Dynamic Imine Chemistry at Complex Double Emulsion Interfaces.

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ABSTRACT: Interfacial chemistry provides an opportunity to control dynamic materials. By harnessing the dynamic covalent nature of imine bonds, emulsions are generated *in situ*, predictably manipulated, and ultimately destroyed along liquid-liquid and emulsion-solid interfaces through simply perturbing imine equilibria. We report the rapid production of surfactants and double emulsions through spontaneous *in situ* imine formation at the liquid-liquid interface of oil/water. Complex double emulsions with imine surfactants are stable to neutral and basic conditions and display dynamic behavior with acid catalyzed hydrolysis and imine exchange. We demonstrate the potential of *in situ* imine surfactant formation to generate complex surfactants with biomolecules (i.e., antibodies) for biosensing applications. Furthermore, imine formation at the emulsion-solid interface offers a triggered payload release mechanism. Our results illustrate how simple, dynamic interfacial imine formation can translate changes in bonding to macroscopic outputs.

Introduction

Double emulsions are of significant importance in food applications,[1] drug delivery,[2-6] and cell-encapsulation.[7] The fabrication of complex emulsions (e.g., oil-in-oil-in-water, water-in-oil-in-water) has primarily focused on microfluidic techniques and phase separation emulsification, with particular interest in developing responsive double emulsion systems.[8-12] For example, responsive double emulsions can provide a detectable optical readout when responding to external stimuli, making them an effective sensing platform technology.[13-15] However, responsive emulsions that rely on pre-made surfactants limit the ability to use biomolecules as surfactants at the interface for biosensing applications. Pre-synthesized amino-acid based surfactants are a step towards functional bio-compatible surfactants; however, they lack the specificity necessary for sensing applications.[16] Although post-emulsification functionalization of responsive biological components has been reported,[17-18] these processes introduce complications and additional preparation steps. An additional issue with pre-made molecular surfactants is that they need to have appreciable solubility in one of the emulsion phases. This solubility requirement complicates the development of more complex surfactants. Generating surfactants from precursors via *in situ* interfacial synthesis during emulsion formation prevents additional manufacturing steps and provides access to surfactants that are ordinarily not used as a result of solubility restrictions or synthetic complexity.

Dynamic covalent bonds provide an avenue to impart materials with responsive behavior. Imine formation, specifically, provides a versatile interface with biological materials through reactions with the primary amines of lysine residues. Imine surfactants can be generated *in situ* by interfacial reaction between an amine and an aldehyde, and we demonstrate herein that single or double emulsions can be synthesized in one-step, bypassing post-emulsification functionalization (Figure 1a).

Reversibility of imine bond formation has proved useful in creating materials with dynamic behaviors including in 2D assemblies,[19-20] biomimicry,[21] systems chemistry,[22] drug/receptor design,[23] micelles,[24-26] and vesicles.[27-28] There have been limited examples of imine surfactant stabilized emulsions.[29-32]Additionally, imine bond equilibria are pH sensitive, allowing for manipulation of the bond and therefore the structures of double emulsions (Figure 1b). Although there are reported examples of *in situ* molecular surfactant generation for single emulsions, including deprotonation of carboxylic acids and metallosurfactants,[33-39] and Pickering single and double emulsions,[30-31] we are unaware of any prior reports of double emulsion fabricated via *in situ* molecular surfactant synthesis.

In this study, we demonstrate the fabrication and manipulation of complex double emulsions as a consequence of *in situ* formation of imines at emulsion interfaces. Imine formation greatly simplifies the fabrication of complex double emulsions, reducing a multi-step process to a single-step 5 sec method. The dynamic nature of this reaction allows the manipulation of emulsions interfaces through pH changes and associated imine exchange. In addition, dynamic imine formation at

the emulsion-solid interface produce reactive wetting and controlled payload release (Figure 1c).

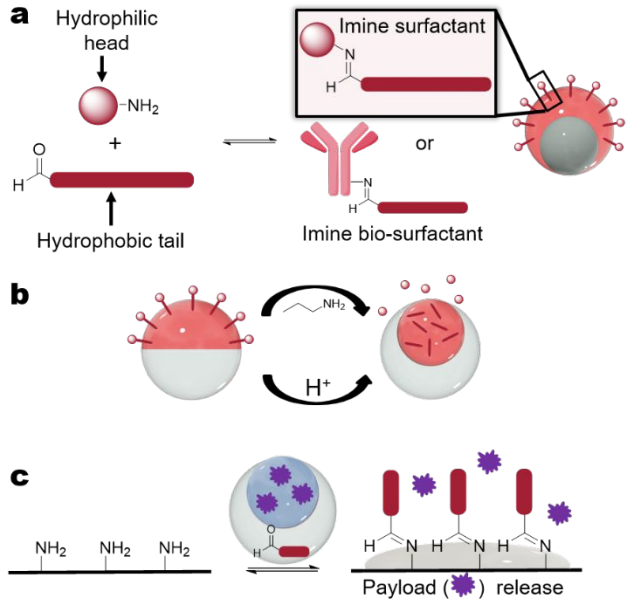

Figure 1: Interfacial imine formation scheme. a) Non-amphiphilic amine/protein and aldehyde reagents in separate immiscible phases to form an imine surfactant through interfacial formation during emulsification; b) By lowering the pH of the system or through imine exchange, the amphiphilic properties of the imine surfactant are cleaved causing changes in morphology or emulsion stability; c) Amine functionalized surface and aldehyde reagent in an emulsion to form an imine functionalized surface and triggered payload release.

