

Programmable Emulsions via Nucleophile-Induced Covalent Surfactant Modifications

Ann Fernandez, ‡^a Cassandra A. Zentner, ‡^b Manisha Shivrayan,^a Emil Samson,^a Suchol Savagatrup,^b Jiaming Zhuang,^a Timothy M. Swager,^{*b} and S. Thayumanavan ^{*a}

^aDepartment of Chemistry, University of Massachusetts – Amherst, Amherst, Massachusetts 01003, United states

^bDepartment of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States

ABSTRACT: Responsive surfactants designed with kinetic control to triggers are rare and offer new opportunities in generating tunable reactive assemblies. In this work, we discuss the design of novel molecular assemblies based on covalently triggerable surfactants programmed to respond exclusively to nucleophilic triggers to cause interfacial alterations. Through a formal S_N2' type Michael addition chemistry, these induced alterations at the interface of single and dynamic double emulsions can be kinetically tuned and brought about by various small molecule nucleophiles and functionalized nanoassemblies to cause macroscopic responses - bursting or morphology changes. In addition, separate responsive modalities can be used to further control the emulsion systems to impart cascade trigger behavior and programmed release applications.

Introduction

One of the most promising methods to create responsive assemblies of vesicles and emulsions is the tunable control over the self-assembly of their amphiphilic constituents. For example, simple changes to pH, temperature, or ionic strength can impact overall strength of surfactants without changes to their structure.¹⁻⁴ By utilizing well-known chemical transformations, more promising control is shown in systems where dramatic changes in surfactant structure dictate their propensity to self-assemble. Specifically, switchable or cleavable surfactants create responsive assemblies with both reversible and irreversible responses.⁵⁻⁸ Such responsive amphiphiles, and the molecular assemblies they form, are useful in many applications including in oil recovery,⁹⁻¹⁰ drug delivery,¹¹⁻¹² biodegradation,¹³ and sensing.¹⁴⁻¹⁵ Small molecules,⁵⁻⁷ polymers,⁷ and Pickering particles⁸ have been designed to fulfil these applications. More sophisticated approaches have utilized external stimuli, such as with light,¹⁶⁻¹⁷ CO_2 ,^{9,18} enzymes,¹⁵ heat induced retro-Diels-Alder,¹⁹ and dynamic covalent chemistry,²⁰⁻²² to trigger changes to surfactant structure.

Although chemical reactions have been utilized for structural changes in surfactant molecules, tunable kinetic control over these reactions and the effect of such control over nanoassembly transformations has been less studied. In one study, the inverse has been demonstrated, where self-assembly is used to impart kinetic control of

the hydrolysis of cleavable ester surfactants of varying alkyl chain lengths.²³ In general, however, responsive surfactant based assemblies are in either the initial state or final state – before and after reaction – providing an “on/off” signal. Conversely, by controlling the rate and extent of reaction, an assembly system under kinetic control can provide varying responses to subtle changes in environment, adding system versatility beyond an “on/off” signal. In this work, we are interested in designing sophisticated single and double emulsion systems with kinetic control of surfactant cleavage in response to small molecule or molecular assembly triggers. For this purpose, we have developed new surfactant molecules that can be programmed to undergo covalent modification by nucleophiles. Specifically, we use triggerable Michael acceptor (TMAC) functionalities as components of head groups in the surfactant molecules that assemble at and stabilize the interface of emulsions. Our group has previously demonstrated kinetic control of TMAC small molecule analogs.²⁴ The hypothesis is that the nucleophile induced modification of the TMAC surfactant will alter its ability to stabilize the emulsion interface, leading to macroscopic changes in the emulsion systems. Although previous work by our group and others have demonstrated surfactant and emulsion responses to nucleophiles, this report provides investigations into kinetic control of the emulsions through surfactant reactivity. To establish this premise, we demonstrate that specific small molecule nucleophiles and nanoassemblies with

nucleophile functionality can affect the stability and the morphology of single and double emulsions, respectively. Further, the tunable reactivity of the surfactants on the microscale can be observed through macroscopic outputs of the emulsion systems, demonstrating the use of reaction kinetics to affect extent of emulsion response. Additionally, we show that these assemblies can be rendered responsive to an unrelated cue by coupling these processes to a nanoassembly that exhibits responsive molecular release features. Collectively, we demonstrate highly controlled and programmable emulsion systems.

Methods/Experimental

Materials: All reagents were obtained from commercial sources and used as received without further purification. Sodium hydroxide (NaOH) was purchased from Macron. Tetrahydrofuran (THF) was purchased from Acros Organic. Thiourea was purchased from Alfa Aesar. Zonyl FS-300 (40 wt% solution, Zonyl), cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), 2-(2-methoxyethoxy)ethanol, p-toluenesulfonyl chloride, concentrated HCl, hexamethylenediamine (HMDA), dodecyl acrylate, 1,4-diazabicyclo [2.2.2] octane, phosphorous tribromide, triethyl amine, pyridine, xylene diisocyanate, 1,1,1-Tris(hydroxymethyl)ethane, 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid N-succinimidyl ester, octyl methacrylate, azodiisobutyronitrile (AIBN), 1,4-dioxane, trifluoroacetic acid (TFA), and acetyl chloride were purchased from Sigma Aldrich. FC-770 was purchased from Synquest. Formaldehyde, methylene chloride, sodium sulfate, hexanes, ethyl acetate, diethyl ether, 4-dimethylaminopyridine, Invitrogen Attofluor Cell chamber, and 3500 MWCO dialysis tubing were purchased from Thermo-Fisher Scientific. *t*-Boc-NH-PEG-Amine (M.W. 5000) was purchased from Laysan Bio. Polydimethylsiloxane (PDMS) Sylgard 184 elastomer kit was purchased from Dow Corning. Milli-Q water from a Barnstead Nanopure Water System (Thermo fisher Scientific) was used for the preparation of the emulsions.

