

Supporting Information

Janus Emulsions for the Detection of Bacteria

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Keywords: *carbohydrates • emulsions • Escherichia coli • Janus particles • sensors*

General Methods and Instrumentation

Hexane, FC770, D-(+)-Mannose, 1-tetradecanol, Concanavalin A, Bovin serum albumin and Zonyl FS-300 were purchased from Sigma-Aldrich. HEPES buffer (1M) was purchased from ThermoFisher. Solvents were purchased from Sigma-Aldrich and used as received.

NMR spectra were recorded using a Bruker Avance 400 MHz NMR spectrometer and were referenced to the proton resonances resulting from incomplete deuteration of NMR solvent (^1H). High resolution mass spectrum was determined with a Waters Q-TOF Micro Mass Spectrometer using electrospray ionization (ESI) ion source. Bright-field images were taken with a Zeiss Axiovert 200 inverted microscope equipped with a Zeiss AxioCam HRc camera.

Synthetic Procedures

ManC14 was synthesized according to a modification of a published method.^[1] D-(+)-Mannose (0.75g, 4.16mmol) was reacted with 1-tetradecanol (10.71g, 0.05mol) in 1,4-dioxane (15ml) containing 96% H_2SO_4 (40 μl) for 12h followed by neutralization and evaporation of volatile compounds. The crude product was purified by column chromatography on SiO_2 (20% EtOH/EtOAc) to obtain the product as a white powder. ^1H NMR (400MHz, CDCl_3): δ 4.83 (s, 1H). ESI-MS: calculated for $\text{C}_{20}\text{H}_{44}\text{O}_6\text{N}$ ($[\text{M}+\text{NH}_4]^+$): 394.3163, found: 394.3170.

Janus Emulsion Assay Preparation

Janus emulsions, composed of equal volumes of hexane and FC770¹ in aqueous continuous phase were fabricated using either bulk emulsification or a microfluidics device, which generates polydisperse or monodisperse droplets respectively. Both methods could generate emulsion assays applicable for qualitative and quantitative detections. Monodispersed emulsion assays were fabricated for Figure 1 for proof-of-concept experiments. However, polydisperse emulsion assays were preferentially used for later experiments because the fabrication process is easier and doesn't require specialized equipment, which is essential for our proposed on-site sensor application. In a polydisperse emulsion assay, smaller droplets tends to be tilted and stick on to the surface of bigger droplets (**Figure S1**). Large Janus droplets (more than 200 μm in diameter) will not tilt because the binding strength between carbohydrate surfactant and lectin does not provide enough force to tilt the particles against gravity. Thus, we deliberately designed the Janus droplets to be in the range of 50-150 μm in diameter to facilitate the tilting motion upon agglutination. However, droplets in different sizes are all connected together with ConA and can be confirmed with the movie provided as supporting information. Thus we observed no differences in agglutination and limits for detection for the droplets within the ranges of diameters reported.

¹ Perfluorinated solvent from 3M.

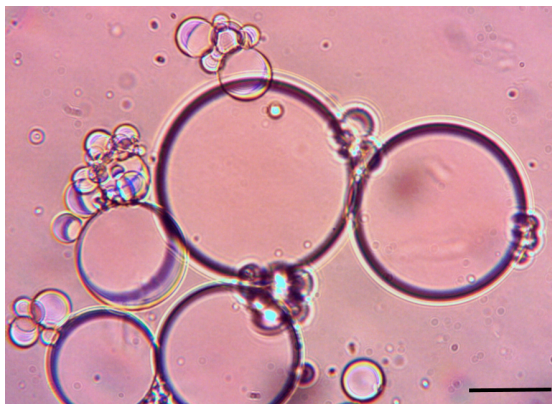


Figure S1 Agglutinated Janus droplets in different sizes.

In both fabrication processes, the hydrocarbon phase (hexane) and fluorocarbon phase (FC770) were mixed and heated above the upper critical temperature to generate a single droplet phase. This droplet phase was then dispersed into the continuous aqueous phase containing the two surfactants to generate single phase emulsions and upon cooling, the hexane and FC-770 phases separated to generate Janus emulsions. The composition of all droplets was identical because every droplet originated from the same single phase.

