An autonomously oscillating supramolecular self-replicator

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A key goal of chemistry is to develop synthetic systems that mimic biology, such as self-assembling, self-replicating models of minimal life forms. Oscillations are often observed in complex biological networks, but oscillating, self-replicating species are unknown, and how to control autonomous supramolecular level oscillating systems is also not yet established. Here we show how a population of self-assembling self-replicators can autonomously oscillate, so that simple micellar species repeatedly appear and disappear in time. The interplay of molecular and supramolecular events is key to observing oscillations: the repeated formation and disappearance of compartments is connected to a reaction network where molecular level species are formed and broken down. The dynamic behaviour of our system across different scale lengths offers the opportunities for mass transport, as we demonstrate via reversible dye uptake. We believe these findings will inspire new biomimetic systems and may unlock nanotechnology systems such as (supra)molecular pumps, where compartment formation is controlled in time and space.

Oscillations involve the periodic fluctuation between two or more distinct states. In addition to being observed in complex biological networks, oscillations are inherent to cellular level functions such as respiration and hormone secretion. Oscillations are also readily seen in macroscopic level phenomena like cardiac rhythms and species populations[1-5]. To observe

oscillatory behaviour in chemical systems, several criteria need to be met. The states need to be metastable, *i.e.* away from thermodynamic minima, and the system must operate out-of-equilibrium, which can be achieved by consuming a fuel[6,7]. Oscillations also require non-linear kinetics provided by feedback processes to escape a steady state[8]. The hope of understanding and harnessing such complex out-of-equilibrium systems has inspired the design of systems which oscillate on the molecular level in inorganic[9-13], biochemical[14] and synthetic organic[15,16] systems.

The design and realisation of supramolecular replicators capable of sustained oscillations would be a major step towards creating systems with life-like complexity[17] as all known life forms operate beyond the molecular scale. It would also give insight into how complex systems could emerge, evolve and exhibit robust lifecycles. The design of supramolecular systems exhibiting opposing feedback processes, which also incorporate non-linear kinetics to prevent reaching equilibrium, is challenging, and the few reported examples of oscillations in supramolecular systems quickly cease over time[18-20]. Molecular events such as the construction of bonds, often occurs on different time scales than processes occurring at greater length scales (such as the self-assembly of compartments, interfacial events and mass transport). Coupling molecular and supramolecular feedback processes to form a sustained supramolecular oscillator would unlock what has been described as a functional 'intermediate' on the chemical evolutionary path to cellular biochemical machinery[17].

Here we present a system that achieves controllable, sustained oscillations of supramolecular compartments using the interplay of molecular events and higher length-scale processes (Figure 1a). Components **1** and **2** react to give metastable **3** which can self-assemble into micellar compartments. As these structures combine self-replication, compartmentalization

and a primitive metabolic cycle they may serve as primitive models of cells, and we show how simple cell-like species can emerge, decay and then repeatedly arise again from an energy consuming network of relatively simple chemicals.

Results

Micelles composed of **3** are capable of encapsulating reagents. This brings the reagents close together, catalysing both the formation and breakdown of the compartment's own building blocks **3**. In doing so, these supramolecular structures induce both positive feedback (autocatalytic formation) and negative feedback (destruction cascade) in the network (Figure 1b). Compound **4** is a breakdown product and recycled back to **1** using a fuel to maintain the system out-of-equilibrium. This system is related to one previously described by our group[21], in which chemical fuelling gave a steady state concentration of metastable **3** (see Figure 1c). We discovered that altering the reaction parameters in this system gave access to a new kinetic regime where it could break out of the steady state and produce oscillations in [**3**] (Fig S28). The thiol used in this preliminary work (1-octanethiol) is capable of producing oscillations but has a less reproducible output than that used here (2-octanethiol, **2a**).