Instrumental: ¹H- and ¹³C-NMR spectra for 2-(2-methoxyethoxy)ethanethiol (EG₂-SH) were recorded on a Bruker Advance DRX 600 spectrometer. The chemical shift data are reported in units of δ (ppm) relative to residual solvent. ¹H and ¹³C NMR spectra of surfactants, polymers, and kinetic studies were recorded on a Bruker DPX-400 MHz or 500 MHz NMR spectrometer and all spectra calibrated against trimethylsilyl (TMS) standard. Mass spectrometric data were acquired using electron spray ionization (ESI) on a Q-tof-micro quadrupole mass spectrometer (Micro mass). Molecular weights of the polymers were measured using gel permeation chromatography (GPC, Waters) with PMMA as standard fitted with a refractive index detector, and THF as eluent with a flow rate of 1 mL/min. Tip sonication for particle preparation was performed with a Q125 Sonica probe sonicator. Dynamic light scattering (DLS) measurements were performed on a Malvern Nanozetasizer-ZS. Fluorescence measurements were performed using a PerkinElmer LS 55 Spectrometer. All optical images reported were taken

on an AmScope B120C-E1 equipped with an AmScope MU1003 camera or collected using NIS-Elements AR software on a Nikon TiE inverted microscope fitted with an Andor Zyla sCMOS camera. Samples were treated with UV irradiation (Black Ray UV lamp, 365 nm, 115 V ~ 60 Hz) or an LED Engin LZ1-00UV00 365 nm LED with a fixed current 700 mA driver (Digikey). Monodisperse double emulsions were prepared using a Dolomite Microfluidic device equipped with two Mitos P-Pumps (Basic), external Remote Chamber (30 mL), and Telos High Throughput Droplet System.

Analyte and Surfactant Synthesis/Preparation: Tetraalkylammonium-12 (TEA₁₂), pyridinium-12 (PYR₁₂), and 4-dimethylaminopyridinium-12 (DMAP₁₂) surfactants and polymeric assemblies Amine-P2 and Acetamide-P2 synthetic procedures are outlined in Schemes S1-2 and Figures S1-20. A modified literature procedure was followed to synthesize EG₂-SH (Scheme S3).²⁵ Amine-P2 and Acetamide-P2 assemblies were prepared by dissolving 0.4 mg of the respective P2 polymers in 200 μ L methylene chloride. 1 mL water was added to the solution and sonicated for 1 min using a probe sonicator and stirred overnight to obtain the Amine-P2 and Acetamide-P2 self-assemblies. HMDA encapsulated in a polymerosome (PAP-HMDA) were prepared following a literature procedure²⁶ by adding 100 μ L of 2 mg/mL HMDA to a 1 mg/mL solution of PAP polymer (M_n = 18,000 g/mol) in water (20 wt% HMDA to PAP), and stirred overnight. The solution was dialyzed against water for 24 h using 3k MWCO membrane. The PAP-HMDA solution was then concentrated to 4 mg/mL using 3K MWCO spin filters. DLS results indicate that the size of PAP vesicles before and after the addition of HMDA is ~200 nm (Figure S46d-e).

Critical Micelle Concentration (CMC): CMC studies were performed by first preparing 4 mL solutions of either TEA₁₂, DMAP₁₂, or PYR₁₂ solutions in deionized water at a concentration of 2 mg/mL. Following this, 100 μ L of 1 mg/mL Nile Red in acetone was added to the aqueous solutions and stirred overnight. The solutions were then filtered across nylon syringe filter with a pore size of 0.45 μ m to remove unencapsulated dye. Fluorescence measurements of the surfactant solutions with encapsulated dye were then performed at various concentrations to calculate the respective surfactant CMC values (Figure S21).

Critical Aggregate Concentration (CAC): CAC values of the amine-P2 and Acetamide-P2 were also determined using encapsulation of Nile Red. To 4 mg of either Amine-P2 and acetamide-P2, 400 μ L acetone was added and sonicated for 5 minutes. Following this, 2 mL deionized water was added dropwise to prepare polymer solutions of 2 mg/mL. Following this, 100 μ L of 1 mg/mL Nile Red in acetone was added to the aqueous solutions and stirred overnight. The solutions were then filtered across nylon syringe filter with a pore size of 0.45 μ m to remove unencapsulated dye. Fluorescence measurements of the polymer assemblies with encapsulated dye were then

performed at various concentrations to calculate the respective CAC values (Figure S22).

Emulsification: Single emulsions were prepared by hand shaking hexanes and surfactant containing aqueous solution (neutral pH) at a 1:4 volume ratio for 10 s. Poly-disperse double emulsions were fabricated according to published literature procedure.¹⁶ A 1:1 volume ratio of hexane and FC-770 were heated above their upper critical temperature (T_c) to form a homogeneous mixture. 25 μ L of the heated hexane/FC-770 mixture was added to 500 μ L of surfactant containing aqueous continuous phase (neutral pH), and vortexed for 5 s to emulsify. Double emulsions were imaged upon cooling and full phase separation of the hexane/FC-770 mixture. Monodisperse double emulsions were fabricated on a Dolomite Microfluidic device with 50 μ m chip. The emulsion system consisted of an aqueous solution of 1 wt% SDS as the continuous phase (neutral pH) and a 1:1 volume ratio of hexanes and FC-770 (heated above their T_c) as the droplet phase. Pressures were controlled at 200 mbar for continuous phase and 100 mbar for dispersed phase during droplet formation through the chip. After double emulsion formation, the continuous phase was exchanged for the desired surfactant system. Monodispersity was retained with solvent exchange.

Testing Setups and General Procedures: PDMS microfluidic device fabrication procedure was modified from a published protocol.²⁷ Briefly, the microchannels were constructed by casting a mixture of 10:1 base:crosslinker onto a reverse mold of SU-8 photoresist on a silicon wafer. After complete curing at room temperature for 48 h, two holes were punched for the inlet and outlet, and the layer was plasma bonded (Harrick Plasma, ambient air) to a thin PDMS layer (1.5 mm thick) with a circular hole (4 mm diameter) to create a holding well for emulsions. The two bonded layers were then transferred and bonded to another layer of flat PDMS bonded to the glass slide for additional support (Figure S33).

PDMS microfluidic device channels and droplet well were first treated with 0.1 M NaOH to increase the hydrophilicity of the walls. Continuous phase and droplets were then added to the well. After the initial morphology was recorded, Pump 2 (continuous phase) flow rate was set to a standard 100 μ L/min and Pump 1 (analyte, 12.5–50 mM) flow rate was adjusted to achieve 0.25 – 1.1 equiv. of analyte to triggerable-surfactant concentration. The analyte and continuous phase meet at the T-junction and start reacting. Continuous flow through the droplet well was sustained for 3 minutes, to completely exchange the original continuous phase in the well with the mixed analyte/continuous phase from the T-junction. After 3 min, both pumps were stopped, and clips placed on the inlet and outlet tubing to isolate the droplet well and to allow further reaction between analyte and triggerable-surfactants without additional flow.

