$ **1**; 3.5 mg/mL) or polystyrene-block-poly(acrylic acid-co-acrylamidophenylboronic acid) ($B(OH)_2$ **2**; 1.2 mg/mL), respectively, were pre-dissolved in the perylene-containing hydrocarbon phase before emulsification.

5. Emission Spectroscopy of Droplet Monolayers.

A bifurcated fiber setup (HORIBA F-3000 Fiber Optic Mount connected to a HORIBA Jobin Yvon Fluorolog-3 spectrofluorometer (model FL-321) equipped with a 450 W Xenonlamp as the excitation source) was used to monitor the emission intensity above the droplet monolayers. A Thermo-Fisher Scientific Invitrogen Attofluor Cell Chamber was used as sample holder for the emission measurements. The bifurcated fiber (NA=0.22) was mounted in a distance of 5 cm above the droplet monolayer. In each experiment, the glass surface of the sample holder was wetted with the surfactant solution (1 mL) before the droplets (40 μL) were deposited into the surfactant solution in the middle of the chamber. Due to gravity, the droplets started spreading and ultimately align in a perfect monolayer. Once the monolayer was formed, the emission intensity of the perylene band at $\lambda = 475$ nm was recorded. All sensing experiments were performed using monodisperse (d = 100 μm) droplets. In principle, the emission read-out was independent from the droplet size (in the size-region of macro-emulsions), however only monodisperse droplets arrange in a perfectly reproducible monolayer on the flat glass surface, thus leading to reproducible emission intensities. Determination of L-curve: For the determination of the light curve (i.e. the emission intensity as a function of the droplet morphology) of the dyed emulsion droplets, 40 μL of pre-fabricated monodisperse droplets were deposited into 1 mL of 0.5 wt. % surfactant solution containing different ratios of SDS and Zonyl. The data points were normalized to the emission intensity of the droplets in the Janus morphology (ratio SDS:Zonyl = 50:50).

6. Carbohydrate Sensing

For sensing of the monosaccharides fructose, glucose, and mannose, and the polysaccharide mannan, complex emulsions functionalized with boronic acids **1** and **2** were used. Since the boronic acids **1** and **2** behave as surfactants, the maximum emission intensity peak on the L-Curve shifts to higher Zonyl/SDS ratios (i.e. less SDS is needed). Before sensing of the mono- and polysaccharides, a L-curve (i.e. the light intensity curve as a function of varying surfactant ratios of SDS and Zonyl) for droplets $B(OH)_2$ **1** and $B(OH)_2$ **2**, respectively were recorded. For sensing of carbohydrates, the ratios of the surfactants SDS and Zonyl in the aqueous phase (DI water: pH < 7) were strategically chosen such that the droplet morphology at the starting point (i.e. no carbohydrates present) resulted in the state of the maximum emission intensity (ratio for $B(OH)_2$ **1**: Zonyl:SDS = 86:14; ratio for $B(OH)_2$ **2**: 100:0). For sensing, vials with the respective 0.5 wt.% surfactant solutions (90:10 and 100:0 respectively) containing the carbohydrates in different concentrations (from 0.1 μg/mL to 5.0 mg/mL) in an overall volume of 1 mL were prepared. Subsequently, 40 μL of pre-formed droplets were added to these solutions. The emulsions were agitated in a Labnet Vortemp 56 incubator (shaking speed of 150 rpm at rt) for 2 h. Then, the solutions were deposited into a Thermo-Fisher Scientific Invitrogen Attofluor Cell Chamber and the emission intensity was recorded as described above. For each sample ten emission spectra were recorded at different positions of the droplet monolayer. This procedure was repeated five times for each carbohydrate concentration. In addition, we tested the resulting changes in droplet morphology (measured as a function of the related emission changes) when adding the carbohydrates D-Fructose and Mannan to our emulsion droplets dispersed in DI water (pH < 7) vs. in PBS buffer solution (~ pH: 7.3-7.6) (Ext. Data. Fig. 6). As revealed by these experiments the pH dependency (in the region of neutral pH) of the carbohydrate recognition is negligible, which we attributed to the particular driving force of a decrease in the interfacial tension of the HC/W interface through the formation of a stronger surfactant, the boronate ester adduct.



Extended Data Figure 6. pH-dependency of carbohydrate recognition in the region of neutral pH: The associated changes in droplet morphology (measured as a function of the emission intensity decrease) upon addition of carbohydrates to boronic acid functionalized emulsion in DI water (pH < 7) vs. in PBS buffer solution (pH ~ 7.4).