In the current work we increased the rates of formation (by increasing the amount of fuel added and the stirring rate) and destruction (by adding DMAP as a catalyst, increasing the stirring rate and the pH) of metastable species **3**. Increasing the stirring rate (from 100 to 800 rpm) enhances mixing between the aqueous and apolar layers. Under these conditions an emulsion is observed, which increases the availability of **2** in the aqueous layer, accelerating both the formation and destruction processes. Also, reactant **2** is more sterically hindered, giving more robust, controllable kinetics. The idea that the metastable surfactant can take up hydrophobic reagents in the micelles is supported by diffusion-order NMR spectroscopy experiments showing clear association between a self-assembled surfactant and the thiol (Fig S26 & S27).

Micelles of **3** can likely also stabilise microdroplets of thiol, based on our prior microscopy work observing these systems at the nanoscale, which found that incubation of the surfactant assemblies with excess thiol caused micelle growth.[22] Combined, these changes result in oscillations in the concentration of **3** as illustrated in Figure 1d, and supramolecular oscillations as shown in Figure 1e (see also Fig S3).

Five stages of an oscillation

At the molecular level, our studies suggest that oscillations in the concentration of **3** can be understood by a mechanism consisting of five different stages **i.-v.** (Figure 1d). **i. formation:** the interfacial reaction between a solution of hydrophilic **1** and phase separated hydrophobic **2** is initially limited by the transfer of **2** into the aqueous layer. **ii. self-assembly**: dynamic light scattering (DLS) and cryo-EM measurements reveal that the product **3** can self-assemble into micelles (see Fig S6, 31 & 32). As the concentration of **3** increases, micelles of **3** are formed which help solubilize **2** and speeds up their own formation. This autocatalytic process[21,23,24] provides positive feedback and results in an exponential increase in micelles. Next, the concentration of **3** increases linearly while the micelles continue to form, likely taking up and stabilizing microdroplets of thiol **2**. Here, the increase in [**3**] is roughly equal to the amount of oxidant fed to the system, which at that point is the limiting factor.

ii. tipping point: Large quantities of **2** are now in the aqueous layer because of the fast stirring and the presence of micelles. The maximum [**3**] is approached as compound **1** becomes increasingly depleted, due to the oxidant supply no longer keeping up with the increasing amount of solubilised thiol. As a result, a breakdown reaction between **2** and **3** dominates, depleting the micelle's building blocks. The breakdown is accelerated by 4-dimethylaminopyridine (DMAP)[25] (*vida infra*), though it is not essential to observe oscillations as we have performed experiments in the absence of DMAP that also oscillate (see Fig S8). **iv. destruction:** key to oscillatory behaviour is a second, counteracting non-linear process, which we propose is provided by the breakdown of the micelles. As the system becomes starved of **1,** for which formation is limited by oxidant supply, **2** can increasingly react with **3**. The destruction of molecular **3**, causes the micelles of **3** to breakdown. As these micelles were solubilizing **2** in their interior, any **2** that was in the interfacial or aqueous layer become increasingly destabilized in these layers and so it engages in the further destruction of molecular **3** and its micellar compartments, propagating the breakdown. **v. induction period:** After the complete consumption of **3**, the excess of aqueous thiol either returns to the apolar layer over time or reacts with regenerated **1**, as the addition of fuel continues. This restores the system to its original conditions, allowing the next oscillation to begin.

Key to this hypothesis is the autocatalytic nature of the destruction, where the decay of some micelles accelerates the breakdown of others, and we sought ways to investigate this process in more detail. In one set of experiments we added decaying micelles (*i.e.* an aliquot from an oscillating reaction that has passed the tipping point) into a separate reaction that was still in the linear growth regime (see Fig S12). This resulted in the immediate, premature decrease of [**3**]. Being able to seed the breakdown step in this way supports the idea that this this an autocatalytic process[24]. This breakdown cascade causes the micelles to rapidly disappear from the system in a delayed, non-linear fashion as is required to obtain oscillations.