Results and Discussion

Phase separation and dynamic double emulsions

A facile fabrication of double emulsions is enabled by thermally induced phase separation.[10] In this method, the dispersed phase, comprising two immiscible oils (e.g., hydrocarbon (HC) and fluorocarbon (FC) oils), is heated above their upper critical temperature. The homogenous (single phase) mixture of HC and FC oils is then emulsified in an aqueous continuous phase containing surfactants. Upon cooling, the two oils separate to yield double emulsions with uniform compositions and a structure determined by the surfactants in the system. In the present studies, HC-surfactants and FC-surfactants in the continuous phase stabilize the HC/water (HC/W) and FC/water (FC/W) interfaces, respectively. Tuning the balance of the interfacial tension (γ) at the water interfaces of HC and FC ($\gamma_{HC/W}$ and $\gamma_{FC/W}$, respectively) using HC and FC-surfactants generate morphologies that range from encapsulated to Janus droplets (Figure 2). The perfect Janus morphology (equal hemispheres) is achieved when $\gamma_{HC/W} = \gamma_{FC/W}$. When $\gamma_{HC/W} >> \gamma_{FC/W}$, a higher surface area at FC/W interface over HC/W is preferred leading to HC-in-FC-in-water (HC/FC/W) encapsulated droplets, and the inverse is necessary to attain FC-in-HC-in-water morphology (FC/HC/W). Adjusting the mass ratio and/or the effectiveness of the two surfactants would lead to changes in the balance between $\gamma_{HC/W}$ and $\gamma_{FC/W}$ and thus the changes in the droplet morphology, thereby creating a dynamic system.

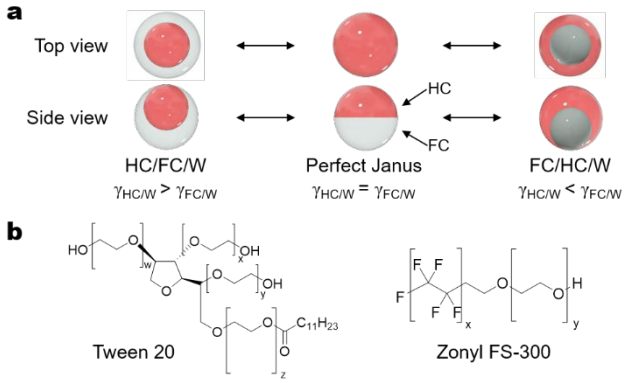

Figure 2. Dynamic double emulsions. a) Morphological changes of dynamic double emulsions of HC (red) and FC (white) with adjustments in γ balance. Top and side view images of the droplets at each morphology are shown. Typical FC solvents used are denser than the HC solvent, thus phases align with gravity; b) Example of commercially available HC and FC-surfactants.

In situ surfactant and phase separation emulsification

We report a new emulsification method that uses a water-soluble amine and oil-soluble aldehyde that undergo a rapid *in situ* interfacial reaction to produce imine surfactants (Figure 3). To achieve exclusive interfacial imine formation, we chose the amine to be soluble only in the water phase, and the aldehyde to be soluble exclusively in only the HC or the FC oil phases. Additionally, the amine and aldehyde reagents were chosen to be non-amphiphilic to prevent inherent surfactant character from generating emulsions without imine formation. As a result, stable double emulsions require imine formation via the *in situ* method.

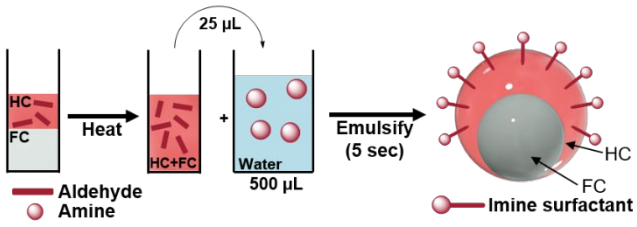

Figure 3. *In situ* formation of imine surfactants and double emulsions. Aldehyde, selectively soluble in HC or FC, is dissolved in the dispersed phases. HC and FC are heated above miscibility and are added to amine-enriched continuous phase. Interfacial imine formation promotes surfactant formation and emulsification simultaneously.

To validate our interfacial reactions, we investigated a proof-of-concept scheme with mono-functional amine polymer monomethoxypolyethylene glycol amine (amine **5**, M.W. 1000) that reacts with FC-soluble compounds **1-2** or HC-soluble carbonyl compounds **3-4** at the FC/W and

