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Interfacial bioconjugation on emulsion droplet for biosensors

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Abstract

Interfacial bioconjugation methods are developed for intact liquid emulsion droplets. Complex emulsion droplets having internal hydrocarbon and fluorocarbon immiscible structured phases maintain a dynamic interface for controlled interfacial reactivity. The internal morphological change after binding to biomolecules is readily visualized and detected by light transmission, which provides a platform for the formation of inexpensive and portable bio-sensing assays for enzymes, antibodies, nucleic acids and carbohydrates.

Keywords

Bioconjugation; Biosensors; Interfacial functionalization; Emulsions; Janus particles

1. Introduction

Biosensors play an important role in medical sciences and drug discovery, with applications in healthcare, pharmaceuticals, food safety and environmental monitoring.^{1,2} Biosensors take on many forms and the selectivity can be enzyme-based,³ tissue-based,⁴ antibody-based,⁵ and/or DNA-based.⁶ However, central to all biosensors is the need for a transduction mechanism coupled to the recognition. Electrochemical,^{7,8} piezoelectric,⁹ and fluorescent¹⁰ signals are attractive because they can be quantitatively detected with inexpensive and compact equipment often without requiring complicated labelling processes. There has not been uniform success in meeting these latter goals and current biosensors have limitations preventing large scale applications wherein high speed and analysis of unprocessed samples are required, such as on-site real-time environmental monitoring and food pathogen detection.

We report herein a method for interfacial functionalization on dynamic complex emulsion droplets for biosensing applications. We have identified dynamic complex emulsion droplets as powerful liquid sensing particles that can be manufactured in large scale and display

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A. Supplementary data

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intrinsic optical properties for the creation of large sensing signals. These properties stem from changes in interfacial tensions or analyte induced organizations of droplets. The first was initially explored with stimulus-responsive surfactants that give dynamic complex emulsions capable of undergoing morphological switching.¹¹ Using pathogen induced changes in the directional alignment of carbohydrate functionalized emulsion droplets, we have reported smartphone detection of *E. coli*.¹² To expand the utility of emulsion droplets assays for the detection of other biomolecules/organisms, expanded method for the integration of recognition or transduction functionality into these systems is needed. One issue is that the general production of precision liquid colloids is carried out by dispersing water insoluble liquids into water containing surfactants that assemble spontaneously at the interface. Hence, to create new generations of functional interfaces, new methods are needed. To this end we have identified trialkylgallic acid (GA) groups as synthetically accessible reactive anchors and have combined these groups with well-known bio-functional schemes based upon *N*-hydroxysuccinimide (NHS) for amines and maleimide (MA) for thiols. A unique challenge in the functionalization of complex liquid emulsion droplets is to affect transformation of morphologies while preserving the overall structural integrity of individual droplets. This delicacy is however a key advantage of liquid colloids that maintains dynamic and compliant surfaces that mimic the properties of natural live cells. The intrinsic morphology-dependent optical properties of emulsion droplets¹³ enable optical biosensors without fluorescent labelling of the target analytes. By design the emulsions are stable under the sensing conditions and we seek to create assays for bacteria, enzymes, antibodies and DNAs that can be used without the need of complex and specialized equipment.

2. Emulsion assays and surfactants design

2.1. Dynamic complex emulsions

Complex emulsions are fabricated at temperatures above the upper critical solution temperature of the internal phases to create materials with precisely determined compositions.¹¹ Specifically, droplets containing equal volume of hydrocarbon (diethylbenzene) and fluorocarbon (HFE7500) liquid are emulsified around 40 °C, which is above T_c (37 °C) in an aqueous continuous phase containing Zonyl FS-300 (hereafter 'Zonyl'), which is a nonionic fluorosurfactant. Surfactants lower the interfacial tension between two immiscible liquids and stabilize emulsion droplets. Droplets containing both hydrocarbon and fluorocarbon will switch morphologies between H/F/W (hydrocarbon-in-fluorocarbon-in-water), Janus, and F/H/W (fluorocarbon-in-hydrocarbon-in-water), with changes in the relative strength of the fluorocarbon and hydrocarbon surfactants (Scheme 1).

