**Accelerated ripening in chemically fueled emulsions**

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Abstract
Chemically fueled emulsions are solutions with droplets made of phase-separated molecules that are activated and deactivated by a chemical reaction cycle. These emulsions play a crucial role in biology as a class of membrane-less organelles. Moreover, theoretical studies show that droplets in these emulsions can evolve to the same size or spontaneously self-divide when fuel is abundant. All of these exciting properties, *i.e.*, emergence, decay, collective behavior, and self-division, are pivotal to the functioning of life. However, these theoretical predictions lack experimental systems to test them quantitively. Here, we describe the synthesis of synthetic emulsions formed by a fuel-driven chemical cycle, and we find a surprising new behavior, *i.e.*, the dynamics of droplet growth is regulated by the kinetics of the fuel-driven reaction cycle. Consequently, the average volume of these droplets grows orders of magnitude faster compared to Ostwald ripening. Combining experiments and theory, we elucidate the underlying mechanism, which could help better understand how cells regulate the growth of membrane-less organelles.
Droplets are crucial for the spatial and temporal organization of the biochemistry in living cells. These droplets, referred to as membrane-less organelles, can compartmentalize chemical reactions, store genetic material, and protect proteins from misfolding in response to environmental stress. Chemical cues within the cell actively control the formation and properties of these droplets. For example, the process of activation of the main constituent proteins of P-granules or stress granules involves a chemical reaction cycle fueled by the hydrolysis of ATP. Theoretical studies have shown that droplets in such chemically fueled emulsions can exhibit a set of different properties compared to emulsions approaching thermal equilibrium. For example, the droplet size within these emulsions can be regulated by the kinetics of the chemical reactions. Similarly, the droplet position and the position of particles within the droplets can be controlled. Finally, theoretical studies have shown that the droplets in these emulsions can spontaneously divide. However, these theoretical predictions lack experimental systems to test them quantitively. Examples of synthetic molecular assemblies regulated by fuel-driven reaction cycles have been described, including chemically and photo-chemically fueled fibers, dynamic vesicles formed by the consumption of ATP, dynamic DNA-based assemblies and colloids. Indeed, such studies result in surprising behavior like dynamic instabilities in synthetic fibers or solutions of nanostructures that oscillate between different colors. However, the field of chemically fueled droplets and emulsions remains mostly unexplored from an experimental perspective. Experimental systems of these emulsions could validate theoretical predictions, demonstrate new physical and chemical behavior, and offer valuable insights into the mechanisms relevant for the regulation of membrane-less organelles.

In this work, we have developed synthetic emulsions regulated by fuel-driven reaction cycles (Fig. 1a). The droplets in these emulsions emerge when chemical fuel is supplied, and they decay when all fuel is depleted. These emulsions show a surprising behavior, i.e., the average volume of droplets in the emulsion increases rapidly. Moreover, we find that the rate of this growth is determined by the kinetic rates of the reaction cycle. The droplet growth shows similarities to Ostwald ripening but is up to two orders of magnitude faster. Similar to Ostwald ripening the average volume of the droplets is a result of the growth of large droplets at the expense of small ones (Fig. 1b). By the quantitative agreement between experiments and theory, we propose a mechanism where the fuel-driven chemical reaction cycle can accelerate the growth of the average droplet volume. The mechanism relies on small droplets dissolving due to deactivation of droplet material while big drops grow due activation of droplet material. This mechanism could be relevant for the control of growth speed and size of membrane-less organelles in living cells and could represent a new strategy of controlling nano-structures out-of-equilibrium.
Results and discussion.

Design of the reaction cycle. All emulsions in this work follow the same experimental design (Fig. 1), i.e., a water-soluble precursor (A) molecule is activated by an irreversible reaction with a fuel.\textsuperscript{28,29} The product of that reaction (B) is less soluble and phase-separates into droplets. Moreover, this product B is intrinsically unstable and spontaneously deactivates via hydrolysis, yielding the original precursor A. That means that the emergence and sustenance of the emulsion require fuel, while the phase-separated droplets will decay and vanish in the absence of fuel. Thus, these emulsions are maintained away from thermodynamic equilibrium and are thus referred to as chemically active emulsions.\textsuperscript{9} Since the water-soluble fuel is required for the activation and water is required for the deactivation, the chemical reaction cycle will predominantly take place in the aqueous phase.\textsuperscript{23}

We consider four reaction cycles that are all driven by the hydrolysis of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as a carbodiimide-based fuel. In all activation reactions, a carboxylate-based precursor A is activated by reaction with EDC (fuel). The activated precursor subsequently reacts with a second nucleophile in order to form the phase-separating product (B). These nucleophiles are an intramolecular carboxylate to form a cyclic anhydride (Cycle 1 and Cycle 2), an intermolecular carboxylate to form the symmetric anhydride (Cycle 3) or NHS to form the NHS-ester (Cycle 4). The deactivation reaction of the metastable products occurs via the hydrolysis of the product. We chose these reaction cycles because of their strongly different hydrolysis rate constant varying by two orders of magnitude (Fig. 2a). The varying rate constants allow, for the first time, an exploration of the effects of chemical rates on the growth behavior of an active emulsion, i.e., the behavior of the droplets in a chemically fueled emulsion.