7. Antibody Immobilization

Similar to the attachment of carbohydrates to $B(OH)_2$ **2**-functionalized droplets, two antibodies, a FITC-dye labeled anti-human IgG-antibody and an anti-*Salmonella* Typhimurium antibody were attached by pre-dispersing the antibodies (35 μg/mL) in the surfactant solution (1.0 mL in PBS buffer solution; pH ~ 7.4) and subsequently adding 40 μL of pre-formed monodisperse droplets. The surfactant solutions contained 0.5 wt.% Zonyl surfactant dissolved in a PBS buffer solution. The emulsions were agitated for 2 h at 150 rpm before recording the emission intensity or recording confocal microscope images. A Nikon A1R Ultra-Fast Spectral Scanning Confocal Microscope with an excitation wavelength of 487.1 nm, and an emission wavelength of 525.0 nm, to capture an image at a specific Z-slice. The fluorescent ring signifies interfacial localization of the FITC-labeled IgG antibodies at the hydrocarbon-water interface.

8. Salmonella Sensing

8.1 General Procedure

In order to monitor the emission intensity increase back to the highest emission intensity upon addition of heat-killed *Salmonella enterica* serovar Typhimurium (HKST) cells, we first functionalized 40 μL of monodispersed droplets with mannan or anti-*Salmonella* Typhimurium antibody, respectively, as described above (35 μg/mL). After agitating the vials for 2 h (150 rpm) HKST cells were added yielding an overall concentration between 0 and 10^8 cells/mL. The solutions were deposited in the incubator again for 2 h (rt, 150 rpm) before the emission intensity was recorded as described above. A total of 5 samples were measured for each individual concentration of HKST cells. In order to monitor the time-dependency of the emission intensity increase, HKST cells (10^5 cells/mL) were added to vials containing droplets pre-functionalized with either mannan or anti-*Salmonella* Typhimurium antibody. The emission intensity of these samples was recorded directly after the addition of the HKST cells, and after 10, 30, 60, 90, 120, 150, 180, and 210 min in order to determine the time-dependency of the droplet response.

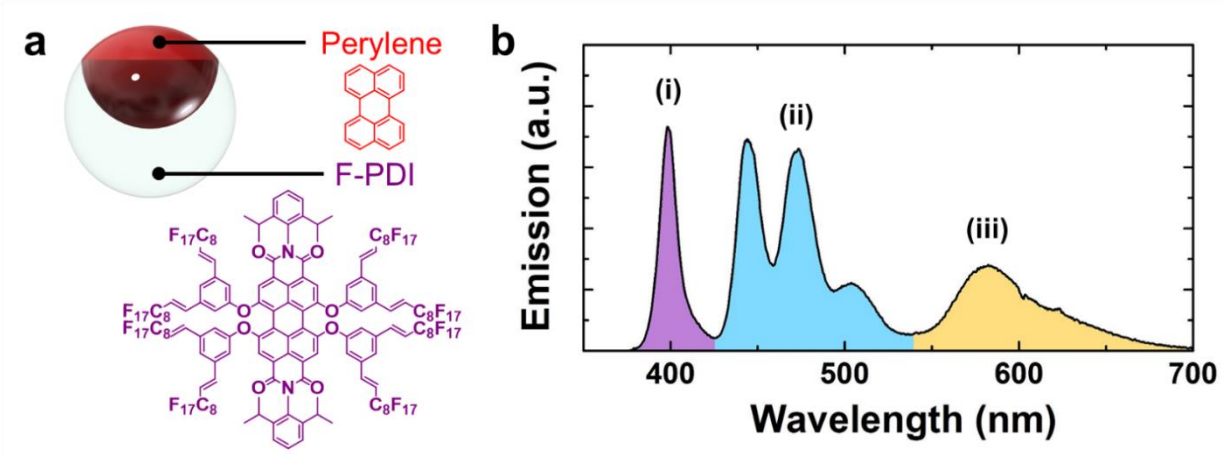
8.2 Preparation of Live Bacteria Samples

Using a sterile inoculating loop, the surface of a frozen *Salmonella enterica* serovar Typhimurium (ATCC 14028) glycerol stock (-80°C) was scratched and streaked across the surface of an LB agar plate. The plates were inverted and incubated overnight (18 hours) at 37 °C. One isolated Salmonella colony from the plate was transferred to a sterile polystyrene culture tube (Fisher) containing 5 mL of LB broth, and the snap cap was applied in the loose/aerobic position. The cells were cultured overnight (18 hours) in an incubator (Innova 4230) at 37 °C with shaking at 160 RPM. The LB broth (BD Difco) was prepared by mixing 25 grams of powder with 1L of nanopure water in a sterile flask. The broth was autoclaved at 121°C for 15 minutes, and cooled to room temperature prior to use. The LB agar plates were prepared by adding 25 grams of LB Broth powder (BD Difco) and 20 grams of agar (Neogen Acumedia) with 1 L of nanopure water in a sterile flask. The media was autoclaved at 121°C for 15 minutes, cooled to 55°C and distributed to 100mm x 15mm sterile polystyrene plates in 25 mL increments. The plates were stored inverted at 4°C. Using 2 mL of the overnight culture, the sample was centrifuged for 10 minutes at 500X. Without disturbing the pellet, the supernatant was decanted, and replaced with 2 mL of PBS resulting in an average concentration of $6.61*10^9$ CFU mL^{-1}. Tablets (Sigma Aldrich) were used to prepare 10 mM phosphate buffered saline (PBS) solutions; the pH was measured to be 7.3-7.6 using a bench top pH meter (IQ Scientific). The process was repeated two additional times to ensure the LB media was effectively substituted with PBS. The PBS substituted suspension was serially diluted 10-fold by transferring 100μL of sample to 0.9 mL of PBS

