Supporting Information

Janus Emulsion Biosensors for Anti-SARS-CoV-2 Spike Antibody

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Materials

All the chemical reagents used were purchased from Fisher Scientific, Sigma-Aldrich, Click Chemistry Tools, Combi-Blocks, TCI, Synquest or BroadPharm. All reagents and solvents were used as received without further purification. Goat Anti-Human IgG antibody, Recombinant human coronavirus SARS-CoV-2 Spike Glycoprotein RBD (Active) and Recombinant Anti-SARS-CoV-2 Spike Glycoprotein S1 antibody were purchased from abcam. The inactivated serum samples from COVID-19 patients and normal patients were purchased from Raybiotech. company.

Instruments

^{1}H-NMR, and ^{19}F-NMR spectra were recorded with an Ascend-600 (600 MHz), JEOL model JNM-ECZ500R/S1 (500 MHz), or Bruker Ascend-400 (400 MHz) spectrometer. Chemical shifts δ are reported in ppm using the residual solvent signals (acetone-d_6: δ_H 2.05 ppm) as an internal reference. Coupling constants *J* are given in Hz and the resonance multiplicity is described as s (singlet), d (doublet), t (triplet), and m (multiplet). Fluorescence spectra were recorded at room temperature using a bifurcated fiber on a Horiba Jobin Yvon SPEX Fluorolog-τ3 fluorimeter (model FL-321, 450 W Xenon lamp). Mass spectra were recorded with a high-resolution JEOL AccuTOF 4G LC-plus equipped with an ionSense DART source, and a Bruker Autoflex Speed MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer) in Reflection Detection mode. Optical images of emulsions were obtained by an inverted microscope equipped with an AmScope camera. SEM images were obtained by a Merlin and Crossbeam 540 Zeiss scanning electron microscopy.

Experimental Procedures

Preparation of Two-Dye Droplets.

Janus Emulsion were prepared using the bulk emulsification method. Sub-PC was dissolved in DEB at concentration of 1 mM and P-TCO was dissolved in DEB at concentration of 1mg/mL. The fluorocarbon dye F-PBI, was dissolved in HFE7500 at concentration of 0.1 mM.

The droplets containing F-BHQ and Lumogen F Orange 240 were prepared by dissolving Lumogen F Orange 240 at 0.1 mM and P-TCO at 1 mg/mL in DEB and F-BHQ at 2 mM in HFE7500.

We used a mixture of 0.5 wt% Zonyl : 0.5 wt% Tween 20 1:1 (v/v) as the continuous phase.

Quantification of Goat Anti-Human IgG antibody and SARS-CoV-2 spike protein RBD.

We use Goat Anti-Human IgG-FITC as fluorophore to quantify these two proteins. The equation of calibration curve (y=159385x-587) is acquired by measuring the fluorescence

spectra (λ_{ex}=490 nm) of Goat Anti-Human IgG-FITC at 0.2, 0.1, 0.05, 0.02 and 0.01 μg/mL, respectively (Figure S1).

For the quantification of Goat Anti-Human IgG antibody, we treat the 50 μm monodispersed droplets (1mg/mL P-TCO dissolved in the hydrocarbon phase) with 60 μL of Tetrazine-Goat Anti-Human IgG (FITC) antibody (1.0 mg/mL) for overnight at room temperature. The droplets are washed with fresh continuous phase for three times and then the number of droplets is counted (2×10^5). After this step, all the droplets are dried in a 4 mL glass vial. 2 mL of DI water is added to re-dissolve the Tetrazine-Goat Anti-Human IgG (FITC) antibody which was conjugated on the surface of droplets. The number of Goat Anti-Human IgG antibody could easily be calculated from the fluorescence spectrum and the equation of calibration curve.

For the quantification of SARS-CoV-2 spike protein RBD, droplets are treated with 60 μL of Tetrazine-SARS-CoV-2 spike protein RBD (0.1 mg/mL) for overnight. Then 30 μL of anti-SARS-CoV-2 spike antibody (1.0 mg/mL) is added to the continuous phase for 2 hours to bind with the SARS-CoV-2 spike protein RBD on the droplets. Finally, 60 μL of Goat Anti-Human IgG (FITC) antibody (1.0 mg/mL) is added to the continuous phase for two hours for binding with the anti-SARS-CoV-2 spike antibody. Droplets are washed with the fresh continuous phase for three times between each step to remove the unreacted reagents. We use same procedure as stated before to re-dissolve the Tetrazine-Goat Anti-Human IgG (FITC) antibody bind to the surface of droplets and quantify the number of Goat Anti-Human IgG antibody FITC. Since there is binding between each protein and antibody, the number of Goat Anti-Human IgG (FITC) antibody equals to or is less than the number of SARS-CoV-2 spike protein RBD on the droplets.

Fluorescence spectra are recorded as shown in Figure S1B. As we know that the molecular weight of Goat Anti-Human IgG antibody-FITC is 150 kDa, we use the published method[1] to calculate the area per Goat Anti-Human IgG antibody on the surface of droplets to be 4300 nm^2/antibody and the area per SARS-CoV-2 spike protein RBD on the surface of droplets to be 6500 nm^2/protein.

Clinical Serum Sample Test.

8 μl of serum is added to 80 μl of continuous phase of Two-Dye droplets for 2h at room temperature. Fluorescence spectra of droplets are recorded after the 2-hour incubation with the excitation at 361 nm. All the measurements are repeated for three times. The original

concentrations of anti-SARS-CoV-2 spike IgG antibody in human sera is calculated with the relative fluorescence intensity at 580 nm, the correlation curve and the dilution factor (ten).

Safety Statement.

No unexpected or unusually high safety hazards were encountered.

Experimental procedures for the Synthesis of Polymerized Droplets

Materials

1H,1H,10H,10H-Perfluoro-1,10-decyl diacrylate was prepared following a previously reported procedure.[2] Trimethylolpropane ethoxylate triacrylate (Mn ≈428 g/mol), 1,4-butanediol diacrylate; phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide (Sigma Aldrich); fluorinated acrylate oligomer CN4000 (Sartomer).