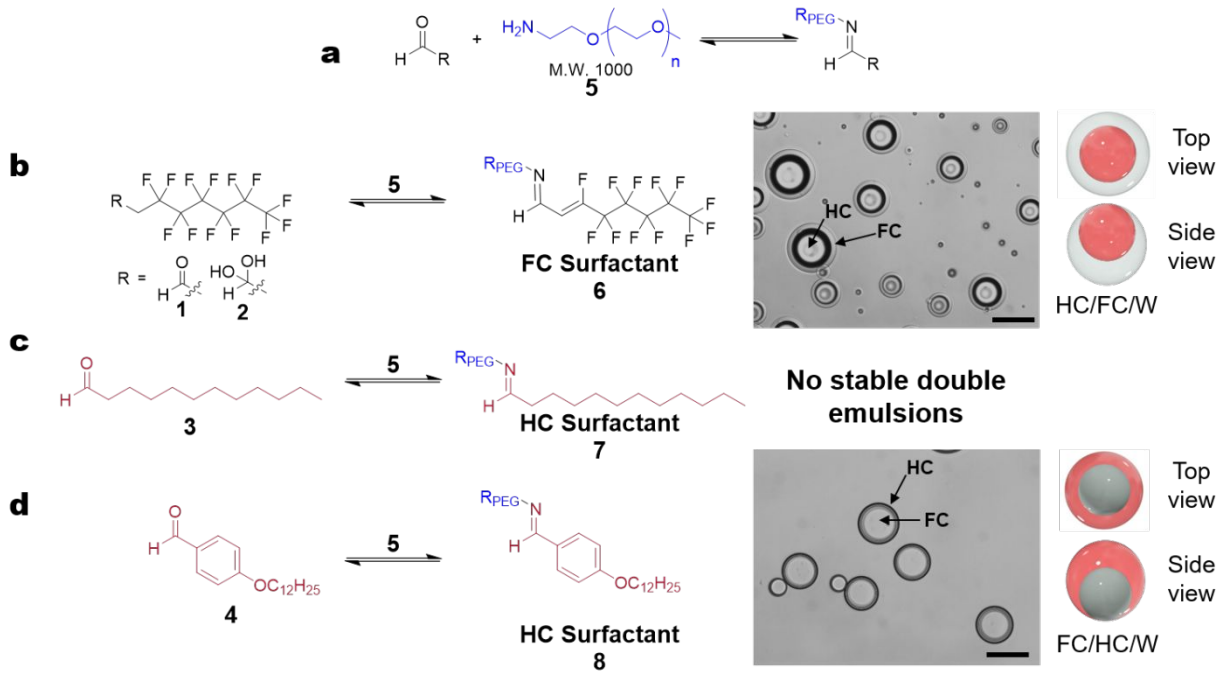

Figure 4: Interfacial imine formation scope and results. a) Overall interfacial imine formation reaction; b) FC-surfactant **6** derived from FC-soluble reagents **1** or **2**. Top view images of HC/FC/W morphology emulsions stabilized by FC surfactant **6**; c) Proposed imine formation at HC/W interface, with HC-soluble reagent **3**, but no stable emulsion droplets obtained; d) HC-surfactant **8** derived from HC-soluble reagent **4**. Top view images of FC/HC/W morphology emulsions stabilized by HC surfactant **8**, with top and side view morphology depictions. Black – FC soluble reagent, red – HC soluble reagents, blue – water soluble reagents. Scale bar = 100 μm

HC/W interfaces (Figure 4). The reactions were initially analyzed by ^{1}H NMR to elucidate the extent of reaction of **1-4** with 2-(2-(2-Methoxyethoxy)ethoxy)ethanamine ($mPEG_3NH_2$) in order to evaluate potential substrates (SI 3.2). Substrates **1-3** showed quantitative conversion to the imine within 30 min, while benzaldehyde **4** showed only 5% conversion in THF-d_8 at room temperature. Although these conditions vary from those at the target interfaces, they do reflect some of the intrinsic reactivity of the components.

We confirmed that the precursor substrates are incapable of stabilizing double emulsions individually by evaluating if the reagents, **1-5**, display inherent surfactant capabilities (SI 3.3-3.4). Substrates **1-4** (at or below 200 mM) and amine **5** (at or below 1 mM) did not exhibit surfactant behavior, and double emulsions formed with these individual components coalesced over the experimental time frame. We subsequently demonstrated the *in situ* generation of surfactant to stabilize emulsion droplets for each reaction in Figure 4. Based on the model studies, **1-4** are expected to form imines by condensation with **5** at either the FC/W or HC/W interfaces. For each emulsification, we reacted 1 mM of amine **5** and 200 mM of HC/FC soluble reagents **1-4** in a neutral pH continuous phase (SI 3.4 for optimization). As expected with the generation of FC-surfactant **6** from **1** or **2** with amine **5**, the diethylbenzene/HFE-7500 droplets adopt a HC/FC/W morphology (FC encapsulating HC in water) that are stable with minimal coalescence for more than 10 months (Fig 4b). Although complete conversion was not observed in the NMR model studies, HC-surfactant generated from benzaldehyde **4** and amine **5** produces stable FC/HC/W morphology double emulsions (toluene:HFE-7500&FC43, 9:1) a result of the *in situ* generation of surfactant **8** (Figure 4d). It is possible only a small amount of surfactant **8** is necessary to stabilize the emulsion, thus the low conversion exhibited in model system is not problematic. Unexpectedly, we did not observe double emulsions using the aliphatic aldehyde **3** and amine **5**. We attribute this result to the instability of imine **7**. Synthetic attempts to pre-make and isolate imine **7** were unsuccessful as a result of its susceptibility to hydrolysis (SI 3.5). Imine **8**, derived from benzaldehyde **4** on the other hand, is hydrolytically more stable than **7** (SI 3.6 & 4.3).