2.2. Surfactant design

Creating surfactant molecules that interact with analytes is the key to create couplings to droplet morphology and orientation. The orientation in the absence of perturbation is typically controlled by the density differences of the internal phases and gravity. The droplets are dynamic lenses and both morphology and orientation produce large optical signals. In our previous studies, we created assays that transduce enzymatic modification of designer surfactants¹⁴ and the binding of bacteria and proteins to carbohydrate surfactants.¹²

Optical transduction in these cases makes use of the light transparency because vertically aligned Janus droplets with internal phases having specific refractive indices. A transparency to highly scattering state is triggered by small distortions in droplet morphology or agglutination (tilting).¹³ To extend these methods, we detail herein a generic surfactant platform, shown in Scheme 2, for droplet bioconjugation with proteins, nucleic acids, and carbohydrates. The three alkyl chains in the tridodecyl gallic acid shown (GA12OH) provide for a robust hydrophobic anchor and the carbonyl based functional site provides for bioconjugation. We also show that the gallic structure is an intrinsic surfactant molecule that provides sufficient stability to prevent speciation of the generic reactive droplets. This design builds on the observation that aromatic rings with peripheral alkane chains organize at the oil-water interfaces to enhance the effectiveness at lowering interfacial tensions.¹⁵

To validate the interfacial behavior of the gallic derived surfactant, emulsions with and without GA12OH in the hydrocarbon oil phase were produced in the same continuous phase (0.01 wt% Zonyl in PBS buffer). The pristine emulsion droplets without GA12OH, are in double emulsion morphology (Scheme 1, H/F/W), namely hydrocarbon-in-fluorocarbon-in-water, whereas the emulsion droplets with 10 mg/mL GA12OH dissolved in the hydrocarbon phase appear in Janus configuration (Scheme 1, Janus). These results confirm that GA12OH is a good surfactant that lowers the surface tension at the oil/water interface. Aside from helping with the control droplet formation, this feature indicates that the carboxylic acid groups are presented at the aqueous interface for chemical modification.

3. Emulsion assays for bioconjugation

3.1. Interfacial functionalization on emulsion droplets

After confirming the surfactant behavior of GA12OH, the chemical reactivity at the droplet interface was studied using the EDC/NHS coupling reaction. As shown in Scheme 3a, GA12OH was loaded into the droplet phase and the droplets adopted a Janus morphology. EDC and NHS solutions were then added in the continuous phase. Fluoresceinamine was used as a model reactant for biomolecules bearing amine groups, and upon addition to the water phase reacts with the *in situ* generated NHS ester. The unreacted fluoresceinamine in the continuous water phase is removed by washing the droplets and a bright green fluorescence was observed at the hydrocarbon-water interface with confocal microscopy. The localized green fluorescence is attributed to the fluoresceinamine-NHS reaction to form a covalent amide bond at the droplet interface.

Another dye, Sulfo-Cyanine 3 amine is separately functionalized to the droplet surface using the same method described above. When two batches of droplets functionalized with different dyes were combined together, no sign of mixing dyes was observed under microscopy even after extended period of time. This further confirms the covalent bond formation at the hydrocarbon-water interface and that we can produce droplets that do not fuse or transfer functional groups between them. This latter feature is particularly important for multiplexed detection schemes.

3.2. GA12-NHS assay for amine conjugation

To investigate the scope of interfacial functionalization, surfactant GA12-NHS was pre-synthesized and dissolved in the droplet hydrocarbon phase (Scheme 3b). Trifluoroethanol was added to the hydrocarbon and fluorocarbon droplet phase to lower the upper critical mixing temperature. After droplets are formed in the water phase, trifluoroethanol partitions into the continuous phase and internal phases then undergo phase separation to produce double emulsions. We expect that the GA12-NHS has some partitioning to hydrocarbon-water interface as a result of its surfactant behavior. The continuous phase was exchanged twice to remove the trifluoroethanol. This is facilitated because the droplets are denser than water and remain on the bottom of flask. The continuous phase solvent exchange does not affect the stability nor the morphology of the droplets. We find that fluoresceineamine functionalization with pre-synthesized GA12-NHS has a higher yield and resulted in 50% more intense fluorescence under confocal microscopy relative to an internal dye reference (described in Section 3.5). As a result, GA12-OH and *in situ* NHS formation is not quantitative.

3.3. GA16-MA assay for thiol conjugation

To implement a maleimide-thiol bioconjugation scheme, GA16-MA (Scheme 3c) was pre-synthesized and loaded into the droplets. The longer hexadecyl chains were needed to increase the surfactant GA16-MA solubility in hydrocarbon phase. BODIPY-FL-Cysteine was used as the reactive model compound for biomolecules bearing thiol groups. Upon addition (Scheme 3c), this dye was covalently linked to the surface of the droplets and bright fluorescence from the BODIPY dye was observed at the hydrocarbon-water interface as shown in Fig. 1. In the control experiments under the same conditions without GA16-MA in the droplet phase, no fluorescent ring was observed by confocal microscopy (shown in Supporting information Fig. S2).