A glass chamber device was fabricated for single emulsion studies without flow. Two pieces of double-sided tape were placed on a glass slide 1 cm apart (height of 70

μ m). A cover slip was placed over the tape to form a thin chamber. In general, 20 μ L of single emulsion droplets were injected into the chamber for all studies. 10 μ L of analyte solution was added to one end of the chamber to allow slow diffusion into the emulsion system.

For studies without flow, double emulsions were tested in an Invitrogen Attotfluor Cell Chamber (Thermo Fisher). In general, 10–20 μ L of double emulsions were placed in 400 μ L continuous phase. After initial morphology was recorded, analyte solution was added, and allowed to sit to track morphology changes overtime.

For all studies presented in this report, the emulsion studies and reactivity were started at neutral pH before the addition of nucleophiles.

Small Molecule Nucleophile Studies: Single emulsion studies: Studies were performed using both the PDMS microfluidic testing device and no flow setups following the general procedures outlined. TEA₁₂, PYR₁₂, and DMAP₁₂ stabilized single emulsions were exposed to analyte solutions at desired equivalences. Additional nucleophile studies and controls are outlined in the Supporting Information (Figure S34–37).

Double emulsion studies: Studies were performed using both the PDMS microfluidic testing device and the no flow setups following the general procedures. TEA₁₂/Zonyl, PYR₁₂/Zonyl, and DMAP₁₂/Zonyl double emulsions were exposed to analyte solutions at desired equivalences. Additional nucleophile studies and controls are outlined in the Supporting Information (Figure S38–42).

Polymeric Assembly Nucleophile Studies – Amine-P₂: Single emulsion studies: Studies were performed using the no-flow setup and general procedure outlined for single emulsions. TEA₁₂ stabilized single emulsions were exposed to 10 μ L of 0.9 mg/mL Amine-P₂ or Acetamine-P₂. Control experiments are outlined in the Supporting Information (Figure S43).

Double emulsion studies: Studies were performed using the no-flow setup and general procedure outlined for double emulsions. To the double emulsions, 20 μ L of 4 mg/mL Amine-P₂ or Acetamide-P₂ in water were added to Zonyl/TEA₁₂ stabilized double emulsion droplets. Control experiments are outlined in the Supporting Information (Figure S44).

Cascade Nucleophile Studies – PAP-HMDA: Single emulsion studies: Studies were performed using the no-flow setup and general procedure outlined for single emulsions. 4 mg/mL PAP-HMDA was exposed to 365 nm UV light for greater than 25 minutes to pre-release HMDA. TEA₁₂ stabilized single emulsions were then exposed to 10 μ L of the pre-released PAP-HMDA solution

following the no-flow general procedure. Control studies are outlined in the Supporting Information (Figure S46).

Double emulsion studies: Studies were performed using the no-flow setup and general procedure outlined for double emulsions with double emulsions stabilized by Zonyl/TEA12. For excitation of PAP, 365 nm LED was used. First, 100 μ L of PAP-HMDA (4 mg/mL) was irradiated for 25 minutes to release HMDA. The entire mixture was then transferred to the microscope chamber containing the double emulsion solution and the morphology tracked overtime (Figure S48). Secondly, 100 μ L of PAP-HMDA (4 mg/mL) was added to the double emulsions and excited in situ. After 5 min of irradiation, the droplets were removed from the 365 nm light, let sit under ambient conditions for 5 min to ensure no morphology change after light removal, after which morphology changes were recorded. The same droplets were excited for an additional 5 min (10 min total), and the process repeated (Figure S49). Double emulsion control studies are outlined in Figure S48.

Kinetics Studies: Kinetics studies were carried out by observing the rate of product formation from TEA12, PYR12 and DMAP12 surfactants with 1.1, 0.5 and 0.1 equiv. of EG₂-SH (5.5, 2.5, and 0.5 mM for TEA12; 4.4, 2.0, or 0.4 mM for DMAP12 and PYR12), using ¹H-NMR. After dissolving the desired surfactant in 500 μ L acetone-*d*₆ and respective amounts of EG₂-SH, the mixture was immediately monitored and then every 15 minutes to study reaction kinetics over a 12 h period and then every 24 h (Figures S23-29). Studies were performed in acetone-*d*₆,

because of complications resulting from precipitation of the product in water. Partitioning studies of the product in water vs. hexanes were additionally performed (Figure S30-31).

Results and Discussion

Surfactants and Emulsion System Design

The structures of the triggerable surfactants, used in this study, are shown in Figure 1a-b. These structures are based on a quaternary ammonium moiety installed at the α -methyl carbon of an alkyl-methacrylate molecule. From a surfactant perspective, the quaternary ammonium moiety serves as the hydrophilic head group and the long alkyl chain as the hydrophobic tail. The inherent Michael acceptor properties of the acrylate moiety is further aided by the quaternary ammonium moiety as the leaving group. The triggering feature of these surfactants is highlighted by the fact that they are susceptible to a formal S_N2' reaction, where incoming nucleophiles would release the quaternary ammonium moiety (Figure 1a). Such a reaction would deprive the surfactant of the charged hydrophilic head group, causing it to alter its interfacial stabilization characteristics. Note also that the nature of the leaving group has a profound influence on the kinetics of the Michael addition reaction. We take advantage of this feature to tune the kinetics of emulsion-triggering with nucleophiles. The generally anticipated reactivity profile is expected to follow the order (from fastest to slowest): tetraalkylammonium-12 (TEA12),