\textbf{Fig. 1. Schematic representation of fuel-driven droplet formation.} \textit{a)} A chemical reaction cycle drives the transition from the precursor to the droplet-forming product at the expense of a chemical fuel (activation). The droplet-forming molecules are unstable and will hydrolyze to the precursor (deactivation). \textit{b)} Schematic representation of the experimentally observed droplet growth of the emulsion. Within minutes, many small droplets ripen to a few large ones.
The chemical reaction cycles depicted in Fig. 2a were used to form emulsions by the hydrolysis of EDC (fuel, Fig. 2b). The precursors in each of the reaction cycles (Fig. 2c) follow a simple molecular design. One domain (depicted in black) consists of a linear or branched aliphatic tail that drives phase-separation. The second domain (in blue) is the reactive domain that comprises one or two carboxylate-groups that makes the precursor well soluble. The loss of the negatively charged carboxylate(s) upon the activation reaction (anhydride or NHS-ester formation) results in a decrease in solubility inducing the formation of the emulsion.
**Reaction kinetics with droplets.** For each of the seven precursors depicted in Fig. 2c, we followed the evolution of the reaction cycle in response to a single batch of the fuel (EDC) by measuring the concentration of precursor, product, and fuel by HPLC (Fig. 3a-b and Supplementary Fig. 2). In all cases, we found that the concentration of the metastable product initially rises, but decays as the system was running out of fuel. We can thus define a growth- and a decay-regime for each of these experiments. In the growth regime, activation dominates, and the product concentration increases. In contrast, in the decay-regime, fuel is depleted, and deactivation dominates.

For each cycle, the concentration of droplet-forming molecules decayed linearly throughout the decay-regime when droplets were present (Fig. 3b and Supplementary Fig. 2). The linear decay is a result of the constant deactivation rate (hydrolysis), which is induced by a self-protection mechanism of the droplets.27,29 Because the droplets do not contain water, the deactivation can take place at the interface of a droplet and on the product fraction that remains in the aqueous phase (i.e., outside of the droplets), which is close to the solubility ($c_{\text{out}}^{(0)}$). The deactivation can thus be described by the effective rate: $r_{\text{eff}} = k_d c_{\text{out}}^{(0)}$. We determined the linear deactivation rate ($r_{\text{eff}}$) and the solubility ($c_{\text{out}}^{(0)}$) of all products (Supplementary Table 2). The combined data allows calculating the deactivation rate constant ($k_d$) of each of the products, which scale over two orders of magnitude from $8.2 \times 10^{-3}$ to $4.9 \times 10^{-5}$ for cycle 1 to cycle 4, respectively (Supplementary Table 2).
Rapid growth of average droplet size when driven by a single batch of fuel. By confocal microscopy and image analysis software, we measured the evolution of the droplet size in response to a single batch of fuel. To measure how the dynamics of the chemical reaction
cycle affect the behavior of the droplets, we performed these experiments on all products listed in Fig. 2c, i.e., the droplets formed by seven precursor-product combinations with vastly different reaction kinetics. For all droplet-forming reaction cycles, confocal microscopy showed that the droplets were present within the first minute after applying the fuel. The rapid formation of droplets points to a low nucleation energy and low capillary length which could be confirmed by theoretical estimates (Supplementary Section 4). Since the lateral resolution of the microscope is around 600 nm, the size of these initial droplets was likely overestimated. Indeed, a cryogenic transmission electron microscopy study showed that the droplets were below the resolution of the microscope (Fig. S8). We thus discarded all data in the first minutes of the experiment but instead focused on the evolution of the droplet volume beyond the 0.2 µm$^3$.

We found that for all products, the average volume of the droplets increased throughout most of the experiment. That included the growth-regime, but surprisingly, also the decay-regime in which the total product concentration was decreasing. For example, in the experiment with C$_8$SA, we found the average volume of the droplets increased for the first 140 minutes to almost 5 µm$^3$ (Fig. 3c-d). After roughly 120 minutes, the droplet volume rapidly decreased, and the droplets disappeared (Fig. 3d).

Interestingly, we found that the higher the deactivation rate, the greater the growth rate of the average droplet volume (Fig. 3f, and Supplementary Table 2). For example, 4-tert-butylphthalic anhydride was one of the fastest deactivating products and the average volume of the droplets in the emulsion was the fastest increasing (Supplementary Fig. 3). In contrast, the average volume of droplets in an emulsion of one of the slowest hydrolyzing products, C$_{10}$SA, increased slowly. The trend of increasing average droplet growth rate with an increasing deactivation rate was observed for hydrolysis rates ranging over three orders of magnitude.

The histograms of the droplet population at different times in the cycle provided clarity on the increase in the average droplet volume (Fig. 3e and SI4). The histograms show that, in the decay regime, the population of small droplets (e.g., below 8 µm$^3$) decreases rapidly. In contrast, the population of large droplets stays relatively constant. Consequently, the average droplet volume increases. However, this increase does not occur due to droplet growth, but because of the selective decay of small droplets. The loss of small droplets is particularly obvious when plotting the total number of droplets against time, which rapidly decayed (Supplementary Fig. 3).