3.4. Controlled conjugation reactivity of the emulsion assays

Our complex emulsion assays rely exclusively on the interfacial functionalization and the recognition characteristics thereof. The droplet interface is dynamic and the morphology switches between H/F/W, Janus, and F/H/W with changes in the interfacial tensions between hydrocarbon-water interface and fluorocarbon-water interface.¹⁴ Another advantage of the emulsion droplets is that they can provide hydrolytic stability for the reactants that are localized in an internal phase that initially doesn't share an interface with water. With controlled activation the reactant can be used for functionalization at later times.

As shown in Scheme 4, GA16-MA and BODIPY-FL-Cysteine was used to demonstrate the controlled interfacial conjugation. Tween 20 was chosen as the continuous phase "activating" hydrocarbon surfactant. Together with Zonyl as the continuous phase fluorocarbon surfactant, it is possible to tune the morphology of the droplets to facilitate maleimide-thiol conjugation. Tween 20 was chosen because of its mild surfactant behavior, which means it will not completely cover the hydrocarbon-water interface but is still able to change the morphology of the droplets. In the H/F/W morphology state, wherein the hydrocarbon oil was encapsulated inside the fluorocarbon phase, GA16-MA is rendered inert. When the droplet morphology was switched to Janus or F/H/W by the addition of

Tween 20, the hydrocarbon phase now has a reactive interface with water and the interfaces are activated for functionalization reactions.

3.5. Quantification of the interfacial conjugation reaction

To further quantify the level of covalent functionalization at the interface, anthracene with different emission wavelength from BODIPY was used as an internal fluorescent reference to indicate the amount of fluorophore functionalized at the droplet interface. As shown in Scheme 4, the droplets with different starting morphology were functionalized with BODIPY using maleimide-thiol chemistry. After the reaction, the morphology of the droplets was tuned to the exact Janus state for confocal imaging by changing the continuous phase surfactant with either Zonyl or Tween 20. The fluorescent intensity of both fluorophores are analyzed through the open access software ImageJ (National Institute of Health, Bethesda, MD, USA; available for download at <https://imagej.nih.gov/ij/>) and the relative intensity ratio between $E_{\text{anthracene}}$ and E_{BODIPY} was plotted against the initial droplet morphology, indicated by relative surfactant ratio f_{Tween} (Fig. 2). The more surface area at the hydrocarbon-water interface during conjugation, the more thiol is functionalized as indicated by the BODIPY fluorescent intensity.

4. Biomolecule functionalized emulsion assays

4.1. Protein A functionalized assay for IgG detection

We have shown that the emulsion droplets containing GA based reactive surfactants can be functionalized with molecules bearing free amine or thiol group. Functionalization with biomolecules is necessary to achieve broad utility in biosensing assays. As a proof-of-concept, we have targeted a Protein A functionalized emulsion assay for the binding of anti-mouse IgG. As shown in Scheme 5, Protein A reacts through amines with Janus emulsion droplets containing GA12-NHS. After functionalization, the emulsion droplets maintained a Janus morphology (Fig. 3a). Addition of FITC (fluorescein isothiocyanate) labelled anti-mouse IgG to the continuous phase results in binding to Protein A on the surface of droplets. This modification resulted in a change in droplet morphology from Janus to a F/H/W double emulsion (Fig. 3b). The binding of IgG to the droplet surface was further validated with confocal microscopy. The bright green fluorescence from the FITC labelled IgG was observed under confocal microscopy and was only located at the hydrocarbon-water interface (Fig. 3c). We rationalize that the large IgG molecule provides additional hydrophilic character, which increases the surfactant strength at the hydrocarbon-water interface, thereby expanding the organic water interface. The GA12-NHS is therefore established as an active biomolecular reactive group for functionalization of the hydrocarbon-water interface.