Pendant drop analysis reveals the impact of imine formation on interfacial tension (Figure 5). The fluorous soluble reagents **1** and **2** are surface active, reducing γ from 43.3 mN/m to 11.1 and 10.40 mN/m respectively when 200 mM of the reagents in HFE-7500 were dispersed in water; however, this interfacial tension reduction is insufficient to stabilize double emulsions. Addition of amine **5** to the continuous phase further reduces γ to 3.84 and 4.35 mN/m by reaction with aldehyde **1** and hydrate **2**, respectively. Similarly, benzaldehyde **4** assembles at the toluene/water interface to reduce γ from 38.3 to 20.4 mN/m, and with the addition of amine **5**, γ is lowered to 7.61 mN/m. Aldehyde **3** showed no surfactant activity, and the addition of amine **5** did not reduce the overall interfacial tension. We concluded that the intrinsic interfacial activity of **1**, **2**, and **4** is an important component to the success of emulsion formation. Reagents that lower γ assemble at the oil/W interface to allow reaction with amine **5**, which is

constrained to the continuous phase. Interfacial organization may in fact promote imine formation and could reconcile the low conversion observed in model NMR studies for benzaldehyde **4** with **5**. The lower propensity to assemble at the interface, along with hydrolytic instability, explains the lack of emulsification with aldehyde **3.**

We observed an interesting fluoride elimination in the reaction of **1** and **2** with $mPEG_3NH_2$, resulting in an α,β-unsaturated imine (SI 3.2). This behavior finds numerous precedents in literature.[40-42] Accordingly, we propose that an amine compound acts both as a base and a nucleophile to yield the α,β-unsaturated imine (SI 3.7, Figure S9). An important characteristic of the proposed mechanism is that formation of the α,β-unsaturated imine is under equilibrium control retaining the desired reversible behavior. Hydrolysis of the system was confirmed by using a model imine which was formed with $mPEG_3NH_2$ and hydrate **2** *in situ* in THF-d_8 (SI 3.8). Hydrolysis to the α,β-unsaturated aldehyde occurs with the addition of water or 1 M HCl, demonstrating the reversibility of the imine bond (Figure S11). The mechanism, hydrolysis, and interfacial generation is further detailed in the SI (SI 3.7-3.11).

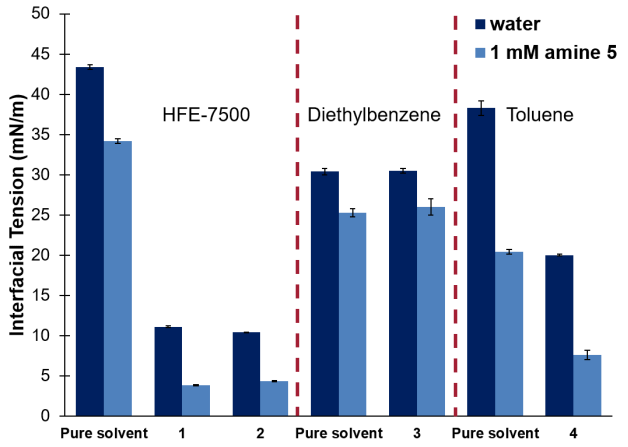

Figure 5: Pendant drop analysis of imine formation. 200 mM solutions of substrates **1-4** in HFE-7500 (**1-2**), diethylbenzene (**3**), and toluene (**4**) were dispersed in water or 1 mM amine **5**. *In situ* emulsification was successful only for substrates that display some surface activity (**1**, **2**, & **4**), reducing interfacial tension in water.

Dynamic nature at liquid-liquid interface

As discussed, the imine bond is dynamic, and changes in pH will impact the formation rates and resulting equilibria. Accordingly, we studied the dependence of *in situ* emulsification on pH. Emulsification with hydrate **2** or benzaldehyde **4** with amine **5** was successful at neutral or basic pH (7-11), and we do not observe stable emulsions at pH < 3. Pendant drop analysis of the reaction showed that at higher pH values, γ was indeed lower (SI 4.1-4.2). At pH 5, double emulsions formed initially, but significant coalescence is observed within one hour. Although low γ was observed in pendant drop analysis for hydrate **2** and amine **5** at pH 5, we concluded that at lower pH hydrolysis leads to coalescence. The pH sensitivity of formation provides further evidence that *in situ* imine formation is crucial to stabilize emulsions.

Changes in the pH affect the imine formation rates and the imine-aldehyde equilibrium. For imine surfactants **6** and **8**, hydrolysis at low pH cleaves the imine and destroys the amphiphilic properties. Disassembly of the surfactants leads to morphological changes when a competing co-surfactant is present, or coalescence/destruction of the double emulsions when no co-surfactant is present. Comparative hydrolysis of imine surfactant **6** and **8** was explored through model imine compounds in THF-d_8 (SI 4.3, Table S2). Based on these studies, we expect a higher hydrolysis rate of imine surfactant **8** as compared to **6**, with the models initially hydrolyzing in 5 min and >1 hr, respectively. Hydrolysis induced morphology changes were studied in a Janus emulsion using imine surfactants **6** and **8** with co-surfactants Tween 20 and Zonyl respectively. The Janus emulsion was generated *in situ* at neutral pH, and then the pH was lowered below 3 by addition of 1 M HCl. As expected, double emulsions stabilized by surfactant **8** and Zonyl displayed instant changes in morphology upon acidification from near perfect Janus to HC/FC/W morphology, thereby signifying the destruction of surfactant **8** (Figure S16). Conversely, when imine surfactant **6** and Tween 20 stabilized Janus emulsions were subjected to the same acidification, small changes in morphology were observed over 5 min (Figure S17). In the absence of co-surfactants, acidified double emulsions stabilized with imine surfactant **8** burst, while imine surfactant **6** stabilized emulsion showed partial destruction with some coalescence (Figure S18-S19). These studies indicate emulsions stabilized by imine surfactant **6** are less sensitive to acidic pH.