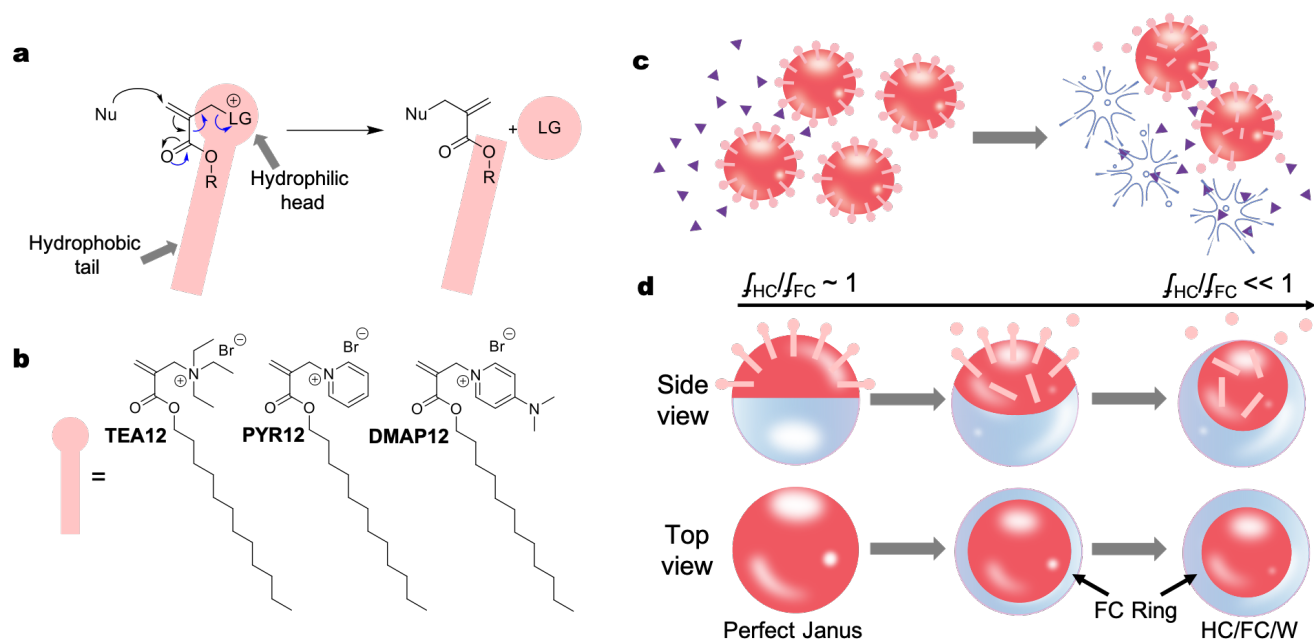


Figure 1. Design of reactive surfactants and emulsions. a) Design of reactive amphiphilic HC-surfactants undergoing nucleophile-triggered cleavage; b) Chemical structures of HC-surfactants in this work; c) Bursting of single emulsions as the result of nucleophile (purple triangles) induced cleavage of charged hydrophilic head groups, thus reducing surfactant strength and destabilization of the HC/W interface; d) Change in morphology of HC (red) and FC (blue) double emulsions from perfect Janus to HC-in-FC-in-water (HC/FC/W) in response to the decrease in mass fraction of reactive HC-surfactant (f_{HC}) to the FC-surfactant (f_{FC}) with the addition of a nucleophile and resulting HC-surfactant cleavage. Changes in morphology are tracked optically by observing the increase in size of the FC-ring. Nucleophile, surfactants, and emulsion sizes are not to scale.

pyridinium-12 (PYR12), and 4-dimethylaminopyridinium-12 (DMAP12).

In systems comprising hydrocarbon-in-water single emulsions (HC/W), the nucleophile-triggered reactions destabilize the HC/W interface to cause emulsions to break and phase separate from the aqueous solution; during this process, the emulsions appear to “burst” to the naked eye (Figure 1c, Video S1). In double emulsions, comprising immiscible HC and fluorocarbon phases (FC) with a FC co-surfactant, destruction of the HC-surfactant would give rise to a clear morphological change (Figure 1d). The morphology of dynamic double emulsions is controlled by the balance of interfacial tensions (γ) at the HC/W and FC/W interfaces, and a Janus state will transform into an encapsulated morphology in response to changes in the relative strength or concentration of surfactants stabilizing one of the interfaces.¹⁶ In a co-surfactant system, Janus morphology ($\gamma_{\text{HC/W}} = \gamma_{\text{FC/W}}$) is achieved with equal amounts of the immiscible liquids and a specific mass fraction of HC-surfactant to FC-surfactant ($f_{\text{HC}}/f_{\text{FC}}$). As the HC-surfactant concentration decreases with nucleophilic addition and the benign FC-surfactant concentration stays constant, the $f_{\text{HC}}/f_{\text{FC}}$ decreases leading to a HC-in-FC-in-W (HC/FC/W) morphology.

We initially determined the concentration of each HC-surfactant to achieve the equivalent $\gamma_{\text{HC/W}}$, thereby eliminating differences in initial emulsion stability in the observed reactivity. Pendant drop studies to determine exact interfacial tensions were not possible due to the

instability of the hexane drops in surfactant solutions over the measurement timeframe, making equilibrium interfacial tensions inaccessible. Although not an absolute interfacial tension measurement, the geometry at the junction between the three phases of double emulsions can be used to determine relative interfacial tension at the HC/ and FC/W boundaries.¹⁶ At a given morphology, the relative ratio of interfacial tensions at HC/W and FC/W interfaces will be consistent across different emulsion and surfactant systems. Therefore, estimations of equal $\gamma_{\text{HC/W}}$ were qualitatively determined through matching morphologies for each double emulsion system. The $\gamma_{\text{FC/W}}$ is held constant across samples and the $\gamma_{\text{HC/W}}$ is adjusted to be equal for each of the three HC-surfactants. To this end, polydisperse double emulsions of hexane/FC-770 in water with uniform configuration were readily obtained by phase separation emulsification.¹⁶ We maintained a constant 0.2 wt% FC-surfactant Zonyl FS-300 (Zonyl) and adjusted the concentrations of the HC-surfactants to produce the perfect Janus morphology, where $\gamma_{\text{HC/W}} = \gamma_{\text{FC/W}}$. Specifically, 5 mM TEA12, 4 mM PYR12, or 4 mM DMAP12 provided the fractions of HC-surfactant to Zonyl ($f_{\text{HC}}/f_{\text{FC}}$) necessary to yield perfect Janus emulsions (Figure S32). These $f_{\text{HC}}/f_{\text{FC}}$ were utilized in all of the double emulsion studies. These concentrations were found to be above the critical micelle concentration for each surfactant, which were calculated to be 0.6, 1.2 and 1.5 mM for TEA12, PYR12, and DMAP12, respectively (Figure S21).

