**2. EXPERIMENTAL PROCEDURES**

**General Procedure for the Preparation of Janus Emulsions.** Janus emulsions were fabricated using bulk emulsification, which generates polydisperse droplets with highly uniform morphology and composition. The disperse and continuous phases consists of 1 mg/mL of PDBCO-PPEG-*b*-PBn or PN3-PPEG-*b*-PBn in DEB and HFE-7500 combined in a 1:1 volume ratio and 0.1 wt% Tween 20:0.1 wt% Zonyl in 1X PBS (10 mM, pH = 7.4), respectively. The disperse phase was heated above the upper critical solution temperature (42 °C) until the two phases become miscible. The miscible disperse phase (30 μL) was rapidly added to 500 μL of warm continuous phase and subsequently emulsified via a vortex mixer at 3500 rpm for five seconds. A 4:6 volume ratio of 0.1 wt% CTAB:0.1 wt% Zonyl in 1X PBS was used as the continuous phase for droplets that will be further modified with DNA.

**General Procedure for Droplet Bioconjugation.** rcSso7d protein or antibody was added to droplet solutions consisting of 30 μL of droplets and 500 μL of continuous phase and incubated overnight on an orbital shaker (100 rpm). After incubation, the continuous phase was solvent exchanged five times with fresh continuous phase solution to remove unreacted rcSso7d protein or antibody. For droplets with additional PEG subunits, poly(ethylene glycol) methyl ether azide (PEG-N3) in 1X PBS (1 mM) was added to the rcSso7d-conjugated droplet solution and incubated overnight on an orbital shaker (100 rpm). The continuous phase was then solvent exchanged five times with fresh solution to remove unreacted PEG-N3.

Droplets functionalized with rcSso7d-IL6 proteins purified via a Ni2+ resin and PEG-N3 utilized 1 nmol of protein and 25 nmol of PEG-N3 in 1X PBS in the bioconjugation step unless otherwise stated. Droplets functionalized with rcSso7d-IL6 proteins that underwent additional FPLC purification and PEG-N3 utilized 0.2 nmol of protein and 25 nmol of PEG-N3 in 1X PBS in the bioconjugation step unless otherwise stated. Droplets functionalized with anti-IL-6 antibodies utilized 0.2 nmol of antibody and 25 nmol of PEG-N3 (when applicable) in 1X PBS in the bioconjugation step.

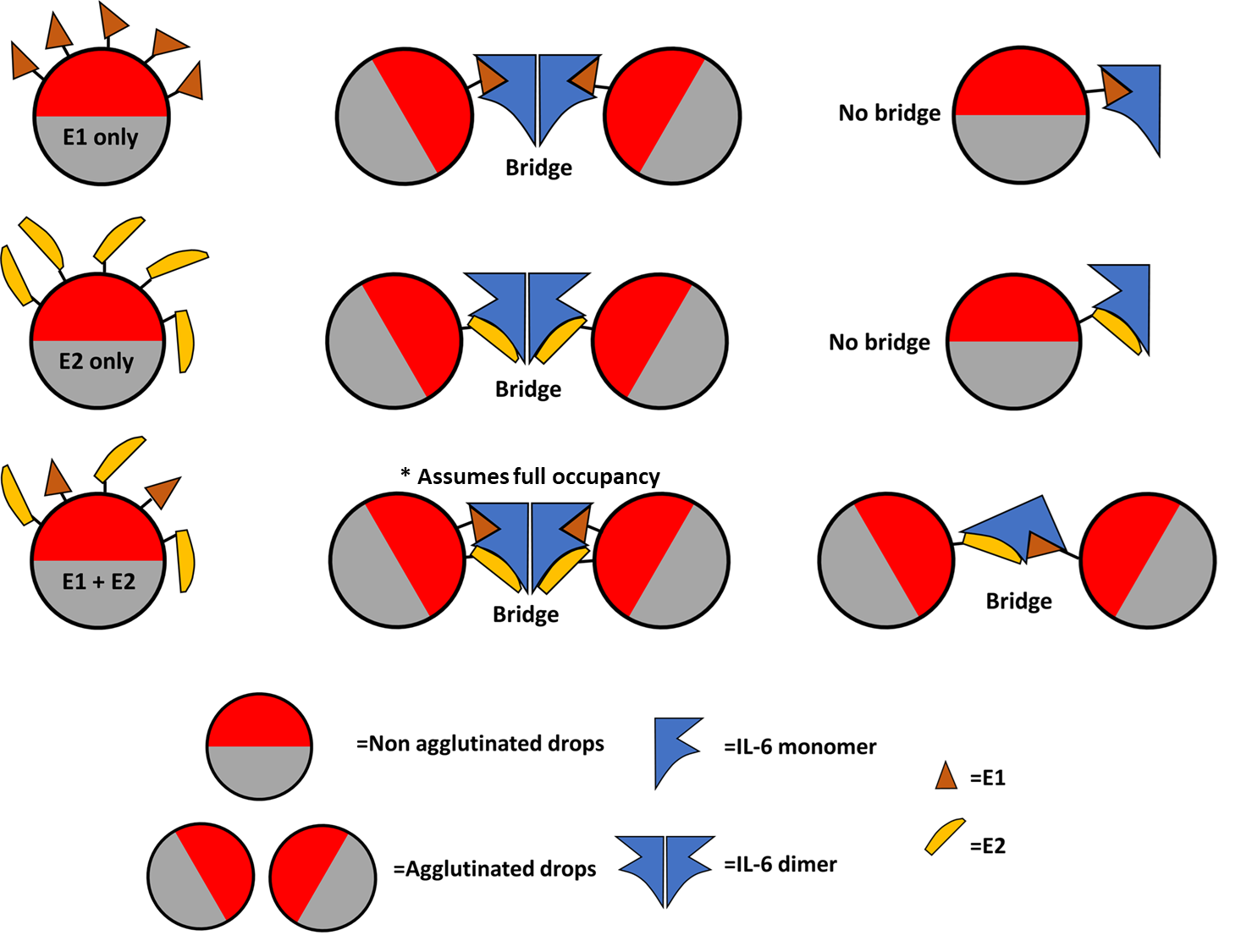
Droplets functionalized with DNA were fabricated as follows: 6 μL of 1 mM 5’-hexynyl-AAA AA-FAM-3’ reconstituted with nuclease free water, followed by 7.5 μL of 2:1 volume ratio of 50 mM THPTA:20 mM CuSO4, and 25 μL of 100 mM sodium ascorbate were added to 20 μL of droplets (with PN3-PPEG-*b*-PBn in the disperse phase) in 500 μL of continuous phase (4:6 volume ratio of 0.1 wt% CTAB:0.1 wt% Zonyl in 1X PBS). The suspension was incubated overnight at an orbital shaker (100 rpm). After incubation, the continuous phase was solvent exchanged five times with fresh continuous phase solution to remove unreacted DNA and excess reagents.