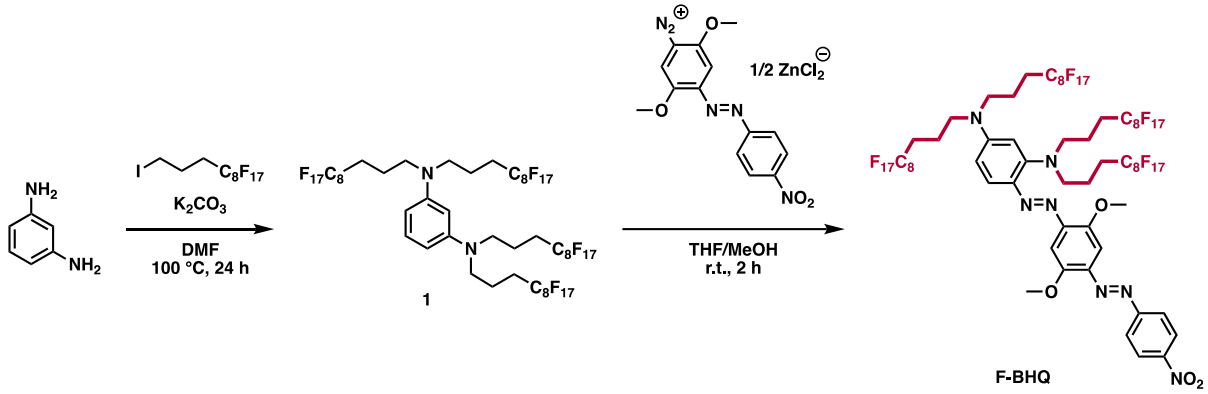
Experimental

Emulsification was conducted by a temperature-induced phase separation method. Emulsions were fabricated using either bulk emulsification or a microfluidic device, which generates polydisperse or monodisperse droplets, respectively. Both procedures allow for the formation of droplets with highly uniform morphology and composition. In a typical bulk emulsification preparation, the disperse phase was heated above their upper critical temperature (Tc) to form a homogeneous mixture. 25 μL of the heated HC/FC mixture was added to 500 μL of continuous phase and vortexed for 10 s to emulsify. Double emulsions were obtained upon cooling using an ice bath. This bulk emulsification method generated polydispersed droplets with diameters ranging from 10 to 200 μm as observed by an optical microscopy. Emulsions were also fabricated using a microfluidic device purchased from Dolomite Microfluidic, which generates emulsion droplets with well-controlled sizes. A Telos 2 Reagent Chip (50 μm) was used, and two Mitos P pressure pumps, one for the dispersed phase and one for the continuous phase, were used for controlling the flow rate. After heating the disperse phase above its upper critical temperature (Tc), the fluids were driven by pressurizing the two individual dispersed and continuous chambers with N_2 providing a pulseless, stable flow to the flow focusing chip (pressures: dispersed phase: 200 mbar; continuous phase: 800 mbar). The dispersed phase was split into two crinkled adjacent flow resistors which provide additional flow stability and mixing. The average diameter of the monodispersed droplets generated from this setup were 50 ± 5 μm as observed by optical microscopy.

As dispersed phase, we used a mixture of fluorinated acrylate oligomer (CN4000), 1H,1H,10H,10H-Perfluorodecyl diacrylate, 1,4-butanediol diacrylate and trimethylolpropane

ethoxylate triacrylate (Mn ≈ 428 g/mol) in a volume ratio of 15:3:14:10. Phenylbis(2,4,6-trimethylbenzoyl)phosphine oxide (1 wt. %) was selected as the photo-initiator. To generate emulsion droplets containing P-TCO for interfacial functionalization, P-TCO was dissolved at concentration of 1 mg/mL. A 1.0 wt. % aqueous solution of sodium dodecyl sulfate (SDS) was used as continuous phase. Morphology change from FC/HC/W double emulsions to Janus was achieved by exchanging the continuous phase to a solution of 1 wt. % SDS/1 wt. % Zonyl = 9.5:0.5 (v/v). Emulsions were irradiated over ice with a 365 nm LED light (4.1 W) for 30 min to generate photopolymerized HC/FC Janus particles.

Experimental Procedure for Synthesis of F-BHQ



Synthesis of Compound 1. A mixture of *m*-phenylenediamine (54 mg, 0.500 mmol), 3-(perfluorooctyl)propyl iodide (1.30 g, 2.21 mmol), and anhydrous K_2CO_3 (304 mg, 2.20 mmol) in DMF (5 mL) was stirred at 100 °C for 24 h. Upon cooling the reaction mixture to room temperature, the residue was dissolved in AcOEt (50 mL) and water (50 mL). The organic layer was separated, washed with water (50 mL × 3) and brine (50 mL), dried with $MgSO_4$, and evaporated to dryness under reduced pressure. The residue was chromatographed on silica gel using hexanes with a gradient of 1% → 10% AcOEt as eluent, and the fraction containing compound **1** (R_f = 0.30 in hexanes, 5% AcOEt) was collected and evaporated to dryness to provide a white solid (190 mg, 0.0975 mmol, 20% yield). ^{1}H NMR (500 MHz, acetone-d_6, 20 °C): δ (ppm) 7.04 (t, J = 8.2 Hz, 1H), 6.26 (dd, J = 8.2, 2.1 Hz, 2H), 6.21 (s, 1H), 3.51 (t, J = 7.2 Hz, 8H), 2.29–2.37 (m, 8H), 1.91–1.97 (m, 8H). ^{13}C NMR spectra could not be obtained due to low solubility. ^{19}F NMR (471 MHz, acetone-d_6, 20 °C): δ (ppm) -81.66 (t, J = 9.8 Hz, 3F), -114.47 (m, 2F), -122.25 (m, 2F), -122.48 (m, 4F), -123.29 (m, 2F), -123.95 (m, 2F), -126.76 (m, 2F). DART MS: m/z calcd. for $[C_{50}H_{28}N_2F_{68} + H]^+$: 1949.1239, found: 1949.1227.

Synthesis of F-BHQ. Compound **1** (80 mg, 0.0411 mmol) was added to a dried flask, and dissolved in acetone (2 mL). Fast Black K salt (50 mg, 0.120 mmol) was added in portions,