The behavior of average population growth at the expense of small droplets shows some reminiscence to Ostwald ripening. Ostwald ripening is a slow process that relies on diffusion of droplet molecules from small droplets to large ones driven by a shallow concentration difference among droplets. As a result, small droplets decay, while big ones
grow (Fig. 5b). While we observed the decay of small droplets, we did not find obvious growth of large ones in our experiments. Moreover, the average droplet volume growth was fast. To put that in perspective, we measured the growth rate of the average volume and compared them to theoretically calculated rates for classical Oswald ripening\(^\text{31}\) (Supplementary Table 3). We found that the growth rate of the average volume was faster compared to Oswald ripening for all chemically fueled emulsions and the difference in growth rates between Oswald ripening and the observed rate was more pronounced with increasing hydrolysis rate constant. For example, the growth rate for the slowest hydrolyzing emulsion (iso-C\(_8\)) was a 1.2-fold faster compared to Oswald ripening. In contrast, the growth rate for C\(_8\)SA was 19 times faster than one would expect based on Oswald ripening. The fastest-growing droplets were formed by 4-tert-butylphthalic anhydride, of which the average volume grew a 95-fold faster compared to Oswald ripening.

**Faster average droplet volume increase when driven by a pseudo-continuous supply of fuel.** In the experiments above, a finite amount of fuel was initially added as a batch resulting in transient droplets of which the average volume rapidly increased. Such experiments contrast living systems where fuel levels are continuously maintained, and reaction cycles may reach a steady state, \textit{i.e.}, activation equals deactivation.\(^\text{32}\) We thus tested the droplets in a steady state, and we used a reaction-diffusion model to calculate the flux of fuel required to reach steady states (Supplementary equation (19) for an estimate). To avoid the need for stirring the sample, which affected droplet behavior, we applied a batch of fuel every few minutes and homogenized the sample by shaking it briefly (Fig. 4a). We used HPLC to confirm the formation of such a steady state in the concentration fuel and product with pseudo-continuous fueling (Fig. 4b and S4). We found that because of the pseudo-continuous fueling, the concentration product would fluctuate around steady state, but fluctuations were never greater than \(\pm 5\%\) of the concentration.

We found that the average droplet volume in the emulsions in a steady state also increased rapidly (Fig. 4c-d). Moreover, the increase was faster compared to the droplets that were batch-fueled (Supplementary Table 3). For example, when C\(_6\)SA was brought to steady state of roughly 7 mM, the average droplet volume increased almost three times faster compared to the batch-fueled experiments and more than 60 times faster compared to classical Oswald ripening (Fig. 4c-e, Supplementary Table 3). The rapid increase of the average volume was further confirmed for other emulsions in a steady state, like C\(_6\)SA and C\(_{10}\)SA (Fig. 4c). Again, we found in these experiments that the average droplet volume growth scaled linearly with the deactivation rate of the reaction cycle (Fig. 4f). For C\(_6\)SA, the rate was the highest, with almost 0.6\(\pm\) 0.2 \(\mu\)m\(^3\)/min. Such a growth rate means that droplets had reached an average volume of 15 \(\mu\)m\(^3\) after a two-hour experiment. In contrast, the lower steady state dynamics of the C\(_{10}\)SA droplets resulted in a much slower droplet growth.
The size distributions of the droplets as a function of time revealed that additional effects that were involved in the growth of droplets in steady state compared to those fueled with a single batch. In the batch-fueled experiments, small droplets were decaying faster compared to Ostwald ripening, but large droplets were not growing significantly (Fig. 3e). In contrast, in the pseudo-continuously-fueled experiments, large droplets were growing while small droplets were decaying fast (see Fig. 4e). The combination of the rapid growth of large droplets and fast decay of small ones further accelerated droplet growth compared to batch-fueled experiments.
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Fig. 4. Behavior of pseudo-continuously fueled emulsions. a) Schematic overview of the experiment: droplets emerge and continuously ripen when pseudo continuously fueled by batches of fuel. b) Concentration profile of total product concentration measured by HPLC approaching a steady. Droplet formation was induced by an initial batch of fuel (10 mM EDC to 10 mM C8), and the system was maintained in steady-state by pseudo continuously fueling with 4 mM EDC/hr. Markers represent data measured by HPLC; the solid red line represents the calculated concentrations using our theoretical model. The growth- and steady-state-regime are shaded blue and red, respectively. c) Micrographs of droplets of C8SA, C8SA, and C10SA in a steady-state by continuous addition of fuel. d) Average droplet volume over time for experiment discussed in a. The markers represent the data as measured by image analysis software in three experiments (n=3), the black line represents the average of those three experiments. The solid red and blue lines represent calculated data for the accelerated ripening and Ostwald ripening, respectively. e) The distribution of droplet volume at 30, 60, and 120 minutes of the emulsion described in a. f) Average volume growth rates against the deactivation rate. The individual data points are shown (dots), their average (markers), and the standard deviation from the average (error bars, N=3).