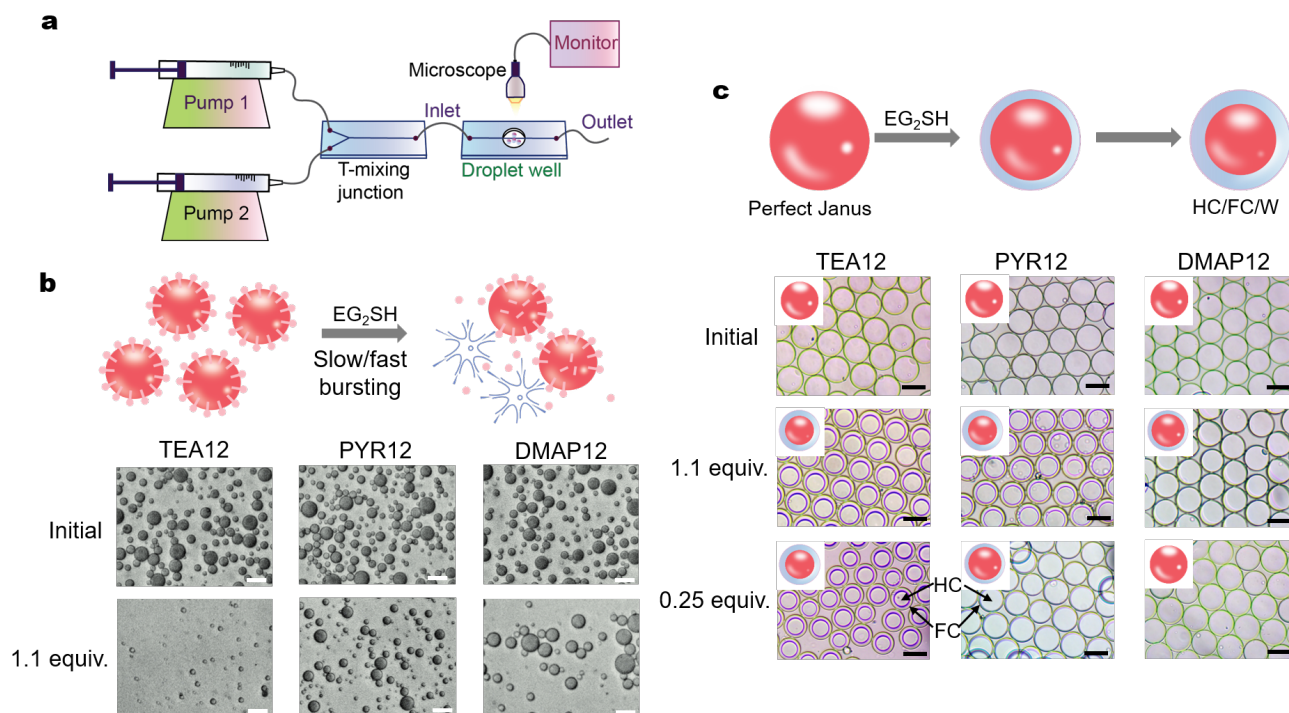


Figure 2. Small molecule trigger studies. a) PDMS microfluidic device for controlled analyte addition; b) Schematic and optical microscopy images of single emulsions stabilized by TEA12, PYR12, and DMAP12 before and after 1.1 equiv. EG₂-SH addition: TEA12 droplets after 8 minutes, PYR12 and DMAP12 droplets after 15 minutes; c) Top-view schematic and optical microscopy images of double emulsion morphology before and after 1.1 equiv. or 0.25 equiv. EG₂-SH addition (1 min after addition). Scale bar = 50 μm .

Small Molecule Nucleophile Studies

As a first step, we validated the strategy and kinetic control with a small molecule trigger: 2-(2-methoxyethoxy)ethanethiol ($\text{EG}_2\text{-SH}$). Using a polydimethylsiloxane (PDMS) microfluidic array device, the emulsions were exposed to precise equivalents of analyte trigger by controlling the flow rates (analyte in Pump 1 and surfactant in Pump 2, Figure 2a & Figure S33). The reactivity of the surfactants is reflected in the time required for single emulsion bursting and the extent of bursting, which depends on the rate of decrease in f_{HC} . Introduction of 1.1 equiv. (5.5 mM) $\text{EG}_2\text{-SH}$ relative to the TMAc surfactant triggered bursting in TEA12 stabilized single emulsions within 45 sec. After 5 min, 90% of the single emulsions had burst and macroscopic phase separation of hexane/water was observed (Figure 2b). PYR12 and DMAP12 displayed slower rates, with the onset of bursting occurring only after 3 min with the addition of 1.1 equiv. (4.4 mM) $\text{EG}_2\text{-SH}$. Also, at the end of 15 min, a significant amount of PYR12 and DMAP12 single emulsions were still intact compared to the TEA12, which had completely burst and phase separated over the same time frame. Control experiments performed with cetyltrimethylammonium bromide (CTAB, 1 mM) stabilized hexane emulsions revealed no response to the addition of 1.1 equiv. (1.1 mM) $\text{EG}_2\text{-SH}$ (Figure S34).

The much greater reactivity of TEA12 is consistent with kinetic studies performed by Zhuang et al. with TMAc-based small molecule reactions with thiols.²⁴ The slower reactivity of the pyridinium-based TMAc molecules is understood based on the resonance stabilization of the positive charge, which results in a weaker leaving group in the formal $\text{S}_{\text{N}}2'$ reaction. In their study, PYR12 reacted slower with thiol compared with DMAP12 contrary to expectations of reactivity based on structure alone. They reasoned that the DMAP base leaving group boosts the nucleophilic reactivity of the thiol and thereby contributed to accelerated reactivity. The reactivity trend of $\text{TEA} > \text{DMAP} > \text{PYR}$ was reflected in kinetic studies performed on the triggerable HC-surfactants (Figure S23-29). Studies in D_2O were prohibitive as a result of insolubility of the product; therefore, experiments were performed in acetone- d_6 . We observed reaction completion within 15 min for TEA12 and 24 h for DMAP12 with 1.1 equiv. $\text{EG}_2\text{-SH}$; However, conversion of PYR12 was only observed after > 2 days. The agreement of the kinetic results demonstrates the added hydrophobic tail does not inherently change the reactivity. Both of these kinetic studies confirmed the superior reactivity of TEA12 demonstrated in the single emulsion system. However, PYR and DMAP did not have differentiated reactivity in bursting studies, contrary to kinetic expectations. The reactivity of PYR vs DMAP leaving groups is greatly dependent on contributions from bases.²⁴ Our emulsion systems likely display different reactivity as a result of the biphasic solvent contributions, wherein the basic leaving groups partition partially into the oil phase, diminishing their contribution to overall reactivity; we, therefore, expect trends more similar to those based on structure alone. We also

note that all emulsion studies are conducted starting at neutral conditions. As Zhuang et al. observed, basic conditions can accelerate the reactivity of the formal $\text{S}_{\text{N}}2'$ reaction.²⁴ In addition, pH conditions can have an impact on nucleophile strength (extent of protonation) and surfactant stability (increased hydrolysis at basic conditions). As a result of the complexity of pH influence on reactivity, pH sensitivity was not investigated in this study.