Although the oscillations may be initially understood as a sequence of five stages, some destruction of species **3** is always expected. To quantify the rate of destruction at different stages of the oscillation we designed an experiment featuring two metastable replicators where one replicator is formed in the normal way while the other would be added by continuous injection (Figure 2a). In the chemically fuelled reaction between phase-separated **1** and thiol **2b**, oscillations of [**3b**] are observed (Figure 2b). The addition of small amounts of **3a** did not have much effect on the oscillations in [**3b**] (see Figure 2b). As the addition rate of **3a** is constant, a linear increase in its concentration would be expected in the absence of any destruction steps. What is instead observed is that [**3a**] oscillates, indicating that **3a** is being consumed in the reaction. The oscillations of **3a** and **3b** are also synchronised allowing us to gain insight into the destruction of **3a**. By comparing the amount of **3a** added to its actual concentration, we can calculate its destruction rate at different points in the oscillations. We found that destruction occurs throughout all stages, and in fact a plot of destruction rate in time shows an oscillating pattern itself. Overall, these experiments show that the rate of destruction is highly correlated to [**1**], featuring a sharp drop in [**1**] as soon as the destruction of **3** overtakes formation, leading to repeating phases where destruction is dominant (shaded areas of Figure 2c).

Molecular level oscillations

Figure 1 shows how compounds **1-5** are involved in the chemically fuelled reaction cycle which generates autonomous oscillations. Changes in the concentration of **3** are strongly coupled to the concentrations of **1** and **4** – as expected to maintain mass balance (Figure 1f). The concentration profiles of compounds **1** (Figure 1f, bottom) and **3** (Figure 1f, middle) are similar but differ slightly in peak shape and the concentration maximum of **1** consistently precedes that of **3**. The concentration of **4** is inversely correlated with **3** (Figure 1f, top), so the concentration of **4** peaks as [**3**] is at a minimum. The concentration of **2** in the aqueous layer also oscillates

in time. (see Fig S18). This is likely due to increased concentrations of **3** taking up the apolar thiol so that it is dispersed into the aqueous layer to give a stabilised emulsion of **2**. This is a temporary state and in the absence of **3**, thiol **2** gradually returns to the apolar layer.

Supramolecular oscillations

While the molecular concentration of **3**, as judged by UPLC analysis of aliquots from the reaction mixture, shows a clear oscillating pattern (Figure 1d), characterizing the real time dynamics of supramolecular architecture requires completely different techniques with high spatiotemporal resolution. To study the self-assembled micelles of **3** (measuring *ca.* 8 nm in diameter, see Fig S6, 31 & 32) we turned to interferometric scattering spectroscopy (iSCAT). iSCAT measures changes to the scattering properties of the glass-water interface caused by the binding of nanoscale particles (*e.g.* micelles) to a microscope slide, enabling quantification of the number of particles for a given time interval[22,26,27]. Monitoring a reaction simultaneously with iSCAT and UPLC, we found the number of micellar particles closely correlates to the molecular concentration of **3**. The micelle count experiences rapid increases and decay that produces clear oscillations over time in phase with the building block (Figure 1e and Fig S1-4). This synchronicity observed between the molecular and supramolecular entities supports the role of the self-assembly in the feedback mechanisms as the oscillations are identifiable at both length scales.

Importance of metastability

The self-assembly and destruction of **3** are key features of our proposed mechanism for oscillations in that both processes initiate non-linear feedback. We performed a control experiment using chemically stable surfactants to test the effect of always having micelles present, independent of the changing concentration of **3**. We found that adding micelle forming sodium dodecyl sulfate or a sulfide (rather than a disulfide) analogue of **3** completely removed

the oscillations, highlighting the importance of chemically metastable species (see Fig S23-24).