followed by MeOH (0.4 mL). The reaction mixture immediately turns purple. The reaction mixture was monitored by TLC and stirred at room temperature for 2 h. Then, the reaction was filtered through a short pad of silica gel with AcOEt as eluent and evaporated to dryness. The residue was triturated twice with hot MeOH (10 mL × 2) to provide **F-BHQ** as a dark purple solid (59 mg, 0.0261 mmol, 65% yield). ^{1}H NMR (500 MHz, acetone-d_6, 20 °C): δ (ppm) 8.46 (d, J = 8.8 Hz, 2H), 8.12 (d, J = 8.8 Hz, 2H), 7.82 (d, J = 9.5 Hz, 1H), 7.56 (s, 1H), 7.40 (s, 1H), 6.68 (dd, J = 9.5, 2.3 Hz, 1H), 6.55 (d, J = 2.3 Hz, 1H), 4.03 (s, 3H), 3.99 (s, 3H), 3.70–3.82 (m, 8H), 2.29–2.46 (m, 8H), 1.96–2.04 (m, 8H). ^{13}C NMR spectra could not be obtained due to low solubility. ^{19}F NMR (471 MHz, acetone-d_6, 20 °C): δ (ppm) -81.65 (m, 12F), -114.40 (m, 8F), -122.25 (m, 8F), -122.47 (m, 16F), -123.29 (m, 8F), -123.88 (m, 8F), -126.77 (m, 8F). MALDI TOF-MS: m/z calcd. for $[C_{64}H_{39}F_{68}N_7O_4 - H]^+$: 2260.1905; found: 2260.1954. Elemental Analysis calcd. for $C_{64}H_{39}F_{68}N_7O_4$ (%): C, 33.98; H, 1.74; N, 4.33. Found: C, 34.32; H, 1.73; N, 4.41. UV/Vis (HFE7500): λ_{max} (log ε) = 333 (4.13), 537 (4.50).

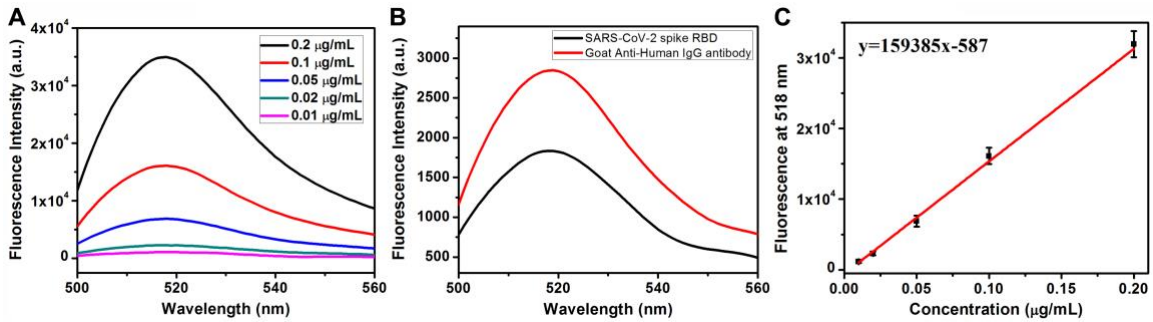


Figure S1. (A) Fluorescence emission spectra (λ_{ex} = 490nm) of Goat Anti-Human IgG-FITC at different concentrations; (B) fluorescence emission spectra (λ_{ex} = 490 nm) of re-dissolved dried droplets; (C) calibration curve (correlation of fluorescence intensity at 518 nm and the concentration of Goat Anti-Human IgG-FITC).

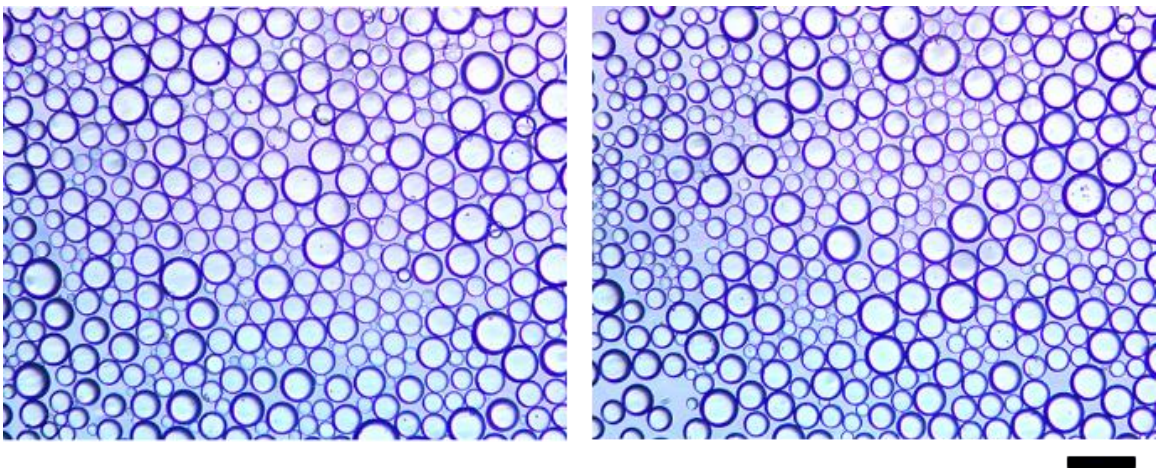


Figure S2. Optical images of P-TCO Janus droplets after bioconjugation with Goat Anti-Human IgG. (scale bar = 50 μm).

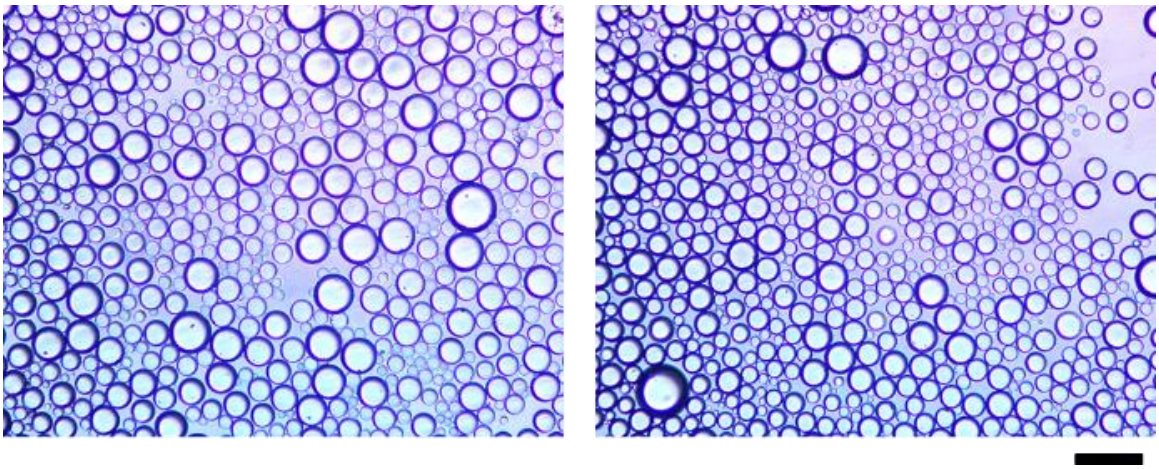


Figure S3. Optical images of P-TCO Janus droplets after bioconjugation with SARS-CoV-2 spike RBD. (scale bar = 50 μm).