Theoretical model of accelerated ripening. To understand the mechanism of rapid droplet growth, we propose a theoretical model that describes phase separation with chemical reactions that can quantitatively describe how the reaction cycle affects the droplet behavior. Our model accounts for phase-separation between product and solvent, diffusion, and all chemical reactions between the fuel F, precursor A, and product B (see Supplementary Information for a full derivation and more details on the model). In our model, fuel (F) is added with a rate \( j_F \) and reacts with a rate proportional to fuel concentration \( c_F \) and precursor concentration \( c_A \) and a rate constant \( k_F \). This reaction leads to the activation of the precursor to form product. The product is spontaneously deactivated with a rate \( k_d \) back to precursor. In addition, the product can diffuse with a diffusion coefficient \( D_B \) among the droplets allowing the droplets to exchange material and thereby undergo Ostwald ripening. In our model, droplets act as sources and sinks of product molecules with rate \( J \) and impose phase separation conditions at the droplets' interfaces (Gibbs-Thomson boundary condition). Thus, outside the droplets, the kinetics of the concentrations \( c_i \) (i = \( F \), \( A \), \( B \)) is governed by

\[
\begin{align*}
\partial_t c_F &= -k_F c_F c_A + j_F , \\
\partial_t c_A &= -\lambda k_F c_F c_A + k_d c_B , \\
\partial_t c_B &= \lambda k_F c_F c_A - k_d c_B + D_B \nabla^2 c_B - J ,
\end{align*}
\]

where \( \lambda \) denotes the yield factor. It reflects that not all fuel molecules actually lead to a product due to the metastability of the O-acylisourea intermediate compound (see Supplementary Eq. (5)). The rate \( J \) determines the total growth of drops, and thereby how the average volume changes with time. Numerical solutions to the model show a good agreement with the concentrations of fuel and total product concentration measured by HPLC (Fig. 3b, and S2).
For batch-fueled experiments \((j_F = 0)\), the model can quantitively describe the fast initial activation of the total product concentration up to its maximum at time \(t_{\text{max}}\), as well as the linear decrease with a slope equal to the effective deactivation rate \(r_{\text{eff}} = k_{d} c_{\text{out}}^{(0)}\) for times \(t > t_{\text{max}}\), where \(c_{\text{out}}^{(0)}\) is the solubility. Due to the good agreement of the model solutions with all experimental traces for total product, precursor and fuel concentrations corresponding to all reaction cycles and the various precursors, we use it to extract the rate constants of the reactions. These rate constants allow us to also quantitatively describe the pseudo-steady state experiments (Fig 4b and S4). In addition, these rate constants are used to theoretically predict the growth of the average droplet volume.

From our model, we derived an approximate mathematical expression of how the average droplet volume changes with time, \(\langle V \rangle(t)\), for both fueling protocols (Supplementary Equation (28, 29)) and valid for \(t > t_{\text{max}}\). This expression reads:

\[
\langle V \rangle(t) = (m_{\text{clas}} + m_{\text{fueled}}) t - \bar{m} t^2, \quad (2)
\]

where \(m_{\text{clas}}\) denotes the growth rate corresponding to Ostwald ripening which depends on the interfacial tension of the product rich-phase coexisting with solvent, the solubility of the product, product molecular volume and its diffusion constant. In separate experimental studies, we have determined those parameters for all considered chemical systems allowing us to estimate the relevance of Ostwald ripening for our fuel-driven systems (see Supplementary Table 2). The additional contributions arising from the fuel-driven chemical reaction cycle are \(m_{\text{fueled}}\) and \(\bar{m}\). Most importantly, our derivation gives explicit expression for contributions to the slope related to chemical fueling as a function effective deactivation rate \(r_{\text{eff}}\). Specifically,

\[
m_{\text{fueled}} = \alpha V_{\text{sys}} r_{\text{eff}}, \quad (3)
\]

where \(V_{\text{sys}}\) is the system size, \(v\) is the molecular volume of product and \(\alpha\) is a numerical prefactor which varies between the two fueling protocols but is the same for all reaction cycles (see Supplementary Eq. (29)). The remaining fuel related growth rate \(\bar{m}\) is zero in the pseudo-continuously fueled mode, while for the one batch mode, it is determined by the amount of initially added fuel (see Supplementary Eq. (30)). Substituting the known parameter values related phase separation and the fuel-driven chemical reactions (see Supplementary Table 2) into Eq. (2,3), we find a good agreement the experimental measurements (red lines in Fig. 3d, 4d, and Supplementary Fig. 3 and 5).

**Mechanism of accelerated ripening.** The agreement between experiment and theory suggests that the mechanism underlying droplet growth in the model coincides with the one in the experiment, i.e., the rapid ripening is based on a combination of Ostwald ripening and the fuel-driven reaction cycle. Ostwald ripening occurs because smaller droplets have a greater Laplace pressure compared to large droplets. This difference leads to a slightly higher concentration of droplet molecules around small droplets compared to large ones (Fig. 5a).
Consequently, a diffusive flux of the droplet molecules from small to big droplets is established that scales with the surface tension. This diffusive flux drives the slow growth of the bigger droplets at the expense of smaller shrinking ones, which eventually dissolve (Fig. 5b). In our chemically-fueled emulsions, a similar concentration gradient emerges between small and big droplets (Fig. 5c). However, besides the diffusive flux among droplets, the chemical kinetics of product activation and deactivation significantly alter the fluxes of product molecules in the system and thereby accelerates droplet ripening.