Control of the oscillations

As the oscillations progress, thiol **2** is converted to hydrophobic disulfide **5**. The ratio of **2**:**5** changes and **5** competes with **2** for uptake into the micelles, decreasing the proportion of thiol in the micelles during later oscillations. At longer times having less **2** in the micelles suppresses the destructive step. This gradually increases the oscillations' period and amplitude as it takes longer to reach the tipping point where oxidative regeneration of **1** can no longer keep up with destruction of **3**. This idea is supported experimentally: when **5** was added at the beginning of the reaction it immediately results in increased amplitudes and periods of the oscillations (see Fig S19-20). Using a large excess of thiol **2**, so that the ratio of **2**:**5** remains high for a long time, mitigates this effect and results in uniform oscillations.

The system is highly tuneable by varying the rate of the destruction of **3** and the oxidation of **4**. Increasing the amount of DMAP increases the rate of destruction of **3** and causes the tipping point (when destruction overtakes formation) to occur earlier, thereby decreasing the period (lifetime of the micelles) and the amplitude (maximum concentration of **3**) (Figure 3a). Increasing the rate of oxidant supply reduces the period of the oscillations and increases their amplitude: more oxidant results in faster amphiphile formation as limiting **1** is produced faster – increasing the slope and peak amplitude (Figure 3b). Increased uptake of **2** due to the higher micelle concentrations also accelerates the autocatalytic formation process such that the tipping point (where oxidation can no longer keep up with thiol uptake) is reached faster, reducing the overall lifetime of the micelles (Figure 3c).

The system shows remarkable responsiveness to changes in oxidation rate within an experiment, allowing the amplitude and period of the oscillations to be controlled in a

predictable and reversible way (Figure 3d). The perpetuation of oscillations is only limited by the amount of available thiol (**2**). By supplying the system with a large excess of **2**, oscillations can be sustained for several days (Figure 3e). The production of waste product **5** (*vide supra*) changes the amplitude and period of the oscillations over the course of days, but changes on the timescale of several hours are negligible and reproducible peaks are obtained.

Coupling oscillations to the uptake of chemicals

Given their role as nano-compartments that enclose **2**, we envisioned the micelles could also uptake other species. To this end, we added the hydrophobic dye indigo to the reaction with the additional goal of achieving a visual readout of the oscillations. By monitoring with UPLC, we could demonstrate that the concentration of **3** and the colour of the reaction are highly correlated (See Figure 4 and Supplemental Video 1). In the absence of micelles, the solution is orange (due to compound **4**). When micelles are formed indigo is gradually taken into the aqueous layer giving a dark purple-brown reaction mixture. When the micelles disappear again, the solution regains its original colour. This allowed us to harness the oscillating supramolecular system to give visual oscillations.

Discussion

Despite the interest in out-of-equilibrium systems, examples of oscillating reactions remain rare. Previous chemical reactions showing sustained oscillations have focussed on molecular scale processes for small molecule organic[15] and biochemical systems[14]. Our system of supramolecular self-replicators is uniquely capable of autonomous oscillations (*i.e.* it does not require coupling to an external reaction) in a small molecular network. Moreover, it is the self-assembly that drives the oscillations, rather than supramolecular events just being a secondary phenomenon. The differing timescales of the molecular and greater length-scale events (supramolecular assembly, mass transport and phase behaviour) are critical in this work in

order to produce oscillations. Hence, it may even be possible to design future systems with an intrinsic delay process, based upon this principle.

This chemical oscillator driven by self-replication of primitive compartmentalized structures, or protocells[28,29], may be relevant to understanding how living systems can emerge from simpler chemistry. For instance, it has been proposed that a key step in chemical evolution is to go from prebiotic chemical oscillators[15] towards complex, living cells[17]. Further, transient phase behaviour like that encountered in this system is now thought to mediate many important biological mechanisms, e.g. protein coacervates and localisation of cell machinery[30-33]. The reaction network of this system displays controlled and sustained oscillations in the concentrations of all key species. Reminiscent of apoptosis (programmed cell death) in real cells[34], a dramatic self-destructive process controls the lifetime and population of the self-assembled structures. These experiments show how protocell-like structures can form spontaneously, repeatedly, and robustly while responding to changes in their environment. This adaptability of the system may be used to allow control over its environment[35-37] and we have shown how oscillations can be coupled to additional processes to control the location and concentration of other compounds. The dynamic behaviour of the system across different scale lengths presents opportunities for mass transport and may have relevance to applications as disparate as drug delivery, signal transduction and the design of functional nanoscale machines which could operate via the directed transport of chemicals, when further spatiotemporal control over these supramolecular assemblies is achieved[38,39].