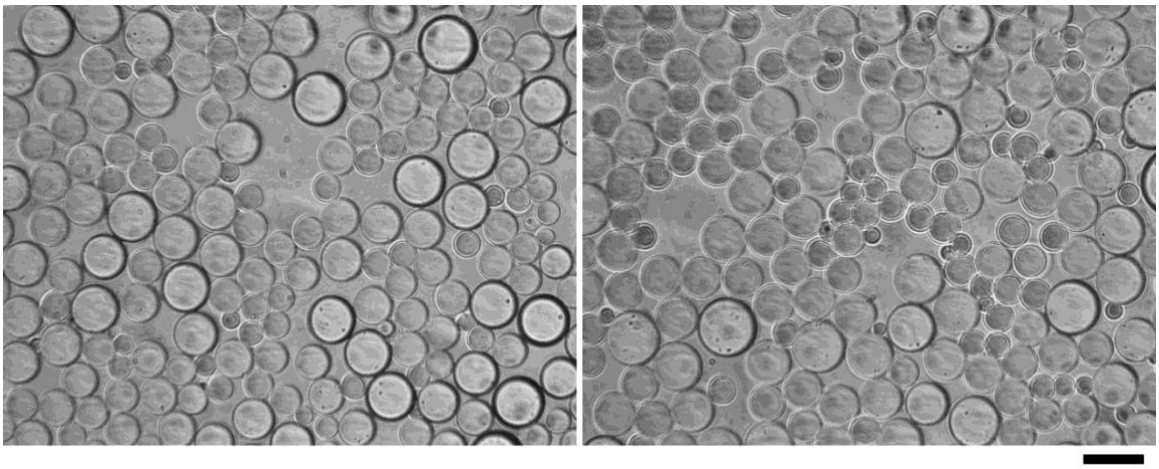


Figure S4. Optical image of polydispersed Janus droplets with addition of serum for 2h. (scale bar = 50 μm).

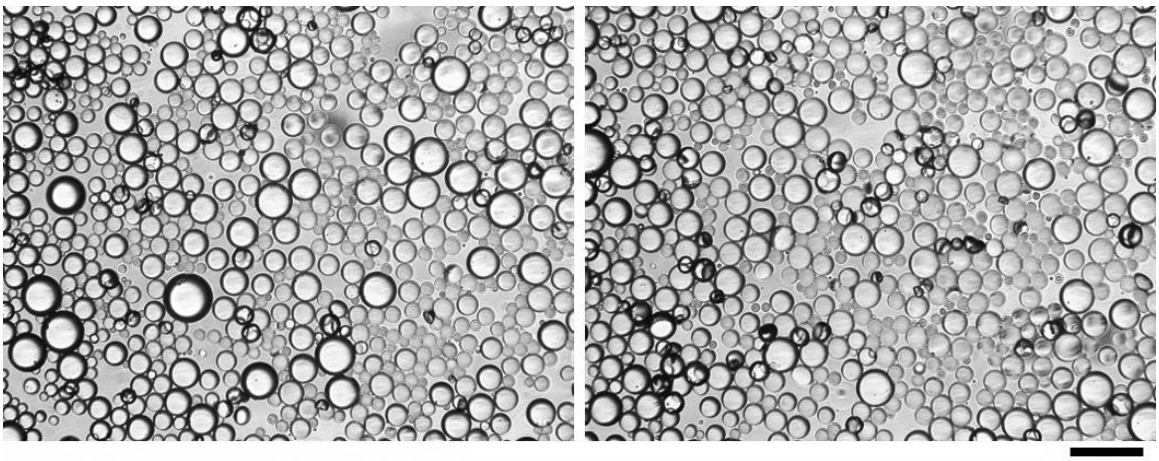


Figure S5. Agglutination assay achieved by adding 60 μg/mL of anti-SARS-CoV-2 spike IgG antibody into the continuous phase of Janus droplets bioconjugated with SARS-CoV-2 spike protein RBD.

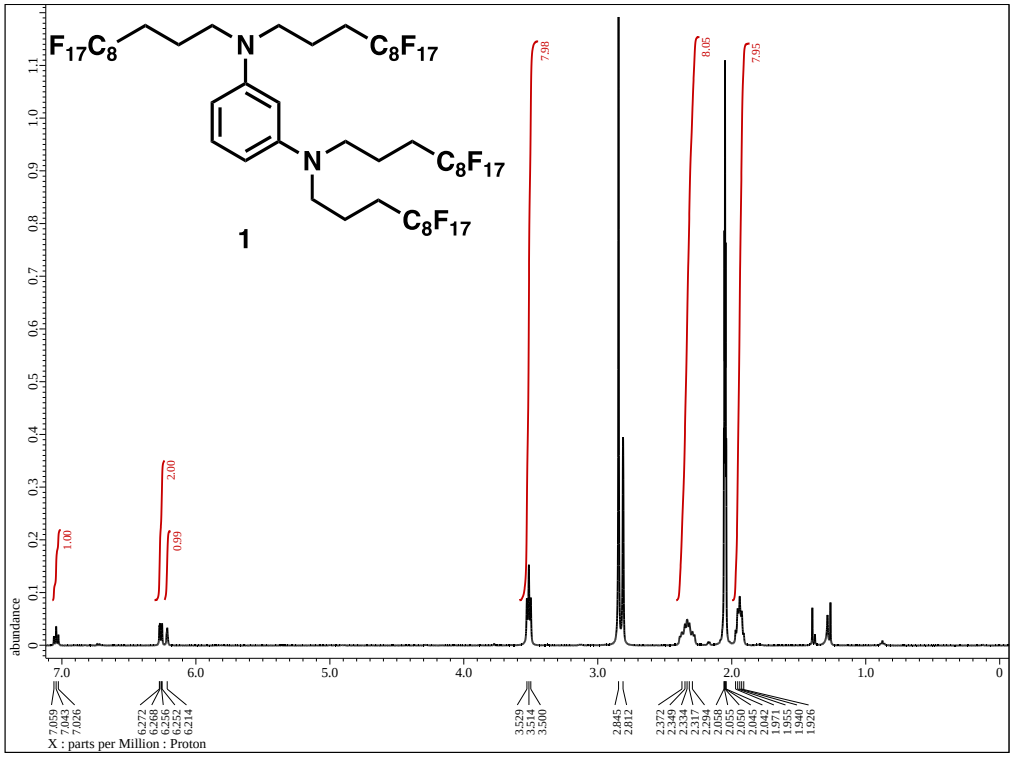


Figure S6. ^{1}H NMR of Compound **1** (500 MHz, acetone-d_6, 20 °C)