As a complement to bursting the single emulsions with the nucleophile-induced reaction at the emulsion interface, we explored the potential morphological changes in double emulsions in response to this process. The rate and extent of change in morphology resulting from a decrease in $f_{\text{HC}}/f_{\text{FC}}$ after nucleophile addition can also be used as a metric to characterize the reactivity in the double emulsion system, as a result of complex double emulsion morphology sensitivity to subtle changes in surfactant environment. Further, any changes in morphology provide an optical readout as a result of HC/FC double emulsion micro-lens properties, which, if desired, can be exploited for sensitive detection of nucleophile triggers.²⁸ In the present studies, the changes were measured by tracking the increase in FC-ring size by eye, which provided valuable information on the macroscopic response to nucleophile triggers. Janus droplets stabilized by TEA12/Zonyl and PYR12/Zonyl rapidly formed encapsulated HC/FC/W double emulsions with the addition of 1.1 equiv. (5.5 mM and 4.4 mM, respectively) $\text{EG}_2\text{-SH}$ within 1 min of flow through the PDMS device (Figure 2c; Figure S38). DMAP12/Zonyl system, however, displayed the anticipated slower reactivity as evidenced by the smaller morphology change after 1 min exposure to 1.1 equiv. (4.4 mM) of $\text{EG}_2\text{-SH}$. The slower reactivity of DMAP12, compared to PYR12, is understood as the electron-donating dimethylamino moiety would offer greater resonance stabilization of the positive charge in the TMAc molecule. Surprisingly though, there was no discernible difference between TEA12 and PYR12, thus we resorted to lowering the concentrations. Indeed, clear reactivity differences between TEA12 and PYR12 were revealed at 0.25 equiv. (1.25 mM and 1 mM, respectively) $\text{EG}_2\text{-SH}$ addition after 1 min of flow; full morphology changes were observed with TEA12/Zonyl stabilized double emulsions, whereas PYR12/Zonyl stabilized emulsions were largely unaffected owing to the lower rate of reaction (Figure 2c). The double emulsion system allowed for clear differentiation in reactivity between the three HC-surfactants, particularly in differing macroscopic responses for PYR12 and DMAP12. In control experiments, no morphology changes were observed after addition of 1.1 equiv. (1.1 mM) $\text{EG}_2\text{-SH}$ to CTAB/Zonyl stabilized double emulsions (Figure S40).

Overall, the reactivity in double emulsions followed the trend: $\text{TEA12} > \text{PYR12} > \text{DMAP12}$, faithfully reflecting the anticipated reactivity based on structure of HC-surfactant. As discussed, the observed difference between kinetic experiments in acetone- d_6 and rate of morphology changes is attributed to reduced contributions from

base as a result of the multi-solvent system. In both single and double emulsion systems, a simple small molecule thiol successfully triggered bursting and morphology changes through the reduction in f_{HC} , with rates in accord with the surfactant reactivity trends. While quantitative kinetic reactivity is not measurable with the macroscopic observations, the emulsion responses are capable of reflecting microscopic reactivity.

To further show the control over the reaction, additional nucleophiles were studied: hexamethylenediamine (HMDA), ethanol, and acetic acid (Supporting information). At 1.1 equiv. HMDA showed similar reactivity to EG₂-SH (Figures S35 & S39). Conversely, weaker nucleophiles such as ethanol and acetic acid are not expected to react with the HC-surfactants quickly, as was demonstrated by a lack of response in both the single and double emulsion systems (Figures S37 and S42). Overall our results demonstrate the control over single and double emulsion systems stability with simple triggers and by tuning the reactivity of the surfactants. We utilized this simple method for the controlled release of a payload to synthesize polyurethane. The encapsulation of a di-isocyanate and subsequent triggered release into a triol containing continuous phase could be used to produce on-demand polymerization in a one-pot system (Figure S50).

Molecular Assembly Nucleophile Studies

Having validated the approach with small molecule triggers, we explored the possibility a 3D-assembly triggering a response in the emulsions systems, thus broadening nucleophile triggers types for potential applications. Studies with small molecule amine, HMDA, showed a similar reactivity profile to EG₂-SH, demonstrating the capability of utilizing amines as trigger. Subsequently, we synthesized an amphiphilic block copolymer that is functionalized with a primary amine moiety at the hydrophilic terminus (Amine-P2, Figure 3). As a result of Amine-P2's amphiphilic nature, the polymers self-assemble in water to form nanoassemblies with an amine-functionalized surface. The resulting assembly of Amine-P2 was found to occur at concentrations of 0.25 mg/mL or greater and formed assemblies of ~150 nm, which is consequently too small to be observed optically in emulsion reactivity studies (Figure S22 and S43). To study these processes, we adjusted our testing setup to allow for direct diffusion-controlled contact of trigger, without flow/continuous agitation. The testbed includes a glass enclosure with an opening on the side to introduce analyte into single emulsion samples, which are less dense than water. An open-air microscope chamber was used for double emulsions that are denser than water. We again validated the new diffusion limited test protocol with EG₂-SH and HMDA to TEA₁₂ stabilized single