For the continuous water phase, two surfactants, ManC14 and Zonyl FS 300, were used to stabilize and generate the Janus emulsions. The two surfactants were dissolved in HEPES buffer solution (10mM, containing 1mM CaCl_2 and 1mM MnCl_2 , pH = 7.5) separately with concentration of 0.0005% and 0.01% by weight, respectively. In both bulk emulsification and microfluidics method, the final volume ratio between ManC14 solution and Zonyl FS 300 solution was kept at 1.2:1 to generate perfectly two-hemisphere Janus emulsions. Janus emulsions were loaded into a stainless steel sample holder with a 1 cm deep well and a 1.5 cm diameter viewing window. We loaded 0.5 mL of mixed surfactant solution containing 30 μL of hexane-FC770 droplet phase into sample holder to effectively create a monolayer of Janus emulsion that covered the whole viewing window. The sample holder and solution of the Janus emulsions were kept below 20°C, the T_c of hexane-FC770 mixture, during the sensing and image acquisition.

Dynamic Nature of the Complex Emulsion Droplets

The Janus droplets described in this work are dynamic. The dynamic morphology change process was detailed in a previous study by our laboratory.^[2] Complex emulsion droplets change their morphology from double emulsion H/F/W to Janus to F/H/W with the addition of hydrocarbon surfactant. The morphology reflects the strength and concentrations of the relative hydrocarbon and fluorocarbon surfactants. The surfactant molecule prefers to reside at the interfaces to lower interfacial tensions thus changing the morphology of droplets. In the context of this work, ManC14 is the hydrocarbon surfactant and Zonyl FS 300 is the fluororous surfactant. If the system lacks the mannose surfactant, the droplets are double emulsions with fluororous phase on the outside encapsulating the hydrocarbon phase (H/F/W, as shown in **Figure S2a**). Addition of the proper amount of the mannose surfactant will transform these droplets from H/F/W to Janus droplets (**Figure S2b**), and excess mannose surfactant can produce F/H/W double emulsions (**Figure S2c**).

Janus Emulsions for the Detection of Bacteria

Janus droplets were intentionally designed as a result of distinct optical behavior. The morphology (double emulsion or Janus) is the direct evidence that the mannose surfactant is active in stabilizing the water/hydrocarbon interface in the Janus particles and is also the anchor for agglutination of droplets

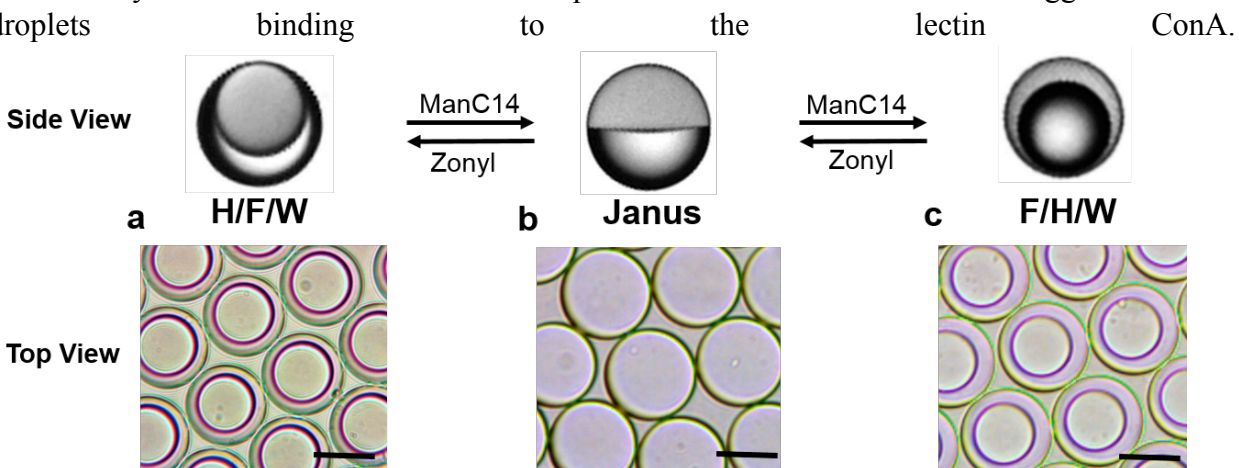


Figure S2. Dynamic complex emulsion droplets. a) H/F/W double emulsion in 0.01% Zonyl FS300 solution. b) Janus emulsion in solution of 0.0005% ManC14 : 0.01% Zonyl = 6:5 (v:v). c) F/H/W double emulsion in 0.0005% ManC14 solution. Scale bars: 50 μ m.