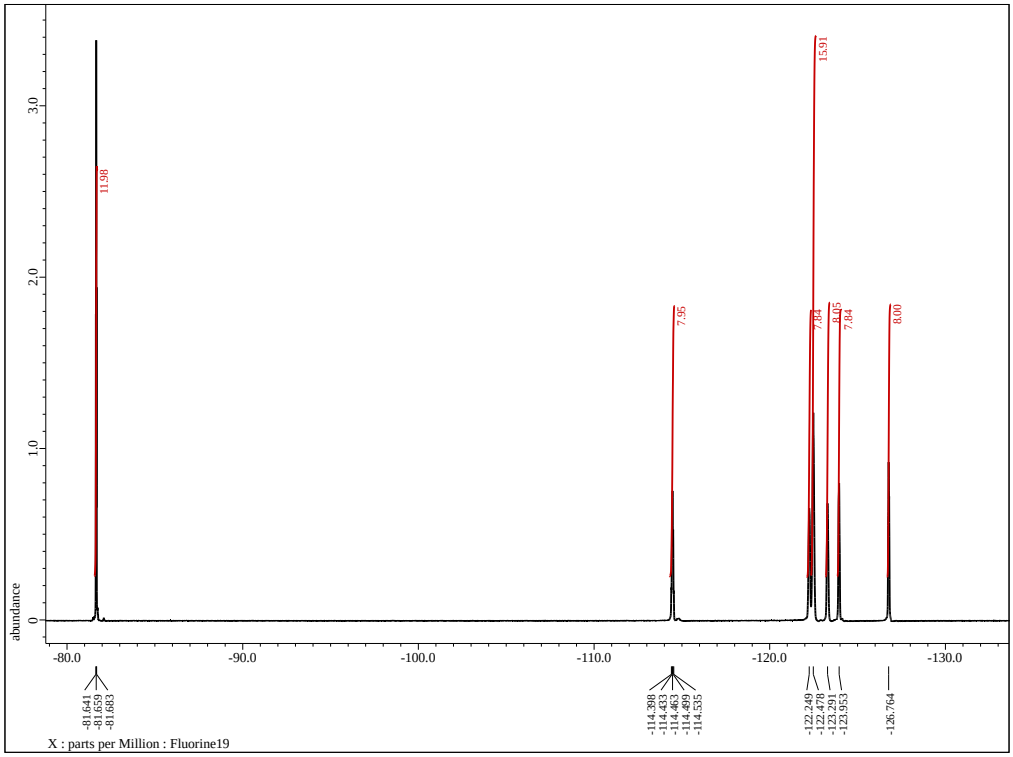


Figure S7. ^{19}F NMR of Compound **1** (471 MHz, acetone-d_6, 20 °C)

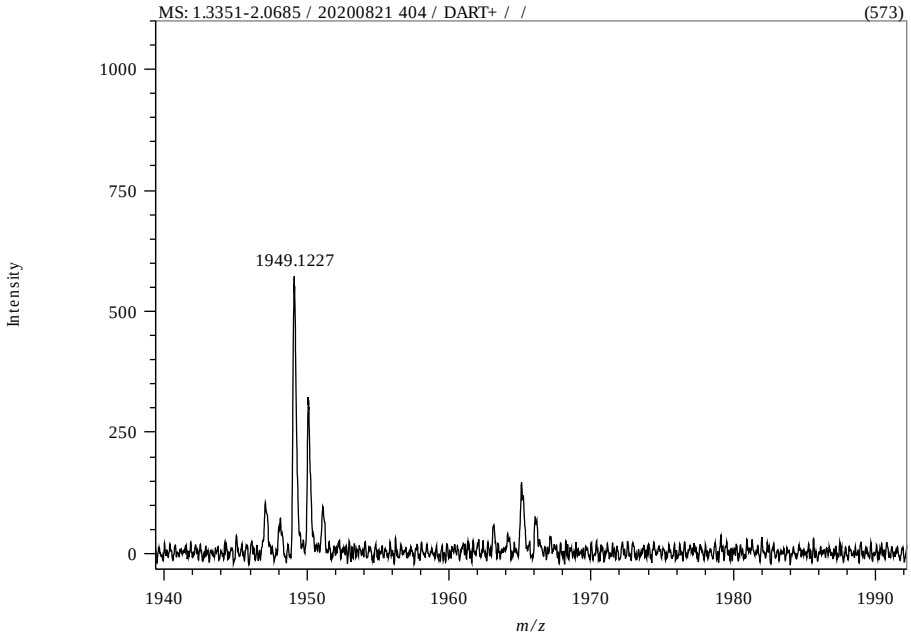


Figure S8. DART MS of Compound **1**.

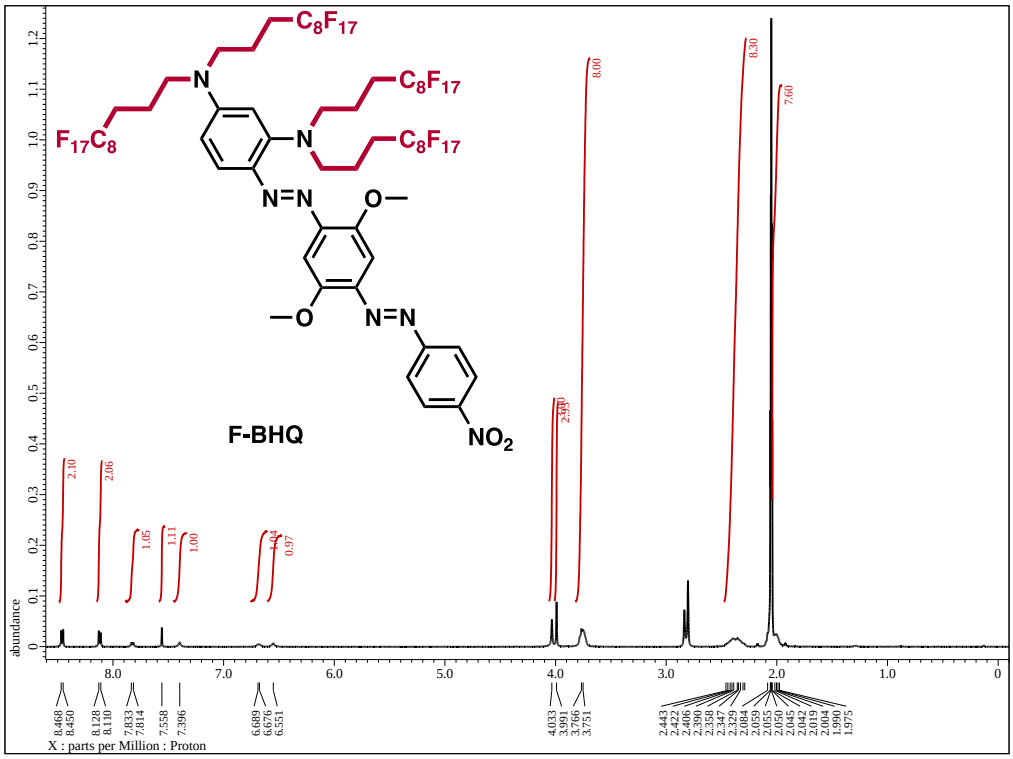


Figure S9. ^{1}H NMR of F-BHQ (500 MHz, acetone-d_6, 20 °C)