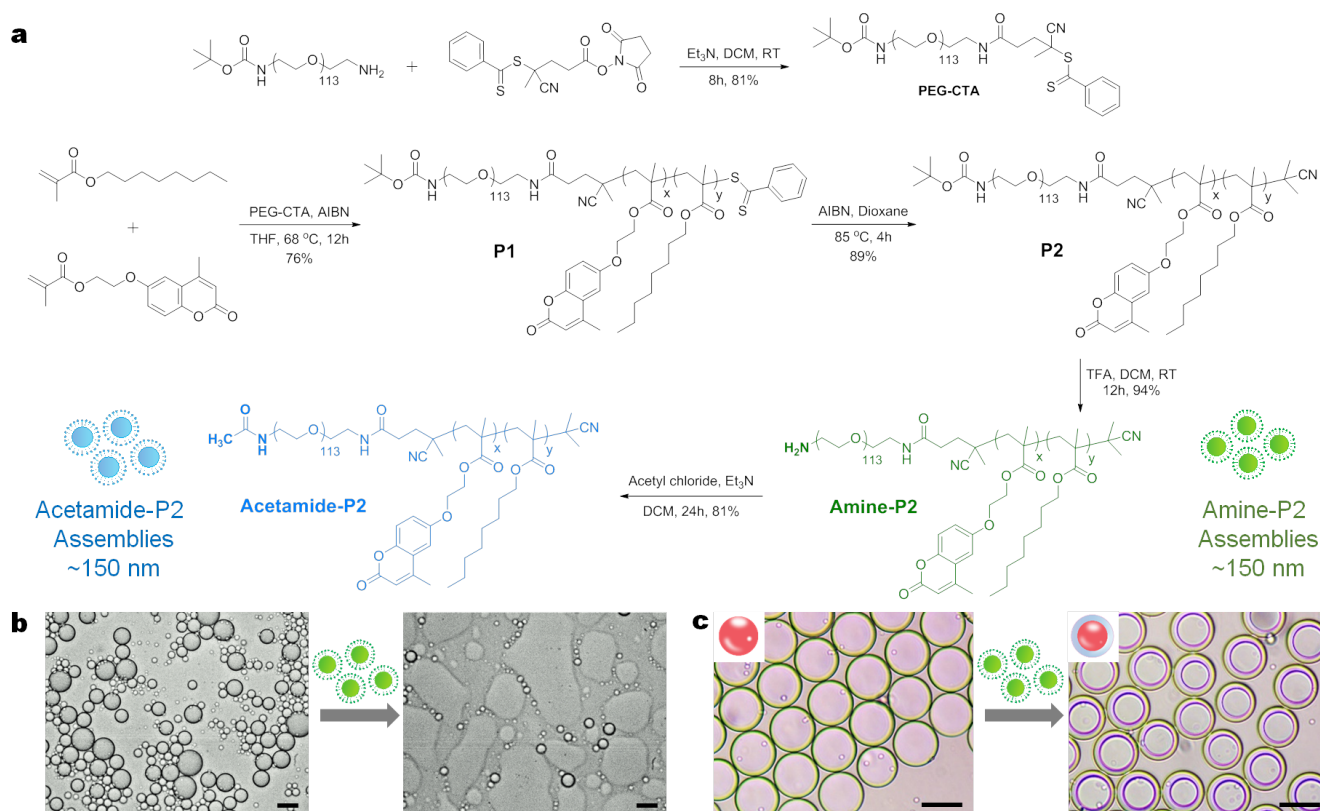


Figure 3. Amine functionalized assembly studies. a) Synthesis, self-assembly, and assembly size of amine and acetamide end-functionalized amphiphilic polymer (Amine-P2 and Acetamide-P2, respectively); b) Optical microscopy images of single emulsion bursting with addition of Amine-P2 after 3 min; c) Top-view optical microscopy images of the morphology changes observed with the addition of Amine-P2 to Janus double emulsions after 3 min. Amine-P2 assemblies are not visible in microscopy images. Scale bar = 50 μm.

emulsions and TEA₁₂/Zonyl stabilized double emulsions and observed instantaneous bursting and morphology changes, respectively (Figure S36 and S41). We then moved on to experiments with Amine-P2 assembly to TEA₁₂ stabilized single emulsions. Within 2 minutes, the onset of bursting was observed with complete emulsion destruction in under 3 minutes (Figure 3b).

Similarly, the morphology of TEA₁₂/Zonyl stabilized double emulsions exhibited complete conversion to the encapsulated HC/FC/W morphology within 3 min due to the decrease in f_{HC}/f_{FC} (Figure 3c). Since the nucleophile-laden ‘attacking’ assembly is amphiphilic in nature, it is possible that simple interfacial contact could cause modifications in the emulsion interfacial stability, without the need for a specific nucleophile-induced modification of the interfacial stability. To test for this possibility, the amine moieties in the Amine-P2 functionalities were converted to the corresponding amide moieties to form Acetamide-P2 assemblies. Acetamide-P2 self-assembles at concentrations of 0.22 mg/mL or greater, with an average size of ~150 nm (Figure S22 and S43). When this assembly, which is identical to the Amine-P2 assembly except

that both the single and double emulsions macroscopic responses are triggered through assembly induced modulation of the TMAc surfactants.

Light Triggered Cascade Studies

We were next interested in evaluating the possibility of causing the transformations in the emulsions by the actuated release of a small molecule from another assembly, thereby modulating emulsion response with an additional control beyond nucleophile addition. To this end, we utilized a recently reported polymerosome, generated through the self-assembly of an amphiphilic diblock copolymer comprising hydrophilic poly(ethylene glycol), an azobenzene linker, and hydrophobic poly(lactide) (PAP, Figure 4a).²⁶ This assembly has been shown to exhibit on-demand release of guest molecules from the lumen of the assembly. The on-demand nature of the process is possible because the membrane barrier is transiently compromised only during the process of light-induced cis-to-trans or trans-to-cis isomerization of the azobenzene unit, but not at the equilibrium state of either of these isomers (Figure 4b). This process represents a far-from-