The dynamic nature of the imine surfactants was further probed through imine exchange. We hypothesized that after *in situ* interfacial formation of surfactants **6** and **8**, an addition of excess propylamine to the continuous phase would lead to an imine exchange. Upon successful exchange, the amphiphilic imines **6** and **8** would generate non-amphiphilic imines **11** and **10** (Figure 6). Such a transformation would then lead to changes in morphology. We validated imine exchange through model solution NMR studies (SI 4.8-4.9). The addition of 10 equiv. of propylamine (relative to amine **5**) to Janus emulsions having surfactant **8** with Zonyl co-surfactant transformed the morphology to the anticipated HC/FC/W form (Fig 6a). No morphology changes were observed in a Janus emulsion stabilized by Tween 20 and Zonyl in the presence of benzaldehyde **4** only, indicating that the morphology changes are a result of imine exchange of imine **8** to form **10** and not simply the formation of imine **10** (Figure S22). Alternatively, surfactant **6** and Tween 20 stabilized droplets exhibited some coalescence and formation of microdroplets within the double emulsions upon addition of 10 equiv. propylamine (Figure 6b). The microdroplets formed by the production of imine **11** contain both a HC and FC component, indicating that **11** behaves as a HC/FC surfactant (SI 4.10-4.13).

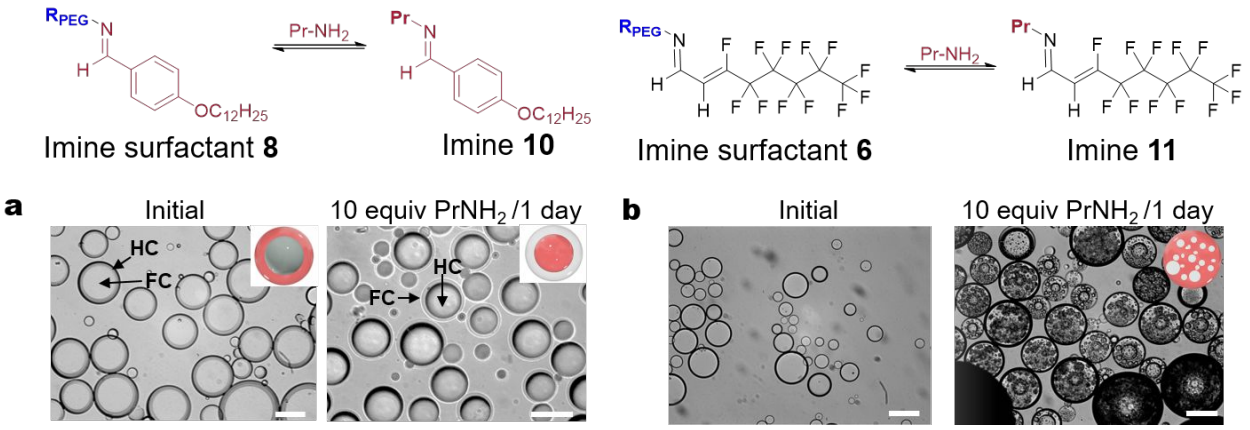

Figure 6. Imine exchange studies in emulsion droplets. a) Toluene/HFE-7500&FC-43 (9:1) droplets stabilized by imine surfactant **8** and Zonyl. Morphology changes from FC/HC/W to HF/FC/W with imine exchange and the transformation of imine surfactant **8** to non-amphiphilic imine **10**; b) Diethylbenzene/HFE-7500 droplets stabilized by imine surfactant **6** and Tween 20. With imine exchange, coalescence and appearance of microdroplets was observed; Scale bar = 100 μm

Bioconjugation at liquid-liquid interface

We were motivated to extend the *in situ* method of double emulsion fabrication to more complex protein-surfactants, as conjugating proteins to double emulsions can be used to generate biosensors for pathogens and viruses.[15,18] In previous methods, proteins were conjugated to the oil/W interface through standard bioconjugation reactions with reactive surfactants on pre-formed droplets. Attaching proteins to oil/W interfaces through this additional fabrication step can pose problems when scaling-up emulsification for commercial applications. We hypothesized that lysine units on the surface of protein would allow for one step *in situ* fabrication via imine formation at the oil/W interface.