First, we describe how the deactivation of product molecules can accelerate the growth of the average droplet volume. The deactivation occurs in the aqueous phase of the emulsion (between the droplets) and can create a local undersaturation, i.e., upon deactivation, the local concentration is slightly lowered below the solubility concentration of the product (Fig. 5c). This local undersaturation is rapidly restored via diffusion of product molecules from one of the surrounding droplets. However, because of the concentration gradient between small and large droplets, the local undersaturation is more likely to be “filled” by products expelled from a small droplet. Deactivation thus selectively decreases the volume of small droplets compared to large droplets. That selective efflux from the small droplets result in an accelerated decay of small droplets compared to Ostwald ripening (Fig. 3d). Moreover, this accelerated decay of the small droplet population result is an increase of the average droplet volume that is faster compared to Ostwald ripening and is regulated by the kinetics of the deactivation: the faster the deactivation, the faster the ripening (Fig. 3f). Finally, once droplet numbers are low and the droplets are on average far apart, diffusion can no more catch up with the deactivation reaction. Consequently, remaining droplets quickly dissolve, and the system becomes homogeneous, which we observed experimentally (Fig. 3c-d).

In the case of pseudo-continuous fueling, both deactivation and activation affect the evolution of the droplets’ size. Small droplets dissolve faster compared to Ostwald ripening because of the mechanism discussed above (Fig. 5c). In addition, the activation reaction continuously activates product in the aqueous phase which is leading to a locally enhanced supersaturation (Fig. 5d). This increase of supersaturation is more likely to be “absorbed” by a large droplet rather than by a small one because products diffuse down the concentration gradients created by the Laplace pressure differences among the droplets. The combined mechanism implies an accelerated decay of small droplets (via deactivation) and accelerated growth of large droplets (via activation). Interestingly, the simultaneous occurrence of both phenomena is visible in the evolution of the histograms, i.e., the population of small droplets vanishes, while the population of large droplets grows (Fig. 4e).

An alternative mechanism for an increase of the average volume is fusion of droplets. However, in all experiments no droplet fusion was observed (Supplementary Figure S7). Sporadically, droplets collided and appeared stuck to one another, but they would eventually
unstick. Consistently, using theoretical estimates, fusion is shown to be too slow to contribute to the ripening rate for the chemical systems considered (Supplementary Figure S7). We also challenged the model assumption that activation and deactivation dominantly occur outside the droplets instead at the droplet interface. In particular, the concentration of product molecules at the droplet interface is rather high compared to the product concentration outside the droplets. However, the rate of hydrolysis events at a droplet interface is still negligible compared building blocks being hydrolyzed in the surrounding corresponding to a typical droplet (Supplementary Section 4).

Our proposed mechanism explains that the average volume of droplets in a chemically fueled emulsion increases faster compared to Ostwald ripening because droplet material is deactivated in the batch-fueled experiments, or both activated and deactivated in the continuously fueled experiments. The mechanism also explains the difference in ripening rates between the two fueling protocols, i.e., ripening is fastest when continuously fueled (Fig. 5e). Our theory suggests that a combination of parameters (activation and deactivation rates, solubility, surface tension, and molecular volume) determine the faster growth of the average volume compared to Ostwald ripening (Supplementary Equation (31)). We combine these parameters into a rescaled effective deactivation rate such that it represents a measure that determines the ripening rate compared to Ostwald ripening. We plot the degree of acceleration compared to Ostwald ripening, i.e., the measured growth rates divided by the theoretical Ostwald ripening rates, as a function of this rescaled deactivation rate (Fig. 5f), and find excellent agreement between experimental data and theory. From this plot, it becomes clear that the ripening of iso-C_{10}, with the lowest rescaled deactivation rate, is almost exclusively driven by Ostwald ripening. In contrast, tBuPA is accelerated by almost two orders of magnitude compared to Ostwald ripening due to its large deactivation rate constant. Finally, the plot demonstrates that continuously fueled emulsions will ripen faster than batch-fueled emulsions by a factor of 2-5.
**Fig. 5. Mechanism of rapid droplet growth.**

**a)** Schematic representation of product molecules (red sphere) around a small droplet (left orange sphere) and large droplet (right orange sphere). Due to a different Laplace pressure, a small droplet is surrounded by a higher concentration of product than a large droplet. Consequently, the droplet population ripens, i.e., big droplets grow, while small drops decay. 

**b)** Due to the concentration gradient described in a, molecules diffuse from small to large droplets. Consequently, the droplet population ripens, i.e., big droplets grow, while small drops decay.

**c)** The deactivation reaction increases the efflux from small droplets.

**d)** In the presence of fuel, the activation of the product leads to additional faster growth of large droplets compared to c.