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Competing Interests

The authors declare no competing interests.

M.G.H. and A.H.J.E. performed the experiments. R.J.H.S. performed preliminary experiments. M.G.H., A.H.J.E. and S.P.F. contributed to designing and analysing the experiments and writing and editing the manuscript. All authors read and approved the manuscript.

Figure 1 | Oscillating components in the out-of-equilibrium, supramolecular reaction network. a, Schematic overview of the oscillating supramolecular system: Components **1** and **2** combine to give self-assembling species **3**, which catalyses both its own formation and its breakdown into components **4** and **5**. Micelles of **3** are believed to take up thiol into their interior and stabilize microdroplets of thiol in the rapidly stirred solution. Compound **4** is recycled by a chemical fuel to maintain the system out-of-equilibrium. **b**, Reaction cycle describing the chemical steps and feedback processes involved in the oscillations: formation, self-assembly, thiol uptake, thiol release, destruction and oxidation (**2a**-**3a**: n=5, **2b**-**3b**: n=7). **c,** Schematic summary of our previously reported chemically fuelled self-replicating system. **d**, Molecular level kinetics, showing oscillations in the concentration of species **3**, and a representation of the stages of an oscillation. **e**, Supramolecular kinetics using iSCAT images of the oscillating system reveal that the number of micelles oscillate in phase to the concentration of **3**. Particles are defined by contrast values, which can be converted into particle mass after calibration (see SI for full data) **f,** Concentration vs time plots of species **1**, **3** and **4.** Lines are drawn to guide

Figure 2 | Probing the destruction reaction in the oscillating system by continuous addition of a preformed second replicator. a, Disulfide **3a** was continuously added via syringe pump to an otherwise normal oscillating reaction where disulfide **3b** was the oscillating replicator. Addition of **3a** at a constant rate allows interrogation of the destruction process alone. The shaded region highlights the destruction events. **b,** Concentrations of **3a** and **3b**, which are respectively added to the system by: continuous injection, and the reaction between **1** and **2b**. While **3a** is being continuously added its concentration oscillates largely in phase with [**3b**]. **c,** The concentration profiles and rate of destruction of **3a** during this reaction**.** (top) The concentrations of **3a** and **1** during this reaction. (bottom) The addition and destruction rates of **3a**. The destruction rate is calculated from the difference between expected concentration (from continuous addition) and the measured concentration. Shaded regions indicate where destruction rate exceeds formation rate. See Figs S13-15 for further discussion. Lines are drawn to guide the eye.

Figure 3 | Effect of the amount of destruction catalyst, oxidant supply and amount of thiol on the oscillations. a, The effect of DMAP concentration on the period and amplitude of the oscillations. Percentages are given relative to the amount of compound **1** at the start of the experiment **b**, The effect of the oxidant supply on the oscillations. **c**, Overall effect of changes in the DMAP concentration and oxidant supply on the appearance of the oscillations. **d**, The period and amplitude of these oscillations can be reversibly altered during an experiment by changing the oxidant flow over time, **e**, By increasing the amount of thiol **2** to 111 eq., the oscillations can be sustained for several days. During this time, the oscillations increase in period and amplitude, which can be attributed to the continuous formation of waste-product **5**. Lines are drawn to guide the eye.