This morphology change from Janus (transparent) to F/H/W (opaque) is easily visualized with the transmission of natural light through thin gravity aligned layers of emulsion droplets without fluorescent labelling.^{12,14} We have shown the change qualitatively using the QR code method before (Fig. 3d) and after (Fig. 3e) binding of IgG to the protein A functionalized emulsions assay. This morphology change is different from the agglutination scheme reported in our previous study,¹² in a way that all the droplets have changed from Janus to F/H/W instead of forming aggregates. The macroscopic change can still be

visualized using the QR code method but not quantitatively with the graphical counting method. Addition optical methods to quantify the morphology changes are the subject of ongoing studies.

To demonstrate there is covalent bond between the droplet and Protein A/IgG complex, additional Zonyl surfactant was added in the continuous phase to force a morphology change from F/H/W to H/F/W. The Protein A/IgG complex is not dislodged from the hydrocarbon-water interface and the added Zonyl produces a deformation (Fig. 4) from a perfect sphere. If the droplet was not functionalized with proteins, the hydrocarbon phase would become an inner phase of a double emulsion, H/F/W. This experiment also shows that the interface was still dynamic after functionalization of either Protein A or IgG. Preserving a dynamic interface is central to sensing opportunities and producing changes in droplet morphology.¹¹

4.2. Nucleic acid and carbohydrate functionalization

To demonstrate the generic bioconjugation capabilities of the emulsion assay, broader types of biomolecules were functionalized to the droplets (Scheme 6). Confocal Images of the functionalized droplets were included in Supporting Information (Fig. S3).

A strand of oligonucleotide 5'-amine C6 linker modified *P1* was covalently functionalized to the droplet surface with GA12-NHS and amine reaction. A complementary strand *P2* bearing a (6-carboxyfluorescein) 6-FAM tag at the 5' was added in the continuous phase. After removing unreacted oligonucleotide by washing, fluorescence from FAM was observed under confocal microscopy at the hydrocarbon-water interface, which indicates the oligonucleotide still maintains the reactivity.

A mannose bearing amine was functionalized to the droplets using NHS-amine chemistry. Concanavalin A (ConA) labelled with FITC was added in the continuous phase. After washing the excess ConA from the mixture, fluorescence from FITC was observed under confocal microscopy, showing the carbohydrate-lectin binding interactions were preserved.

5. Conclusion

We have designed an emulsion assay capable of bioconjugation using NHS-amine or maleimide-thiol chemistry.

This assay has provided a generic platform for functionalization of biomolecules to the emulsion droplets as biosensors with antibodies, enzymes, nucleic acids and carbohydrates. The dynamic interface and droplet morphologies enable the controlled interfacial reactivity. The detection of IgG with protein A functionalized droplet assay was demonstrated using morphological changes that are easily detected with natural light transmission. Quantitative optical methods and sensing of other targeted biomolecule are the subject of ongoing studies.

6. Experimental section

6.1. General methods and instrumentation

Diethylbenzene (DEB), 2-(trifluoromethyl)-3-ethoxydodecafluorohexane (HFE7500), hydroxylamine, trifluoroethanol, phosphate-buffered saline (PBS, pH = 7.6) (1M), Tris buffer (pH = 8.0), HEPES buffer (pH = 7.6), Zonyl FS-300, fluoresceinamine, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), Concanavalin A-FITC, anti-mouse IgG-FITC and Protein A were purchased from Sigma-Aldrich. BODIPY FL L-Cysteine was purchased from ThermoFisher. Sulfo-Cyanine3 amine was purchased from Lumiprobe. Solvents were purchased from Sigma-Aldrich and used as received. Oligonucleotides were purchased from Integrated DNA Technologies (IDT) and used without further purification.

EDC and NHS were dissolved in PBS at 1 M and prepared fresh before each usage. Continuous phase surfactants were prepared as stock solution in PBS, including 0.1 wt% and 0.01 wt% Zonyl FS-300 as the fluorocarbon surfactant and 0.1 wt% Tween 20 as the hydrocarbon surfactant. Hydroxylamine was prepared as 1 M solution in PBS to quench unreacted NHS groups at the droplet interface.

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). Confocal microscopy images were taken at room temperature with Nikon A1R Ultra-Fast Spectral Scanning Confocal Microscope.

6.2. Emulsion assay preparation

6.2.1. Bulk emulsification for polydispersed complex emulsion droplets—

Complex emulsions, composed of equal volumes of diethylbenzene and HFE7500 in aqueous continuous phase were fabricated using bulk emulsification, which generates polydisperse droplets (20–100 μm in diameter). In this process, the hydrocarbon phase (DEB) and fluorocarbon phase (HFE7500) were mixed and heated above the upper critical temperature (around 40 °C) to generate a single droplet phase. This single droplet phase was then dispersed into the aqueous phase containing the continuous phase surfactants to generate single phase emulsions and upon cooling to room temperature, the DEB and HFE7500 phases separated to generate complex emulsions. The composition of all droplets was identical because every droplet originated from the same single phase.¹¹

A generic assay contains 0.5 mL of continuous phase and 20 μL droplet phase.