**e)** Theoretically calculated average droplet volumes as a function of time for C₈SA subjected to Ostwald ripening (blue), accelerated ripening when fueled with a single batch (red), and accelerated ripening when fueled continuously (dark red). The products are grouped per reaction cycle (blue for cycle 4, orange for cycle 3, green for cycle 2, and red for cycle 1). The individual data points are shown (dots), their average (markers), and the standard deviation from the average (error bars, N=3).
Conclusion

In this work, we described a new behavior in chemically fueled emulsions, i.e., the increase of the average droplet volume is regulated by the kinetics of the underlying chemical reaction cycle. We designed four chemical reactions cycles with reaction rates that span over three orders of magnitude in order to quantitatively understand the behavior, and we find that the faster the reaction cycle, the greater the acceleration of average droplet volume. Given the simplicity of the mechanism, we anticipate that the behavior can be observed in other fuel-driven assemblies. For example, accelerated growth could be observed in membrane-less organelles, provided that protein activation and deactivation occur outside of the organelle (e.g., by kinases that operate in the cytosol, but not in the organelle). The mechanism could thus help to explain how the growth of membrane-less organelles is regulated. Our theoretical model further validates the generality of the accelerated ripening. Moreover, we believe the mechanism will hold for crystal growth or the synthesis of nanoparticles, which are also subjected to Ostwald ripening. Such accelerated growth will be particularly powerful for the synthesis micro- and nanostructures of tunable size. For example, once an emulsion has reached a certain average droplet size via the accelerated ripening, the droplets can be polymerized.

Methods

Materials. 4-Morpholineethanesulfonic acid (MES) buffer, pentanoic acid, hexanoic acid, 2-hexenyl-succinic anhydride, 2-octenyl-succinic anhydride, 2-decenyl-succinic anhydride, t-butyl-phthalic anhydride, 4-ethylctanoic acid, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS) and Nile Red were all purchased from Sigma-Aldrich and Alfa-Aesar unless indicated otherwise. The precursors C₆SA, C₈SA, C₁₀SA, and isoC₁₀, were synthesized by hydrolysis of their corresponding anhydrides. The anhydrides were suspended in demineralized water and hydrolyzed over 48 hours while stirring the samples. The absence of anhydride was assessed by HPLC. The samples were freeze-dried and stored at -20 ºC until further use. Their purity was assessed by and Electrospray Ionization-Mass Spectroscopy (ESI-MS) and HPLC.

Sample preparation. We prepared stock solutions of the precursors by dissolving it in MES buffer and subsequently adjusting the pH to 6.0. We used 200 mM of MES buffer for precursor concentrations of 10 mM or less. For higher precursor concentrations, we used a buffer concentration of 500 mM. We used the precursors at the following concentrations: 300 mM C₅, 100 mM C₆, 10 mM of C₆SA, 10 mM of C₈SA, 7.5 mM of C₁₀SA, 10 mM of tBuPA, and 5 mM of isoC₁₀. We prepared the stock solutions of EDC by dissolving the EDC powder in demineralized water, typically, at 1.0 M EDC. We initiated the batch-fueled experiments by the addition of various batch-sizes of EDC to the precursor solution. In the case of isoC₁₀, the
addition of fuel was accompanied by the addition of 5 mM of NHS. We initiated the pseudo-
continuously fueled experiments by the addition of an initial amount of EDC fuel. Specifically,
50 mM of EDC was added to 10 mM of C6SA, 10 mM of EDC was added to 10 mM C8SA, 10 mM 
EDC was added to 7.5 mM of C10SA, and 1 mM of EDC (with 5 mM of NHS) was added 
to isoC10. 15 minutes after the first batch of fuel, EDC was provided with a flux of 55 mM/h, 4 
mM/h, 0.6 mM/h, and 0.05 mM/h, respectively, for C6SA, C8SA, C10SA, and isoC10. We added 
fuel by four batches per hour. The mixture was gently shaken after each addition.

**Analysis of the kinetics of the chemical reaction cycles.** The concentration profiles of the 
chemical reaction networks were monitored over time by means of analytical HPLC. A 1.5 mL 
sample was prepared as described above in a screw cap HPLC vial. The reaction solution was 
stored at 25 ±1°C solution, and samples of the solution were directly injected without further 
dilution. All reagents in the reaction cycle were separated using linear gradients of water and 
acetonitrile. See supporting information for more details.

**ESI.** ESI-MS measurements were performed using a Varian 500 MS LC ion trap spectrometer. 
The samples were diluted in acetonitrile and injected into an acetonitrile carrier flow (20 
μL/min).

**Confocal Fluorescence Microscopy.** Confocal fluorescence microscopy was performed on 
a Leica SP5 confocal microscope using a 63x oil immersion objective. Samples were prepared 
as described above but with 2.5 μM Nile Red as a dye. 12-20 μL of the sample was deposited 
on the PEG-coated glass slide and covered with a 12-mm coverslip. Every minute a 4096 x 
4096 pixel² image was acquired of an area that covered 246 μm x 246 μm. Each experiment 
was performed in triplicate (n=3). More details can be found in the Supporting Information.

**Image analysis and droplet volume and growth rate calculations.** Images were analyzed 
with ImageJ’s “analyze particles” package, from which we derived the volume of each droplet. 
We then calculated the average droplet volume per micrograph and binned several 
micrographs that were taken with one-minute intervals. The standard deviation was calculated 
between the three replicates of the experiments (n=3). From this dataset, the histograms of 
the droplet distributions were also calculated. We calculated the droplet growth by measuring 
the slope of the droplet volume over time for each individual experiment. Then, the average 
growth rate and the standard deviation between the three replicates were calculated (n=3). 
The droplet number was also obtained from the image analysis. Similar to the average droplet 
volume, the droplet number was obtained by binning all the image analysis droplet data in two-
minute bins (for C6, C8SA, and tBuPA) and five-minute bins (for C6, C8SA, C10SA, and isoC10) 
to ensure a sufficient droplet number per unit of time. The standard deviation was calculated 
between 3 experiments (n=3).