Figure 4 | Supramolecular oscillations can be coupled to a secondary process. Supramolecular assemblies of **3**, when present, solubilize the hydrophobic dye indigo so that it changes the reaction mixture from orange to brown. When the supramolecular structures disappear the indigo phase-separates, restoring the orange colour. In this way, oscillations in [**3**] are translated into visual oscillations (see Supplementary Video 1). Lines are drawn to guide the eye.

Methods

General oscillation experiment procedure. Data for figure 1d were obtained according to this general oscillation experiment procedure. A solution of compound **1** (23 mg, 0.058 mmol) and DMAP (10 mg, 1.4 eq.) in 4 mL TRIS buffer (0.5 M, pH 9.00) was stirred at 800 rpm (oval stirring bar, *L*= 10 mm, ø= 5 mm) in a 10 mL round bottom flask at 40°C. Thiol **2a**, 2-octanethiol (500 µL, 2.87 mmol, 50 eq.) was carefully placed on top of the aqueous layer and the addition of hydrogen peroxide (1.12 M, 45 µL/h) by syringe pump (AL-1000, WPI) was immediately started. The reaction was regularly sampled (every 3 mins) and analyzed as described in the UPLC section. Note: for the experiments of figure 1f, 10 mg DMAP and 0.56 M H_2O_2 (45 µL/h) was used, while 180 µL of 2-decanethiol (**2b**) instead of (**2a**) was added. Using thiols **2a** and **2b** results in similar oscillations with regards to surfactant **3a** and **3b**, but concentrations of **1** are higher and more easily monitored when using **2b**.

Reaction monitoring and UPLC analysis. Aliquots were regularly taken by carefully extracting 10 µL of the aqueous layer by microsyringe. The aliquot was immediately added to 0.50 mL of a quench solution (maleimide, 62 mM, 3:1 H_2O:MeCN). The concentration of UV active components of the reaction were monitored using a Waters Acquity ultra performance liquid chromatography (UPLC) H-Class system with photodiode array (PDA) detector and Acquity UPLC BEH C18 column (130 Å, 1.7 µm, 2.1 mm × 50 mm). A mixture of H_2O:MeCN: TFA (2% v/v in H_2O) was used as mobile phase with a gradient of 93:2:5 → 0:95:5 over 2.40 min. Instrument control and data processing were performed using the Empower 3 software. Peak areas were obtained by integration at λ = 240 nm. Concentrations of all monitored components were calculated based on their individual calibration described in the SI.

DMAP loading variation experiments. Data for figure 3a were obtained according to the general oscillation experiment procedure using 4 mL of a stock solution of compound **1** (57.5 mg, 0.145 mmol) and 2, 20, 200 and 400 mol% DMAP (0.35, 3.5, 35.4, 70.9 mg) in 10 mL of TRIS buffer (0.5 M, pH 9.00), thiol **2a** (90 µL, 0.52 mmol, 9 eq.) and hydrogen peroxide (0.56 M, 45 µL/h). Reaction were regularly sampled and analyzed as described in the UPLC section.

Oxidant supply variation experiments. Data for figure 3b were obtained according to the general oscillation experiment procedure using 4 mL of a stock solution of compound **1** (57.5 mg, 0.145 mmol) and DMAP (12.5 mg, 0.7 eq.) in 10 mL of TRIS buffer (0.5 M, pH 9.00), thiol **2a** (either 90, 180 or 360 µL, amount of thiol used was proportional to oxidant concentration) and hydrogen peroxide (either 0.56, 1.12 M or 2.24 M, 45 µL/h). Reaction were regularly sampled and analyzed as described in the UPLC section.

***In situ* oxidant supply variation experiments.** Data for figure 3d were obtained according to the general oscillation experiment procedure using 4 mL of a stock solution of compound **1** (57.5 mg, 0.145 mmol) and DMAP (25 mg, 1.4 eq.) in 10 mL of TRIS buffer (0.5 M, pH 9.00), thiol **2a** (500 µL, 2.87 mmol, 50 eq.) and hydrogen peroxide (0.56 M, flow rate switched between 45 and 135 µL/h). The reaction was regularly sampled and analyzed as described in the UPLC section.