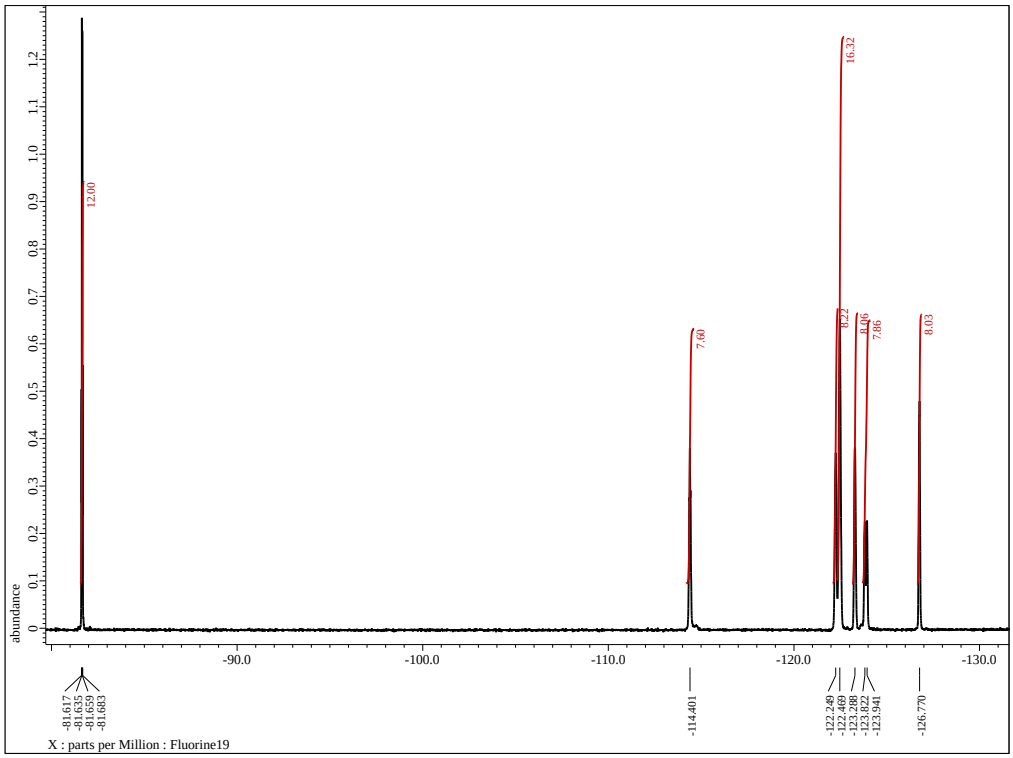


Figure S10. ^{19}F NMR of F-BHQ (471 MHz, acetone-d_6, 20 °C).

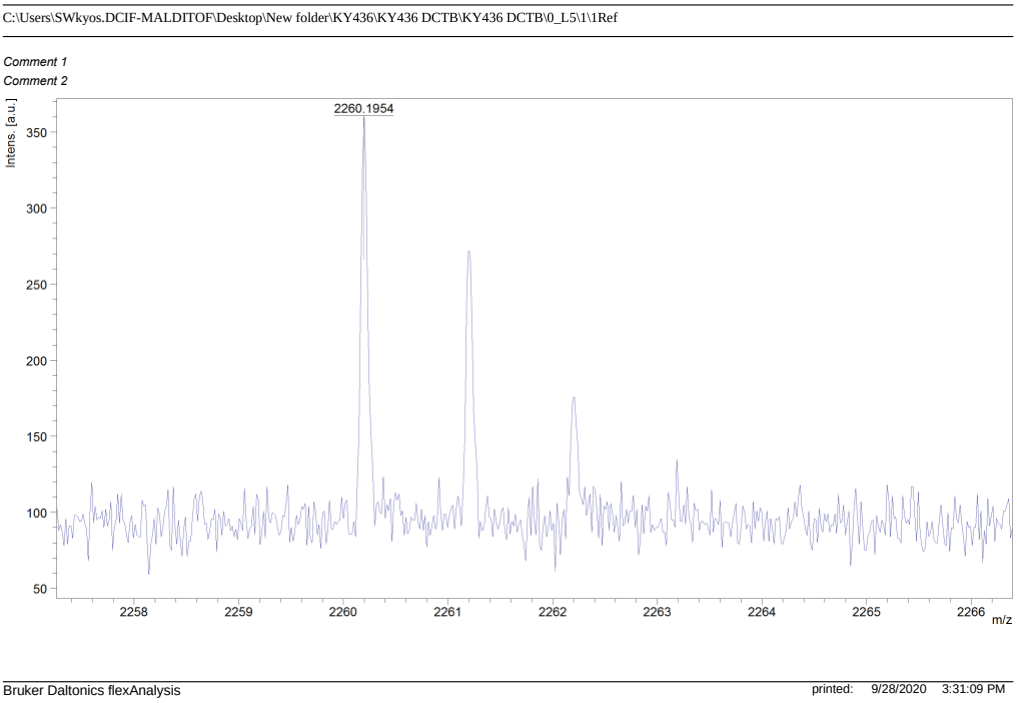


Figure S11. MALDI TOF-MS (Matrix: DCTB) of F-BHQ.