**Theoretical model and classical Ostwald ripening.** Our reaction-diffusion model accounts 
for phase-separation and the fuel-driven chemical reactions. The underlying equations have
been analyzed numerically and also used to find an explicit expression for the average volume as a function of the experimental parameters (e.g., solubility, surface tension, and diffusion constant). More information can be found in the SI. For our emulsions, we also calculated fusion rates and the Ostwald ripening rates based on theory from Lifshitz and Slyozov.31

Data availability
The data that support the findings of this study are available from the authors on reasonable request.

Acknowledgments.
J.B. is grateful for funding by the Technical University of Munich – Institute for Advanced Study, funded by the German Excellence Initiative and the European Union Seventh Framework Programme under grant agreement n° 291763. M.T.S. acknowledges the European Union’s Horizon 2020 Research and Innovation program for the Marie Sklodowska Curie Fellowship under grant agreement n° 747007. Financial support for C.W. comes from the German Research Foundation (DFG) through Transregional Collaborative Research Center (SFB/Transregio) 235 Project 16.

Author Contributions.
J.B. and M.T.S. designed the experiments. J.J. and C.W. worked out the theory. M.T.S, C. W., F.S., H.P., B.R. J.M.G. carried out the experiments. J.B. and C.W. wrote the manuscript.

Competing Interests statement.
The authors declare no competing interests.

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Supplemental Information:

Accelerated ripening in chemically fueled emulsions

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1. Materials

4-Morpholineethanesulfonic acid (MES) buffer, pentanoic acid, hexanoic acid, 2-hexenyl-succinic anhydride (C₆SA), 2-octenyl-succinic anhydride (C₈SA), 2-decenyl-succinic anhydride (C₁₀SA), t-butyl-phthalic anhydride (tBuPA), t-butyl-phthalic acid, 4-ethyloctanoic acid, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxsuccinimide (NHS) and Nile Red were all purchased from Sigma-Aldrich and Alfa-Aesar unless indicated otherwise. The precursor for C₆SA (2-hexenyl-succinic acid), C₈SA (2-octenyl-succinic acid), C₁₀SA (2-decenyl-succinic acid) and tBuPA (t-butyl-phthalic acid) were synthesized by the hydrolysis of the commercially available anhydrides (see protocol below).

Synthesis of 2-hexenyl-succinic acid, 2-octenyl-succinic acid, 2-decenyl-succinic acid, and 4-tert-butylphthalic acid.

![Synthesis diagram]

The corresponding anhydrides (typically ~300mg of tBuPA, C₆SA (n=3), C₈SA (n=5) and C₁₀SA (n=7), ±1 mmol) were suspended in 30 mL demineralized water. The samples were gently stirred and allowed to hydrolyze over the course of 48 h. Then, the absence of anhydride was assessed by High-Performance Liquid Chromatography (HPLC). These solutions were freeze-dried to yield the precursors as a white powder. Their purity was assessed by High-Performance Liquid Chromatography (HPLC, Thermo Fisher Dionex Ultimate 3000, Hypersil Gold 250 x 4.8 mm), ¹H-NMR and Electrospray Ionization-Mass Spectroscopy (ESI-MS).

2. Experimental Methods

Sample preparation. Stock solutions of the precursors were prepared by dissolving the precursor in MES buffer, after which the pH was adjusted to pH 6.0. The concentration of MES was adjusted depending on the precursor concentration. For precursor concentrations of 10 mM and below, 200 mM of MES buffer was used. For higher precursor concentrations, a buffer concentration of up to 500 mM was used. Stock solutions of EDC were prepared by dissolving the EDC powder in demineralized water. Typically, stock solutions of 1.0 M EDC were used freshly. The reaction cycles were started by addition of the high concentration EDC to the precursor solution. All experiments were carried out as close to 25 °C as possible.

Batch-fueled kinetic experiments. Each batch-fueled experiment was carried out by addition of a single batch of EDC to different precursors of certain concentration: 300 mM C₅, 100 mM C₆, 10 mM of C₆SA, 10 mM of C₈SA, 7.5 mM of C₁₀SA, 10 mM of tBuPA, and 5 mM of isoC₁₀. In the case of isoC₁₀, the addition of fuel was accompanied by 5 mM of NHS. All experiments were carried out at 25 ±0.5 °C.

Continuously fueled kinetic experiments. Continuously fueled experiments were performed for C₆SA, C₈SA, and C₁₀SA. An initial amount of EDC fuel was added to stock solutions of the precursors. Specifically, 10 mM of EDC was added to 10 mM C₆SA; 10 mM EDC was added to 7.5 mM of C₁₀SA; 50 mM of EDC was added to 10 mM of C₆SA. 15 minutes after the first batch of fuel, EDC was provided with a flux of 55 mM/h, 4 mM/h, and 0.6 mM/h respectively for C₆SA, C₈SA, and C₁₀SA. These fluxes
were added in batches 4 times per hour. The mixture was gently shaken after each addition. All experiments were carried out at 25±0.5 °C.