Sustained oscillations experiment. Data for figure 3e were obtained according to the general oscillation experiment procedure using 4 mL of a stock solution of compound **1** (57.5 mg, 0.145 mmol) and DMAP (10 mg, 0.6 eq.) in 10 mL of TRIS buffer (0.5 M, pH 9.00), thiol **2a** (1.10 mL, 6.3 mmol, 109 eq.) and hydrogen peroxide (0.56 M, 45 µL/h). The reaction was regularly sampled and analyzed as described in the UPLC section.

Reversible dye uptake experiment. Data for figure 4 were obtained according to the general oscillation experiment procedure using 4 mL of a stock solution of compound **1** (57.5 mg, 0.145 mmol), DMAP (6.25 mg, 0.35 eq.) and indigo (2.5 mg, as a suspension) in 10 mL of TRIS buffer (0.5 M, pH 9.00), thiol **2a** (500 µL, 2.87 mmol, 50 eq.) and hydrogen peroxide (0.56 M, 150 µL/h), at room temperature. The reaction was regularly sampled and analyzed as described in the UPLC section. A video recording of the experiment displaying visual oscillations may be found in the Supplementary Information.

DLS analysis. Analyses on the aggregation behaviour of compounds **3a**, **3b** and the structural mimic of **3** (compound **6**) were performed using a Malvern Zetasizer Nano ZEN5600 model system recording particle and molecule size. Instrument control and data processing were performed using Zetasizer software. Measurements were repeated thrice for every concentration within a dilution series, using an equilibrated heating probe at 25 °C and setting the appropriate parameters for water. All samples were prepared using 0.5 mM TRIS buffer (pH 9.00) and were filtered using a microfilter (pore size 0.22 µm) prior to measurement. Disposable plastic cuvettes were used with 1.0 mL of sample solution.

Interferometric scattering microscopy (iSCAT) experiments. Experiments were conducted according to the general oscillation experiment procedure using 4 mL of a stock solution of compound **1** (57.5 mg, 0.145 mmol) and DMAP (12.5 mg, 0.7 eq.) in 10 mL of filtered TRIS buffer (0.5 M, pH 9.00, microfilter, pore size 0.22 µm), thiol **2a** (180 µL, 1.0 mmol, 18 eq.) and hydrogen peroxide (0.56 M, 120 µL/h). The reaction was regularly sampled and analyzed as described in the UPLC section. At the same time, samples were collected for iSCAT by carefully extracting 1.0 µL of the aqueous layer and diluting this by a factor of 500 using filtered TRIS buffer (0.5 M, pH 9.00). The diluted samples were immediately analyzed using iSCAT, for which 30s videos were recorded. Data was recorded using a previously described set-up[1] and particle numbers quantified as reported earlier[2].

Ring tensiometry measurements. Ring tensiometry measurements were used to calculate the critical micelle concentration (CMC) of compounds **3a**, **3b** and **6** in TRIS buffer (0.5 M, pH 9.00). A dilution series of each surfactant was prepared and the surface tension of each sample recorded at room temperature using a Krus K10ST tensiometer. Surface tension is plotted against ln([surfactant]), with the concentration at which the surface tension no longer decreases corresponding to the CMC. This can be calculated using the intercept of two lines on the curve.

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2. Lebedeva, M.A., Palmieri, E., Kukura, P. & Fletcher, S.P. Emergence and Rearrangement of Dynamic Supramolecular Aggregates Visualized by Interferometric Scattering Microscopy. *ACS Nano* **14**, 11160-11168 (2020).

Data availability

Correspondence and requests for materials should be addressed to S.P.F. The source data underlying Figs. 1b, 2b, 3a-e and 4 are provided as a Source Data file