6.2.2. GA12OH assay preparation—To generate emulsion droplets containing GA12OH for interfacial functionalization, GA12OH was dissolved with gentle heat at 10 mg/mL in DEB. Polydispersed complex emulsion droplets were fabricated via bulk emulsification described above with 0.01 wt% Zonyl as the continuous phase surfactant. 25 μL of EDC solution was added to the emulsion and reacted for 15 min followed by addition of 25 μL of NHS solution. The reaction was set at room temperature for 1 h on a rocker

(Rocker II from Boekel Scientific). The resulting assay is in equivalent to a GA12-NHS assay.

6.2.3. GA12-NHS assay preparation and fluoresceinamine functionalization—

GA12-NHS was dissolved at 10 mg/mL in DEB. Trifluoroethanol was added at 10% in volume to the hydrocarbon and fluorocarbon mixture to decrease the mixing temperature. A mixture of 0.1 wt% Zonyl: 0.1 wt% Tween 1:1 (v/v) was used as the continuous phase to increase the hydrocarbon-water surface area for bioconjugation. After emulsification, the continuous phase was exchanged twice with the same mixture of 0.1 wt% Zonyl: 0.1 wt% Tween 1:1 (v/v) to remove the trifluoroethanol from the emulsion assay.

Fluoresceinamine was prepared as 0.5 mg/mL solution in PBS. 25 μ L of the fluoresceinamine solution was added to the GA12-NHS assay and reacted at room temperature overnight on a rocker. The continuous phase was washed 5 times after reaction to remove the excess amount of dye before imaging.

Sulfo-Cyanine 3 amine was prepared as 1 mg/mL solution in PBS. 25 μ L of the Sulfo-Cyanine 3 amine solution was added to the GA12-NHS assay. The reaction was carried out overnight at room temperature. Two vials containing different dye functionalized droplets were combined together. The mixture was settled for 48 h before imaging.

6.2.4. GA16-MA assay preparation and BODIPY-FL-Cysteine

functionalization—GA16-MA was dissolved at 10 mg/mL in DEB and the assay was prepared using the same method as described for the GA12-NHS assay.

BODIPY-FL-Cysteine was first dissolved in PBS at 1 mg/mL and activated with TCEP. The activated dye solution become bright green within 15 min and 25 μ L of the activated dye solution was added to the GA16-MA assay. The continuous phase was washed 5 times after overnight reaction to remove excess amount of dye before imaging.

6.2.5. Protein A functionalization and IgG detection—Protein A was dissolved at 0.5 mg/mL in PBS buffer. 25 μ L of the Protein A solution was added to a GA12-NHS assay and reacted overnight at room temperature on a rocker. The reaction was stopped with 25 μ L of hydroxylamine solution to quench any unreacted NHS groups at the droplet surface. The continuous phase was washed with surfactant solution for three times. 25 μ L anti-mouse IgG with FITC label from Sigma-Aldrich was added to the Protein A functionalized assay and reacted for 2 h. The continuous phase was then washed with surfactant solution for five times to remove unreacted IgG before imaging. For the QR code detection method, the emulsion solution before and after IgG addition was images in a stainless steel chamber with a glass bottom. The droplets naturally sit at the bottom of the chamber due to gravity and cover the glass bottom. The QR code was printed on a piece of paper and placed under the chamber.

6.2.6. Oligonucleotide functionalization—GA12-NHS assay was prepared as described in the previous section. 3 μ L of oligonucleotide *P1* with a sequence of 5'-NH₂-(CH₂)₆-TTT TTT TTT T AGA GTT GAG CAT-3' at 2 mM in PBS solution was added in

the continuous phase. The conjugation reaction was carried out overnight at room temperature. The reaction was quenched with addition of 100 μ L of 1 M Tris buffer solution. 3 μ L of complementary strand of oligonucleotide *P2* with a sequence of FAM-5'-TTT TTT TTT T ATG CTC AAC TCT-3' at 1 mM in PBS solution was added. The solution was heated up to 50 °C and held for 15 min using a water bath. The emulsion assay was then allowed to cool down to room temperature and the continuous phase was washed 5 times to remove the unreacted oligonucleotide. During the annealing process, the hydrocarbon and fluorocarbon phase will become miscible and form one droplet phase once heated above the upper critical temperature, but the mixture will phase separate and form complex emulsions again after cooling down.¹¹ This process is completely reversible and the droplets retain their original size.