Bulk Emulsification for Polydispersed Janus Emulsions

To generate Janus emulsions via bulk emulsions, we began by preparing an equal-mixture of hexane and FC770 with a total volume of 1 mL in a 5 mL glass vial. The mixture initially formed an immiscible solution at room temperature. The vial containing the mixture was then heated to above the T_c (20°C) using a standard heat gun until the mixture is miscible. In another 5 mL glass vial, 1 mL of the continuous phase containing surfactants was also heated to the same temperature as the vial containing hexane-FC770 mixture. This precaution will mitigate the phase segregation of hexane and FC770 upon addition before emulsification. 50 μ L of heated and miscible hexane-FC770 mixture was then injected into the heated continuous phase via a pipette. The Janus emulsions were then generated by shaking the vial using a vortex mixer at 3000 RPM for 5 seconds. The solution of emulsions was then cooled down below T_c using an ice bath. This method of bulk emulsification generated polydispersed droplets with diameters ranging from 30 to 200 μ m as observed by an optical microscope.

Generation of Monodispersed Janus Emulsions via Microfluidics

Focused Flow Droplet Generator chip (channel width = 100 μ m, channel depth = 20 μ m, tip width = 10 μ m, glass) from Micronit was used to generate monodispersed droplets. Harvard Apparatus PHD Ultra syringe pumps were used to inject the outer phase (continuous phase) and inner phase (droplet phase). The flow rates were 50 μ L min^{-1} for the continuous phase and 30 μ L min^{-1} for the droplet phase. The microfluidic setup was heated above the T_c of the inner phase solution using a heat lamp. Janus emulsions were then cooled below T_c to induce phase separation. The average diameter of the monodispersed droplets generated from this setup were 60 ± 10 μ m.

ConA Sensing

Monodispersed or polydispersed Janus emulsions used for sensing experiments were fabricated using methods described above. ConA was dissolved in HEPES buffer solution (10mM, containing 1Mm CaCl₂ and 1mM MnCl₂, pH = 7.5) with various concentrations and used as the analyte. 10 μ L of ConA solution was added using a micropipette to the sample holder containing Janus emulsions. Solution was then swirled gently and agglutination of Janus emulsions were observed within seconds.

A control experiment for specificity was performed in the presence of free mannose. ConA was pre-incubated with 1mM D-(+)-Mannose in HEPES buffer (10mM, containing 1Mm CaCl₂ and 1mM MnCl₂, pH = 7.5) for 30min before injected into the Janus emulsion assay. The pre-incubation of ConA with mannose inhibits the binding of ConA to the Mannose functionalized Janus Droplets and agglutination was not observed (Concentration of ConA tested in the range of 0.01-0.2mg mL⁻¹).

For the morphology conversion experiment in the presence of agglutination, 100 μ l of 5 wt% Zonyl FS 300 solution was added to the sample holder after Janus droplets were agglutinated with addition of 10 μ L of 1mg/mL ConA solution. The movie is recorded in real time to show the forced morphology change by adding excess amount of fluorous surfactant. A control experiment with Triton X-100 as the hydrocarbon surfactant was performed for comparison. 0.01% Triton X-100 and 0.01% Zonyl in volume ratio of 5:1 was used to make a control sample of Janus emulsion assay. The same amount of ConA was added and no agglutination was observed. 100 μ L of 5 wt% Zonyl FS 300 solution was added to the control assay and the morphology change was symmetrical as shown in the movie.

We performed the qualitative analysis using the QR code from unmagnified images taken from the smartphone. The distance from the phone to the analysis chamber containing the Janus emulsions was approximately 10 cm. The exact distance was calibrated by the image processing software by using the known dimension of the QR code (1 cm \times 1 cm). The binary response we measured was whether the QR code could be read via the software. If the QR code is readable, the Janus emulsions are not agglutinated, and vice versa.

For quantitative analysis, images were recorded before and after adding ConA solution using an inverted microscope with 4x magnification. The image processing software then pre-processed the captured images by transforming them into greyscale images and adjusting the brightness and contrast to the reference image of blank analysis chamber. Using the pre-processed images of 4 \times magnification, the program first applied the adaptive thresholding algorithm to distinguish the darker edges of the Janus emulsions from the droplet complexes with tilted particles. More specifically, the program ignores the edges of the droplets that have inherent low-light intensity and only seeks the area of droplet complexes. We then set a threshold that areas with light intensity of less than 45% of the brightest regions will be considered part of the droplets complex. From this information, the area occupied by the droplet complexes was then calculated (**Figure S3**).