We functionalized droplets with four antibodies – anti-β-actin-FITC, avidin antibody, salmonella antibody, and listeria antibody – to determine the fidelity of the *in situ* method to generate antibody-decorated emulsions. Droplets of diethylbenzene/HFE-7500 with hydrate **2** were reacted with each antibody in 10 mM HEPES buffer, with the expectation that a FC-surfactant would form, thereby giving a HC/FC/W morphology (Figure 7a, SI 6.1). All four antibody systems gave non-spherical droplets upon cooling with incomplete phase separation of the HC and FC phases and strings of bound droplets that are unusable for biosensors. It appears that antibody-antibody interactions, such as those exploited in indirect ELISA techniques,[43] may be responsible for the agglomeration that prevents well behaved phase separation for precision droplets. In addition, antibodies with multiple lysine residues can serve as cross-linkers between individual droplets. To circumvent this behavior, amine **5** was added as a co-functionalization agent to disrupt these undesired processes (Fig 7b, SI 6.1). Similarly, when Tween-20 was instead employed as a co-surfactant during emulsification with *in situ* antibody-imine surfactants, perfect Janus emulsions were obtained (Figure S30). We confirmed that the antibodies are bound at the surface using confocal microscopy and anti-β-actin-FITC stain, with and without added amine **5** or Tween 20. In the absence of amine **5** or Tween 20, a higher density of the antibodies is observed at the defects, indicating that a local overabundance of antibodies is responsible for the non-spherical droplets and phase-separation issues. Although a small decrease in fluorescence was observed in the presence of amine **5** or Tween 20, the multiple primary amine units on each antibody ensures successful conjugation (Figure S30). Importantly, anti-β-actin-FITC was observed only at the FC/W interface, showing the successful conjugation of antibody to hydrate **2**. Avidin, salmonella, and listeria antibodies exhibited the same behavior as anti-β-actin (Figure S30).

We endeavored to ascertain if binding fidelity is retained after random attachment via *in situ* imine formation, which is vital to employing antibody-decorated double emulsion as biosensors. Accordingly, we studied the response of salmonella antibody surfactant stabilized Janus emulsions to heat killed salmonella typhimurium (HKST). In our previous work, Janus emulsions with salmonella antibody displayed morphology changes in response to HKST employing a surfactant cleavage scheme,[15] here we instead demonstrated agglutination in response to HKST. Through the binding of antibodies on different individual droplets to the same HKST cells, emulsions tilt and agglutinate (Figure 7c). As a result of selective antibody functionalization, the FC/W interfaces orient towards each other with pathogen binding. Upon agglutination, the originally optically clear emulsions become opaque, allowing simple "on/off" analysis with a QR code and smartphone reader for the presence of HKST (Figure 7c & S31).[14] Binding affinity was retained with *in situ* surfactant formation, paving the way towards biosensor fabrication with this new technique.