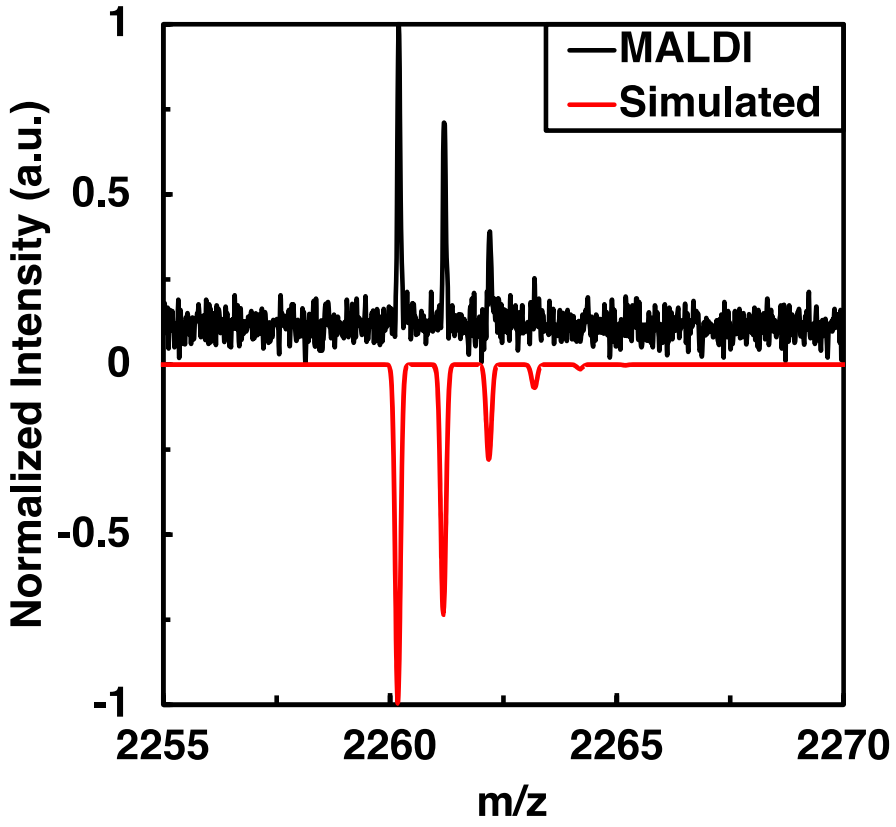


Figure S12. MALDI TOF-MS Isotope Distribution Comparison of F-BHQ.

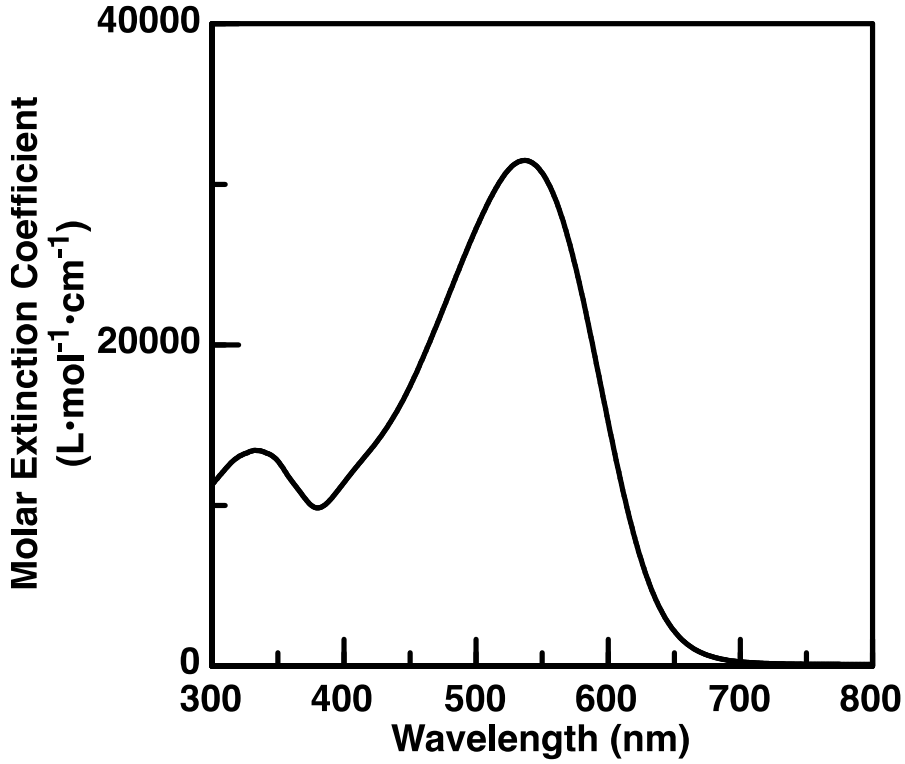


Figure S13. UV/Vis absorbance spectra of F-BHQ (solvent: HFE7500).

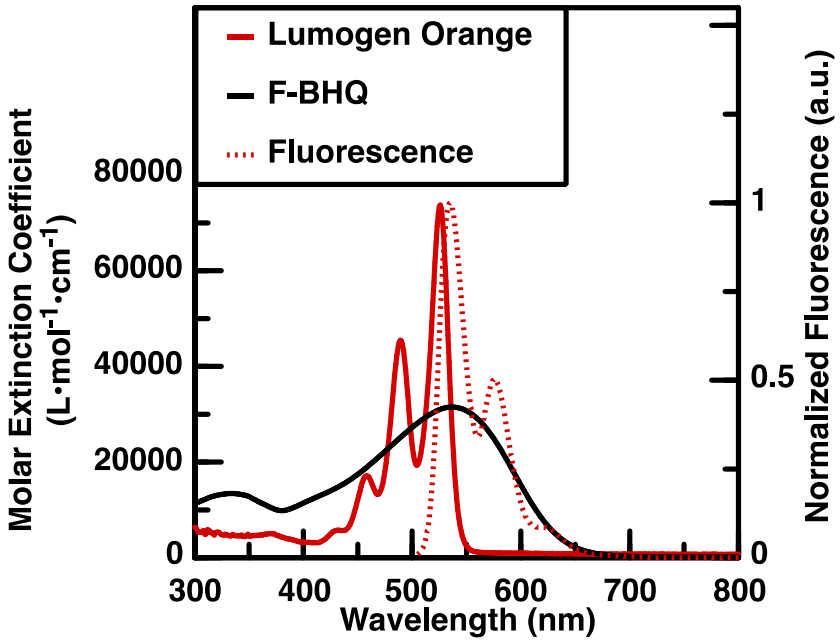


Figure S14. UV/Vis spectra of Lumogen F Orange 240 in diethylbenzene and F-BHQ in HFE7500, overlaid with fluorescence specrum of Lumogen F Orange 240 in diethylbenzene.