6.2.7. Carbohydrate functionalization—GA12-NHS assay was prepared as described in the previous section. Man-C5-NH₂ was dissolved in PBS at 1 mg/ml. 50 μ L of Man-5-NH₂ solution was added in the continuous phase and reacted overnight at room temperature. The reaction was quenched with 100 μ L of 1 M Tris buffer solution. 10 μ L of 1 mg/mL FITC labelled Con A in HEPES buffer was added and reacted for 30 min. The solution was then washed 5 times to remove the excess lectin before imaging.

6.2.8. Droplet morphology tuning—The droplets morphology can be tuned by using different compositions of the hydrocarbon/fluorocarbon surfactant mixture. After bioconjugation of the droplets shown in previous sections, addition of hydrocarbon or fluorocarbon surfactant change the morphology of the droplets because the droplets interface maintains a dynamic equilibrium. Droplets are considered to be in the perfect Janus state when there is a single circle to be focused under microscope (Fig. 4a).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Confocal microscopy images of cysteine-BODIPY functionalized droplets using interfacial maleimide-thiol chemistry. Scale bar in 50 μm . a) Confocal z-stack images of emulsion droplets containing GA16-MA after covalent dye functionalization, 10 $\times$ magnification. b) Confocal cross-section of the droplet containing GA16-MA after covalent dye functionalization, 20 $\times$ magnification.

Fig. 2.

The ratio of emission intensity at the interface (E_{BODIPY}) and inside ($E_{\text{anthracene}}$) relative to the continuous tween 20 surfactant concentration. The higher concentration of tween, the more surface area for the hydrocarbon-water interface.

Fig. 3. Protein A functionalized emulsion droplet for the detection of anti-mouse IgG. Scale bar in 50 μm . a) Microscope image of Protein A functionalized droplets in Janus morphology. b) Microscope image of droplet in F/H/W after IgG bind to protein A. c) Confocal cross section image of droplets with IgG at the hydrocarbon-water interface, in F/H/W morphology. d) Protein A functionalized transparent Janus droplets enables scanning of the QR code. e) After IgG binding to protein A, F/H/W emulsions droplets become less transparent and blocks the QR code information.

Fig. 4. Images of Zonyl forced droplets after IgG binding to protein A. Scale bar in 50 μm . a) Zonyl forced deformation of emulsion droplet on the side under microscope. b) Confocal z-stack images of deformed droplets showing covalent bond formation at the droplet interface.

Scheme 1.

Dynamic complex emulsions morphology change. The red phase is the hydrocarbon oil and the grey phase is the fluorocarbon oil. The color is used hereafter only for display purpose. From left to right, showing the increased strength of hydrocarbon surfactant or the decreased strength of fluorocarbon surfactants. H/F/W as in hydrocarbon-in-fluorocarbon-in-water and F/H/W as in fluorocarbon-in-hydrocarbon-in-water.

Scheme 2.
Structures of GA12OH surfactant with three segments.

Scheme 3.

Interfacial functionalization on the Janus droplet. The red phase is the organic oil and the gray phase is the fluorinated oil. a) *In situ* formation of GA12-NHS at droplet interface and subsequent amine conjugation. b) Pre-synthesized GA12-NHS was dissolved in the droplet hydrocarbon phase and preferably located at the hydrocarbon-water interface after trifluoethanol diffuses out to the continuous phase, followed by interfacial amine conjugation. c) Pre-synthesized GA16-MA for interfacial thiol conjugation. FL in the schemes indicates generic fluorophores.

Scheme 4.

Dynamic emulsion droplets with controlled reactivity. Droplets start in different morphology and functionalized at the hydrocarbon-water interface. Droplets were then tuned to the Janus morphology for imaging. Higher intensity in green channel indicates higher level of functionalization at the interface.

Scheme 5.

Droplet functionalization with protein A and detection scheme with immunoglobulin (IgG).

Scheme 6.

a) Bioconjugation with oligonucleotide followed by hybridization. b) Bioconjugation with mannose derivative for the binding of concanavalin A.