that served as the stock solution for live-cell detection. The cell suspensions and dilutions were enumerated by a 6 x 6 drop plate procedure[31]. In brief, LB plates were removed from the refrigerator and allowed to air dry and to warm to room temperature with the lid off in a laminar flow hood. Each suspension and dilution was refluxed mixed ten times using a pipette, and tips were changed between samples. 250µL of each dilution was added to the first column of a 96-well plate. 180µL of LB broth was distributed to subsequent wells in the row. A ten-fold serial dilution for each sample was prepared by adding 20µL of sample from the i^{th} column to the i+1 column within the same row of the plate. Each dilution was reflux mixed ten times using the pipette, and tips were changed between dilutions. Using a multichannel pipette, 6 replicates of 7 µL (this volume ensured the drops would not merge on the surface of the agar) from 6 of the target dilutions were deposited on the surface of the LB plate. The plates were allowed to air dry before they were inverted and incubated at 37°C for 12 hours. Following the incubation, the numbers of colonies were counted using a microscope (Stemi DV4; 32X magnification). Colonies were counted within drops which were sufficiently concentrated, but did not generate a confluent film. The average number of colonies for each dilution (n=6) was used to determine the number of colony forming units/unit volume: $CFU/mL = \frac{Average\ \#\ of\ colonies\ (CFU)}{drop\ volume\ (mL) * dilution}$.

8.3 Live-Cell Detection

For live-cell detection, we prepared polydisperse droplets containing two dyes, perylene (1.5 mM) in the hydrocarbon phase and a fluorinated perlylene bisimide (0.5 mM) in the F-phase in order to generate a ratiometric optical read-out excluding size effects arising from polydisperse droplets. The droplets also contained both boronic acids, $B(OH)_2$ **1** and **2** in order to generate a maximum emission intensity in a 100% Zonyl surfactant solution (0.5 wt.%). After preparation of the emulsion, the droplets were functionalized with mannan (500 mg mL) by adding 100 µL of a 5 mg mL^{-1} stock solution to 900 µL of the Zonyl surfactant solution containing 40 µL of droplets. The solutions were stirred for 2 h prior to measuring the decreased emission intensity. In order to determine the emission intensity change upon addition of live bacteria, 50 µL of the bacteria stock solutions (PBS or chicken exudate) were added to the samples containing the 40 µL of droplets and put into the incubator for 2 h at RT with shaking at 15 RPM. Subsequently, the samples were poured into the sample holder in order to measure the emission intensity ratio. A total of 3 individual samples were measured for each sample containing live Salmonella cells.



Extended Data Figure 7. Ratiometric emission read-out. (a) Schematic diagram of the double-dyed complex emulsions used for ratiometric read-out. The hydrocarbon phase (HC, red) contains a perylene dye and the fluorocarbon phase (FC, white) contains a fluorinated perylene diimide dye (F-PDI). The synthesis of F-PDI was reported previously[30]. (b) A representative emission spectrum measured from polydisperse complex emulsions containing perylene in the HC-phase and F-PDI in the FC-phase. The excitation wavelength is shown at 400 nm (i). The emission from the perylene dye (ii) shows the three characteristic peaks at 445, 475, and 500 nm. And the emission from the F-PDI is shown at 580 nm. We used the emission intensities at 475 nm and 580 nm for the ratiometric read-out.

8.4 Preparation of Chicken Exudate Samples

Irradiated chicken exudate samples[32] were diluted 2x by mixing 30 mL of chicken exudate wash with 30 mL of PBS to simulate a whole bird or poultry parts rinsate sample to simulate an original procedure of the Food Safety and Inspection Service[33]; buffered peptone water was substituted with PBS because the samples were evaluated without enrichment. Similar to PBS substitution, the 2 mL of the overnight culture was centrifuged 500X for 10 minutes. Without disturbing the pellet, the supernatant was decanted, and replaced with 2 mL of PBS. The process was repeated, as previously described, however on the third wash the supernatant was replaced with the poultry rinsate (2x diluted exudate). The substituted suspension was serially diluted 10-fold by transferring 100µL of sample to 0.9 mL of simulated poultry rinsate (2x diluted exudate) to provide stock solutions for the sensing of live-bacteria. Measurements and enumeration was performed according to the above described procedures.

9. References

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