**General Procedure for Agglutination Assays.** For each sensing experiment, the glass surface of a Thermo-Fisher Scientific Invitrogen Attofluor Cell Chamber was wetted with surfactant solution prior to depositing the droplets. For single droplet variant agglutination studies, 14 μL of droplets were used. For two droplet variant studies, 7 μL of each type of droplet variant was used. Once the droplets were deposited onto the imaging dish, the droplets were spread out across the dish. Depending on the experiment different volumes of analyte (IL-6 or CRP) was added to the experimental setup (total continuous phase volume = 500 μL). Specifically, 10 μL of human serum and 104 μL of 2.4 mg/mL CRP were added as analyte for the correspond control experiments; a 0.1 w/v% solution of BSA in the continuous phase was prepared for the 1 mg/mL BSA control experiment. If necessary, analyte solutions were diluted with 1X PBS prior to addition. The droplets were incubated for specified amounts of time and orientational changes were recorded using an inverted microscope.

For assays included in the response curves, 475 uL of continuous phase followed by 14 uL of droplets was added to each imaging dish. To keep the surfactant concentration constant across each assay, the corresponding amount of IL-6 was added followed by a few microliters of 1X PBS. Ideal images should have droplet covering the entire window; however, as the droplets agglutinate together, there are often sparse regions in the dish. Therefore, for the overnight images, the dish is sometimes tilted slightly (<10°) to fill in the sparse regions.

*Potential Modes of Binding*

**Figure S1.** Representative image of potential binding schemes of singly (E1/E2 only) functionalized droplets which can only productively bind the dimer of IL-6 on both sides resulting in a bridge between droplets, and doubly (E1+E2) functionalized droplets which can productively bind both the dimer and monomer to promote agglutination. \* indicates that a productive bridge could be formed with only one binder on each side of IL-6, but can potentially be bound by four total binders.



**Microscope Images.** Optical images of the agglutination studies were collected using an inverted microscope equipped with an AmScope camera. Side-view images of the droplets were collected using a custom-built horizontal microscope with an Olympus 50x objective, a Thorlabs tube lens (effective focal length = 200 nm), and an Allied Vision Prosilica GT camera. The droplets were deposited into a 0.1 mm demountable quartz cuvette (Starna Cells, Inc.) and illuminated from the side using a Fiber-Lite M1-152 lamp for bright field images with a white background. Confocal fluorescence images of the droplets were collected on a Zeiss LSM 700 Laser Scanning Confocal Microscope at 10% power. Fluorescence microscope images were taken on droplets functionalized with 0.5 and 2.8 nmol of FITC-labeled E1 and FITC-labeled E2, respectively, which were added to 20 and 30 μL of droplets, respectively.