Janus Emulsions for the Detection of Bacteria

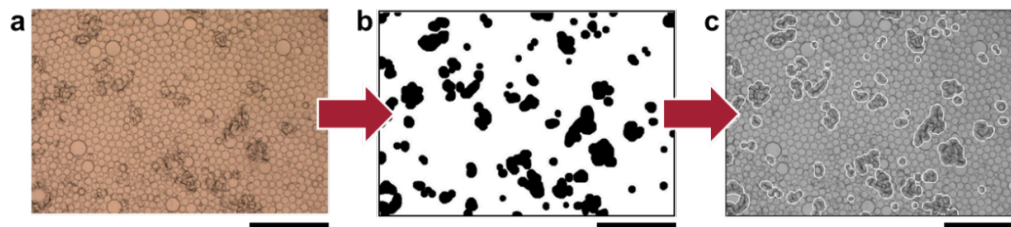


Figure S3. Algorithm for image analysis. The image processing program evaluated the raw image (a) by applying adaptive thresholding algorithm to distinguish area with higher transparency from opaque regions (b). The final locations of the agglutination were highlighted (c). Scale bars: 500 μ m.

Generic Protein Perturbation Experiment

ManC14 functionalized Janus emulsion assay with the addition of non-mannose binding protein bovin serum albumin (BSA). 10 μ L of BSA solution (in comparable concentration with ConA) was added using a micropipette to the sample holder containing Janus emulsions. The final concentration of BSA is 0.02, 0.12 and 1mg/ml. No emulsion agglutination was observed even after extended time period. Additionally, the existence of BSA will not affect the binding between ConA and the Janus emulsions. The agglutination behavior using ManC14 emulsion assay with ConA, BSA and ConA with the existence of BSA is shown in **Figure S4**.

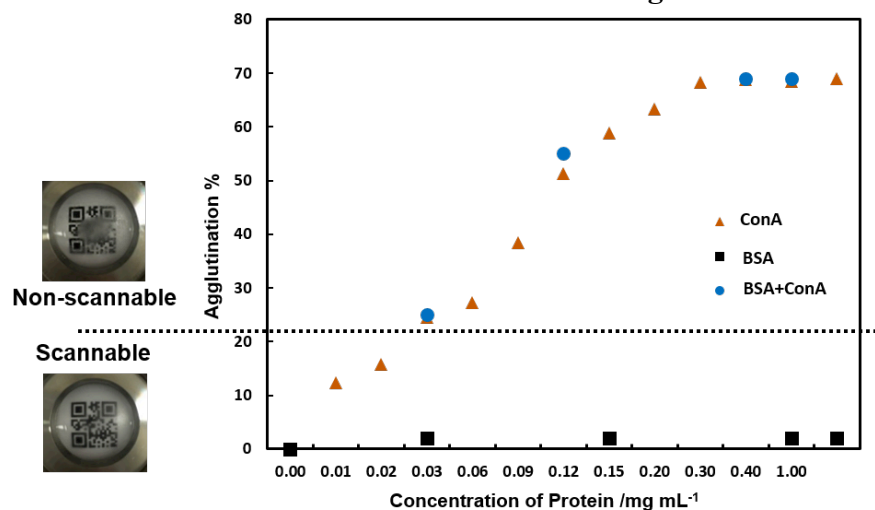


Figure S4. Agglutination behavior of ManC14 emulsion assay with two proteins ConA and BSA. Agglutination valued with QR code detection method.

E.coli Sensing

Bacterial strain bearing a mannose-binding protein (ORN178) and a mutant strain lacking the mannose binding domain (ORN 208) were grown in LB media overnight at 37°C until they reached an approximate OD₆₀₀ of 1.0 (10⁸ cells). The culture was then centrifuged and cells washed twice with HEPES buffer. Live *E.coli* test was conducted by adding a 10 μ L aliquot of these cells to the Janus emulsion assay. The mixture was incubated for 48 h at 37 °C before agglutination was observed under microscope.

Janus Emulsions for the Detection of Bacteria

To speed up the agglutination, both ORN 178 and ORN 208 strains were centrifuged and suspended with 4% paraformaldehyde for 1h. The suspension is then centrifuged to pellet the cells. The supernatant solution containing paraformaldehyde was discarded, and the cells were washed three times with HEPES buffer. The bacteria was then diluted to various concentration for testing. A 10 μ l aliquot of these treated cell suspension was each added to Janus emulsion assay followed by 2 h with robotic shaking under room temperature (25 °C).

Discrimination between the targeted strain, ORN 178, and the control strain, ORN 208, using a smartphone and QR code detection method is shown in a video provided in the Supporting Information. Agglutination was also observed under a microscope (**Figure 5**) to confirm that agglutination is responsible rendering QR code unscannable. Janus emulsion assays without ManC14 (Triton X100 was used as hydrocarbon surfactant) will not agglutinate.

In the video of *E.coli* sensing test, a control experiment was carried out using ManC14 emulsion assay with buffer. Tests with ORN 178 and ORN 208 strains used the exact same ManC14 emulsion assay.

Janus Emulsions for the Detection of Bacteria

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