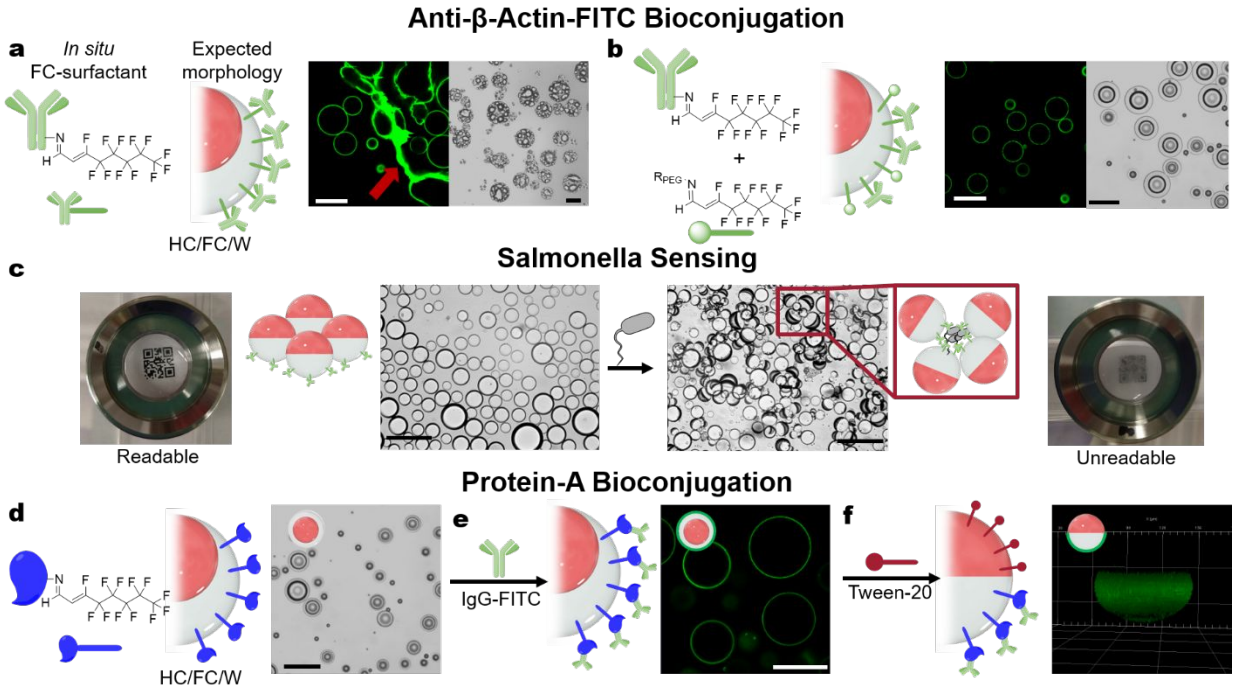

Figure 7: *In situ* generation of FC-surfactants with proteins. a) FC-surfactant generated with anti-β-actin-FITC and hydrate **2**, expected emulsion morphology, confocal image (left), and optical image (right) of non-spherical behavior. Red arrow highlights the string of droplets created by interactions between the antibodies; b) FC-surfactants with hydrate **2** and either anti-β-actin-FITC or amine **5**, which stabilize the emulsion interface, expected morphology, confocal image (left), and optical image (right); c) Agglutination of salmonella antibody functionalized emulsions in response to 10^8 HKST cells/mL. Macroscopically, the droplets changed from transparent to opaque, rendering a QR code unreadable upon agglutination; d) FC-surfactant generated with Protein-A and hydrate **2**, the resulting morphology, and optical image; e) Confocal image of Protein-A conjugated emulsion tagged with IgG-FTIC antibody; f) 3D side view image of selective Protein-A attachment in a Janus emulsion stabilized by Protein-A surfactant and Tween 20. Scale bar = 100 μm

We have examined other proteins to study how size and lysine content impacts bioconjugation. While keeping protein concentration constant, emulsions were successfully generated with hydrate **2** and proteins comprising a broad range of sizes and lysine content (SI 6.6, Table S3). While size did not impact success, proteins with higher surface lysine density (> 0.3 lysine units per kDa) enabled successful *in situ* emulsification. To demonstrate selective protein conjugation at the FC/W interface, Protein A decorated droplets were successfully fabricated and then tagged with complementary binding antibody IgG-FITC and exhibited interfacial attachment, as observed by confocal microscopy (Fig 7d-e, SI 6.8). The morphology was then adjusted with Tween 20 to obtain a near perfect Janus morphology to demonstrate selective protein conjugation at the FC/W interface (Figure 7f). Protein-A double emulsions were stable to the exchange of the continuous water phase to HEPES buffer in the absence of additional surfactants or proteins, thereby signifying the strong association of the new imine surfactant to the interface. Furthermore, the continuous phase could be reused in up to three successive trials without degradation of performance, allowing for the exhaustive use of precious reagents. Finally, the retention of enzymatic activity of human carbonic anhydrase (hCAII) was probed to determine if *in situ* formation has an impact. To this end, the catalytic hydrolysis of 4-nitrophenyl acetate by hCAII was studied before and after emulsification, with no decrease in activity observed (Figure S33). *In situ* imine formation with biomolecules proves to be a quick, robust route to biosensors.

Emulsion-solid interfacial imine formation

The control of interfacial tension through imine bond equilibria provides control for the formation, manipulation, and destruction of double emulsions at the oil-water interfaces. However, no spatial control is achieved as a result of diffusion of the amine through the continuous phase. The ability to manipulate emulsions through dynamic imine bond formation can be further extended to the emulsion-solid interfaces, where spatial control is introduced. Imine formation at solid interfaces has been explored for biomolecule adhesion,[44-45] quantification of amine density,[46] adsorption of gaseous aldehydes,[47] and in Pickering emulsions.[30-31] We hypothesized that interfacial imine formation between an amine-functionalized surface and an emulsion containing reactive aldehydes would generate a locally hydrophobic surface that causes a triggered wetting of the emulsions (Figure 8a). Such controlled wetting via interfacial reactions has potential applications in programed payload release from emulsions.

We initially evaluated imine formation at the emulsion-solid interface using single emulsions containing hydrate **2** on various amine coated surfaces. Poly-lysine and (3-aminopropyl)trimethoxy silane (APTMS)-functionalized

glass were used for the amine sources (Figure 8b). The poly-lysine surface was chosen as a direct connection to our protein studies performed at the oil/W interface. If the imine interfacial reaction occurs, the amine surface would transform into a fluorous-functionalized surface, which should then wet the FC emulsion (Figure 8c). The amine surfaces were submerged in Zonyl-containing aqueous solution, and single emulsions of hydrate **2** in HFE-7500 (200mM) were placed on top of the substrates. For both amine substrates, instant wetting of the FC emulsion occurred, signifying imine formation (SI 7.3). There was no evidence of wetting in control studies with pure HFE-7500 on an amine surface, and additionally hydrate **2** enriched emulsions do not wet unfunctionalized glass (Figure S34). The increase in hydrophobicity with imine formation was confirmed by contact angle measurements before and after emulsion destruction (Table S4). Interestingly, reactive wetting induced by *in situ* imine formation displays instant, complete bursting of the emulsions within a couple minutes, whereas slower, non-reactive wetting is observed on more hydrophobic fluoro-functionalized glass surface over 10 min (Figure 8d). As a result, reactive wetting through imine formation disrupts the emulsion interface to a greater extent than pre-made R_f-functionalized glass.

The selective spatial destruction and improved triggered wetting on amine surfaces has utility in payload release. In a double emulsion system, a payload can be protected within the inner phase of the double emulsion and upon contact with an amine surface emulsion bursting will release the payload (Figure 8e). To demonstrate the potential of payload release, double emulsions were fabricated with hydrate **2** in diethylbenzene/HFE-7500 (stabilized by Zonyl), where diethylbenzene is treated as the payload. Upon contact with APTMS surface, the double emulsions burst. The FC (dyed with a fluorous-perylene dye[48]) wets along the now fluorous-functionalized surface, releasing diethylbenzene (Fig 8f, SI 7.5). We envision this method could be expanded to selective wetting between droplets decorated with antibody along the FC/W interface and surfaces with pathogens. Through the binding interaction, the surface hydrophilicity could change, causing wetting and the payload release of therapeutics. Further, through imine triggered wetting and patterned amine surfaces, interesting opportunities in spatial control of soft material placement are possible.