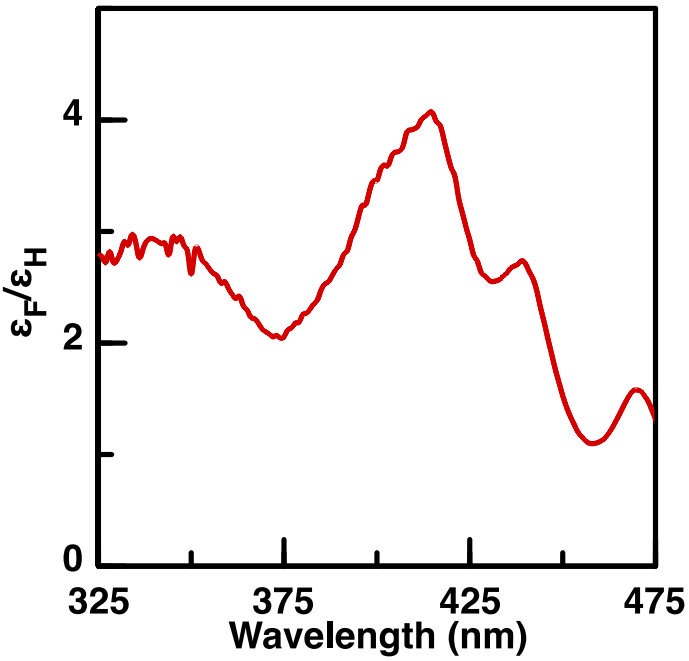


Figure S15. Ratio of molar extinction coefficient of Lumogen Orange (ε_H) and F-BHQ (ε_F).

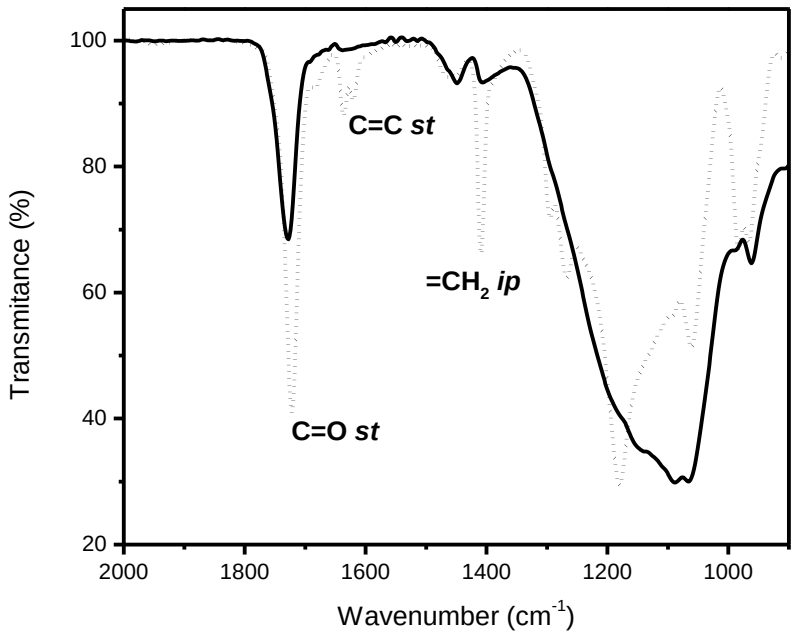


Figure S16. ATR FTIR spectra of the monomer mixture (dotted line) and the photopolymerized solid Janus particles (solid line).

Table S1. Detail information about the clinical samples.

Sample	Age	Sex[a]	COVID-19 test result	Days since symptom onset
P1	92	F	positive	33
P2	78	F	positive	34
P3	76	F	positive	33
P4	76	F	positive	34
P5	60	F	positive	33
P6	50	M	positive	34
P7	18	F	positive	18
P8	62	M	positive	34
P9	56	F	positive	34
P10	44	M	positive	25

[a]F stands for female, M stands for male.

Table S2. Quantification of sensitivity and specificity of the test.

Test	Specificity (%)	Sensitivity (%)	Positive sample number	Negative sample number
Agglutination assay	100	100	10	5

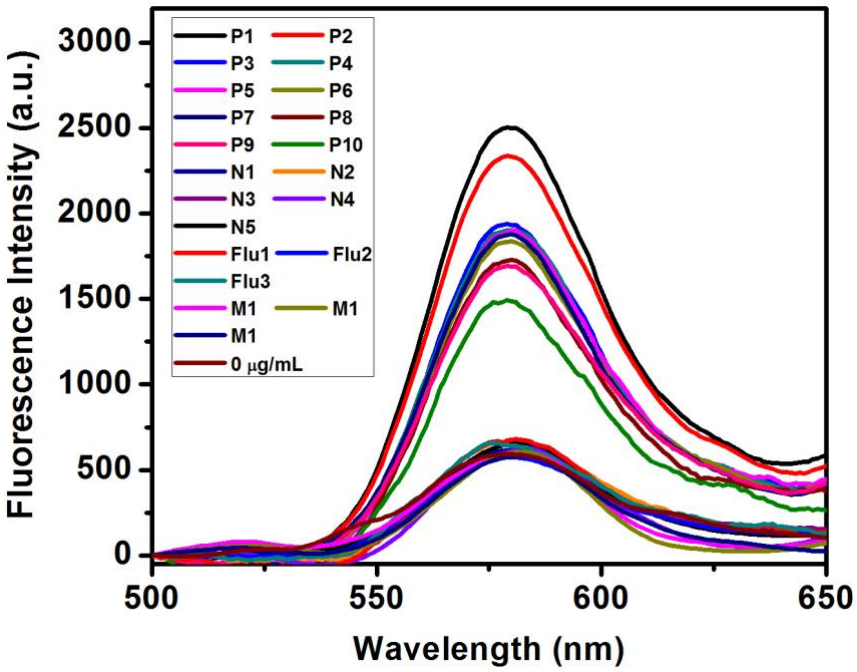


Figure S17. Fluorescence spectra (λ_{ex}=361nm) of 1:1 mixture of dye containing fully bioconjugated Janus droplets with addition of human sera. P1 to P10 stands for sera from COVID-19 patients, N1 to N5 stands for sera from normal patients who are negative for SARS-CoV-2, Flu1 to Flu3 stands for sera from normal patients who are negative for SARS-CoV-2 but have anti-Influenza antibody and M1 to M3 stands for sera from normal patients who are negative for SARS-CoV-2 but have anti-MERS-CoV spike antibody.

References

1. Li, J.; Savagatrup, S.; Nelson, Z.; Yoshinaga, K.; Swager, T. M., Fluorescent Janus Emulsions for Biosensing of Listeria Monocytogenes. *Proc. Natl. Acad. Sci. U.S.A.* **2020,** *117* (22), 11923-11930.

2. Zarzar, L. D.; Sresht, V.; Sletten, E. M.; Kalow, J. A.; Blankschtein, D.; Swager, T. M., Dynamically Reconfigurable Complex Emulsions Via Tunable Interfacial Tensions. *Nature* **2015,** *518* (7540), 520-524.