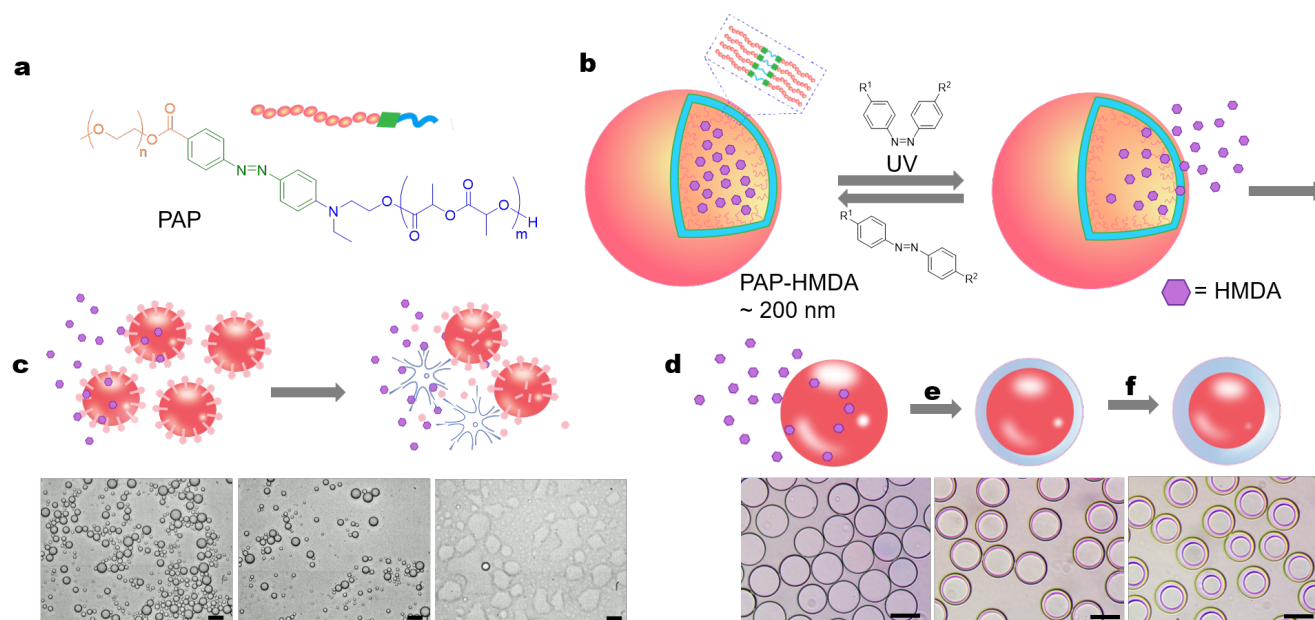


Figure 4. Controlled on-demand triggered release and response. a) Amphiphilic diblock copolymer (PAP) with hydrophilic (orange) and hydrophobic (blue) units linked with an azobenzene unit (green); b) PAP self-assembles into a vesicle (~200 nm in size) for UV-triggered release of HMDA (purple hexagon); c) Schematic and optical microscopy images of single emulsion bursting after pre-release of HMDA. Images from left to right – initial, 2 min and 3 min after addition; d) Top-view schematic and microscopy images of the morphology changes with in situ irradiation of PAP-HMDA with e) 5 min of irradiation and f) 10 total min of irradiation. PAP vesicles are not visible in microscopy images. PAP, HMDA, and emulsion size in schematic representations are not to scale. Scale bar = 50 μ m.

for the nucleophilic functionality, was introduced to either the single or the double emulsion systems, no bursting or morphological changes were observed (Figure S43-S44). Since these experiments were performed above the CAC of Amine-P2 and Acetamide-P2, it is likely that induced changes in the emulsions are a result of assembly interaction with the surfactants at the emulsion interface and/or in the bulk solvent, rather than the reaction of free polymer chains in solution. These results confirm

equilibrium behavior of the polymerosome, as the molecular release is observed only in the presence of an active energy source. We were interested in investigating the possibility of bringing this module into our system, to generate a cascade effect. For our experiments, we loaded PAP assemblies with HMDA as the guest molecule (PAP-HMDA). Mixing the PAP-HMDA assembly with the TEA₁₂-stabilized emulsions did not cause any bursting or morphological changes (Figure S46 and S48). However,

when the same PAP-HMDA assembly was first exposed to light for ~25 minutes, bursting and morphology changes indeed occurred in the single and double emulsion systems, respectively (Figure 4c, Figure S48). PAP assemblies are ~200 nm in size, and therefore are not visible in the microscope images. To further validate the response seen, HMDA at low equiv. was studied with TEA₁₂ emulsions. At equiv. similar to those expected to that which is released, morphology changes and bursting was observed (Figure S45 and S47).

To better demonstrate the cascade process, PAP-HMDA was first introduced to the double emulsion system and then photoisomerized in situ, the release behavior and cascade effect can be concurrently observed. We expect the double emulsions to exhibit morphology changes only upon exposure to UV-light and by removing the UV source, no additional changes in morphology should occur. This modulation of an on-off morphology change with UV-light exposure cycles was faithfully demonstrated. Upon exposure to UV-light for 5 min, the double emulsions underwent morphology changes and did not change further upon resting in ambient conditions for 5 additional minutes (Figure 4d-e). An additional 5 min of UV-light exposure resulted in a further change in morphology, which is demonstrated by an increase in the FC-ring size in the optical micrographs (Figure 4f). Under ambient conditions without any light exposure, no additional morphology changes were observed (Figure S49). Put together, these results show that an in situ actuatable assembly can be combined as a module to bring emulsion sensitivity to an entirely different stimulus (light). A lack of response in the emulsion systems upon the addition of PAP-HMDA before irradiation confirms the encapsulation of the nucleophile within the PAP-HMDA vesicles and far-from-equilibrium release of the nucleophile in the presence of light (Figure S48).

Conclusions

In this work, we have designed covalently triggerable surfactants, based on TMAc chemistry that respond to specific nucleophilic stimulus and utilized them as interfacial stabilizers of single and double emulsions. We have shown that through rational design of the surfactant molecules, specific nucleophiles triggers reduce surfactant strength through the loss of charged hydrophilic head group, thus destabilizing HC/W interfaces. The destabilization of HC/W interfaces causes single and double emulsions to burst and undergo morphological changes, respectively, providing macroscopic response to micro-scale reactivity. Importantly, the structure of the surfactant molecules imparted tunable kinetic control on the emulsions response through changes in leaving group ability. Further, we demonstrated that both small molecule and nanoassembly nucleophiles can be utilized as stimuli. Finally, by combining TMAc-functionalized emulsions with an orthogonally responsive modality, a cascade response was observed wherein light-induced release of nucleophile guest from a nanoassembly resulted in emulsion response. Overall, a formal Michael-type S_N2'

reaction has been conveniently utilized to create programmable emulsion systems with significant control. Such emulsion programming capability has implications in sensing, delivery, adhesion, and self-healing applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Synthesis and preparation of analytes; ¹H-NMR kinetic studies; Microfluidic device design; Nucleophile screening; Control experiments for small molecule, molecular assembly, and light triggered studies. (PDF)

AUTHOR INFORMATION

Corresponding Author

*thai@umass.edu

*tswager@mit.edu

Author Contributions

‡These authors contributed equally.

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ABBREVIATIONS

TEA₁₂, tetraalkylammonium-12; PYR₁₂, pyridinium-12; DMAP₁₂, 4-dimethylaminopyridinium-12; PAP, PEG-azo-PLA; HMDA, hexamethylenediamine; EG₂-SH, 2-(2-methoxyethoxy)ethanethiol; CTAB, cetyltrimethylammonium bromide.

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