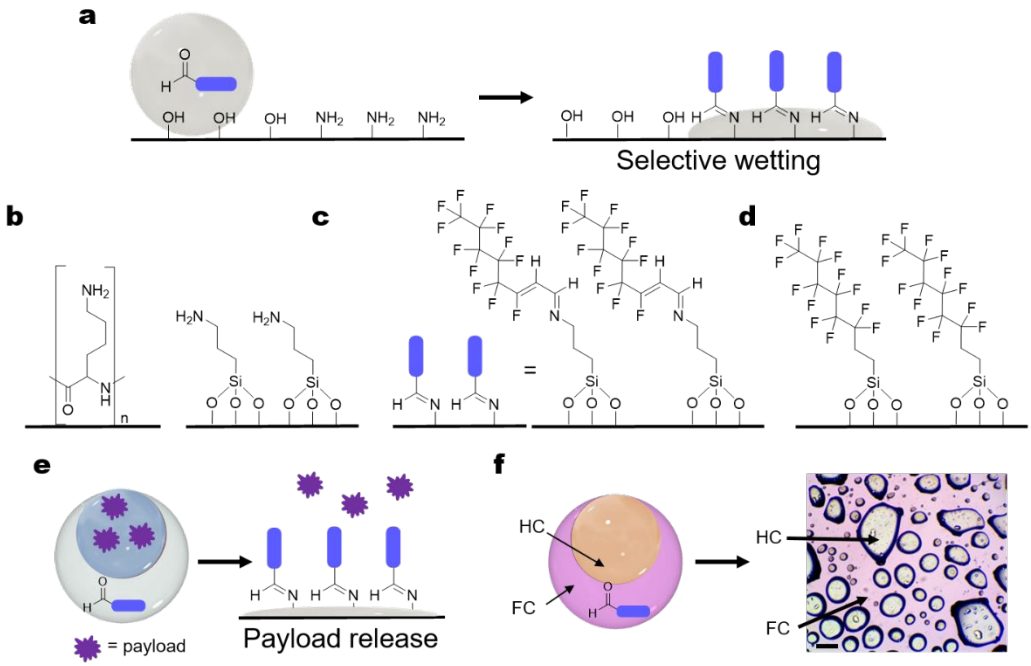

Figure 8: Imine-formation induced selective wetting. a) Selective wetting on amine functionalized surface through imine formation in single emulsions; b) Amine-functionalized substrates: poly-lysinse (left) and APTMS surfaces (right); c) APTMS surface after reactive wetting with hydrate 2; d) (tridecafluoro-1,1,2,2-tetrahydrooxyl)trichlorosilane funtionalized glass surface; e) Scheme of double emulsion payload release; f) Hydrate 2 enriched diethylbezene/HFE-7500 double emulsion bursting on an APTMS surface

Conclusion

We have developed a one-step double emulsion fabrication method to access complex surfactants via *in situ* imine surfactant generation with protein-conjugation. We have shown that successful interfacial imine formation relies on aldehyde reagents that are surface active, allowing for efficient interfacial reaction with aqueous confined amines. In addition, by changing the solubility of the aldehydes (HC or FC-soluble) we generated double emulsions with predictable and varying morphologies. We have further demonstrated selective conjugation of proteins via *in situ* formation. We reported the ability to manipulate emulsions by perturbing the equilibrium with acid or imine exchange at the oil/water interface and the site specific destruction of emulsions through imine formation at the emulsion-solid interfaces. The microscopic control at emulsion interfaces achieved through imine bond equilibria creates programmable

functional materials. We have demonstrated the application of this method towards the rapid generation of biosensors and will endeavor to apply this method to new pathogen targets. Additionally, we are exploring the use of coalescence and microdroplet generation shown with imine exchange as a programmable trigger. As we have shown, the imine bond is a versatile building block in responsive materials.

ASSOCIATED CONTENT

Supporting Information. Instrumentation and materials; Synthesis and characterization of 4-dodecoxy-benzaldehyde; Emulsification techniques; Characterization of *in situ* imine formation; Model NMR studies of imine formation, hydrolysis, and exchange; Multivalent amine substrate for *in situ* imine formation; Biological amine emulsification techniques and analysis; Sensing and enzymatic activity studies; Surface functionalization; Surface wetting technique and characterization. The Supporting Information is available free of charge on the ACS publication website at DOI:

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ACKNOWLEDGMENT

This work was supported by the National Science Foundation Center for Chemical Innovation Grant #1740597, Center for Autonomous Chemistry (CAC). We thank our colleagues in the CAC for helpful discussions. Specifically, we thank Sanjana Gopalakrishnan of the Rotello group (UMass Amherst) for discussions on our emulsion-surfaces studies. We also thank Nathan A. Romero and Suchol Savagatrup for insightful comments and Kosuke Yoshinaga for the fluorous-perylene dye.

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