**HPLC.** The concentration profiles of the chemical reaction networks were monitored over time by means of analytical HPLC (HPLC, Thermo Fisher Dionex Ultimate 3000, Hypersil Gold 250 x 4.8 mm). A 1.5 mL sample was prepared as described in a screw cap HPLC vial. Samples of this solution were directly injected without further dilution and all compounds involved were separated using linear gradients of water and acetonitrile. The following gradients were used. Method 1: water: acetonitrile gradient from 40:60 to 2:98 in 8 min. Method 2: water: acetonitrile gradient from 98:2 to 2:98 in 12 min. Method 3: water: acetonitrile gradient from 98:2 to 2:98 in 13 min. All methods were followed by 2 min 98% acetonitrile. Calibration curves for EDC, precursor and products were prepared in triplicate in order to quantify the compounds over time (λ= 220 nm was used for all of them except for tert-butylphthalic acid, in this case the emission was recorded at λ= 254 nm). See Extended Data Table 1 for all data.

**ESI.** ESI-MS measurements were performed using a Varian 500 MS LC ion trap spectrometer. The samples were diluted in acetonitrile and injected into an acetonitrile carrier flow (20 μL/min).

**UV/Vis Spectroscopy.** The UV/Vis measurements were carried out using a Multiskan FC (ThermoFisher) microplate reader. Samples were directly prepared into 96 well-plate (tissue culture plate non-treated) as described above. All these UV/Vis measurements were performed in triplicate. Data was measured at 600 and 400 nm at 25°±0.5 °C.

**Confocal Fluorescence Microscopy.** Confocal fluorescence microscopy was performed on a Leica SP5 confocal microscope using a 63x oil immersion objective. Samples were prepared as described above but with 2.5 μM Nile Red as a dye. 12-20 μL of the sample was deposited on the PEG-coated glass slide and covered with a 12-mm coverslip. Samples were excited with 543 nm laser and imaged at 580-700 nm. The solutions were replaced every five minutes for the batch-fueled experiments and every 15 min for the pseudo-continuous fueled experiments. Every minute a 4096 x 4096 pixel² image was acquired of an area that covered 246 μm x 246 μm. Each experiment was performed in triplicate (n=3). In the case of tBuPA, the large anhydride droplets were pulled down by gravity, and stuck to the surface of the glass slide. In order to circumvent this problem, the anhydride droplets were immobilized using 0.5% wt of agar-agar gel. 50μL of a hot solution of agar-agar 2% wt was added over 150 μL of the sample of interest. The mixture was gently shaken and deposited on the PEG-coated glass as previously explained. This process was repeated every 5 minutes.

**Image analysis.** The micrographs acquired by confocal microscopy were analyzed with ImageJ’s “analyse particles” package. The software overlays the droplets with a perfect circle and measures its circumference. The measured circumference for each droplet was then converted to the droplet volume.

**Calculation of the average droplet volume.** The droplet volumes were averaged per unit of time by binning all the image analysis droplet data in two-minute bins (for C₅, C₆SA and tBuPA) and five-minute bins (for C₆, C₉SA, C₁₀SA and isoC₁₀) to ensure sufficient droplets per unit of time. These experiments were carried out in triplicate (n=3). From the three combined datasets, the histograms of the droplet distributions over time were also calculated.

**Calculation of the average droplet volume growth rate.** The droplet growth rate was calculated by measuring the slope of the droplet volume over time for an individual experiment. The experiment was

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1 Tena-Solsona, M. et al. Non-equilibrium dissipative supramolecular materials with a tunable lifetime. *Nature Communications* **8**, 15895 (2017).
repeated three times. Then the average growth rate and standard deviation from the average was calculated (n=3).

**Calculation of the droplet number.** The droplet number was also obtained from the image analysis. Similar to the average droplet volume, the droplet number was obtained by binning all the image analysis droplet data in two-minute bins (for C₅, C₆SA and tBuPA) and five-minute bins (for C₆, C₈SA, C₁₀SA and isoC₁₀). The experiment was carried out in triplicate (n=3).

**Reaction-diffusion model for a homogeneous system.** To calculate the concentration of each reactant (precursor, EDC, O-isoacylurea and product) and the change of droplet volume as a function of time, we derived a reaction-diffusion model that accounts for phase separation and chemical reaction kinetics. The model is an expansion of a previously described chemical reaction model. For details on the mathematical derivation, see section "Theoretical Model" below. To extract the kinetic parameters, we have derived a simplified version of this reaction-diffusion model in the limit of small supersaturation where the concentration outside is homogeneous and equal of the solubility \( c_{\text{out}}^{(0)} \). This homogeneous reaction-diffusion model is equal to the phenomenologically formulated model described previously.²,³ The concentrations in this model were calculated on the basis of five differential equations that described the following five chemical reactions:

- **Reaction 0**: Reaction of EDC with water, *i.e.*, the direct hydrolysis (\( k_0 \), first order rate constant):

  \[
  \text{N}^\text{+} \text{C} = \text{N} \quad + \quad \text{H}_2\text{O} \quad \rightarrow \quad \text{N}^\text{+} \text{C} = \text{N}.
  \]

- **Reaction 1**: Reaction between EDC and the precursor, *i.e.*, the activation of the precursor (rate constant \( k_F \), second order reaction):

  For reaction cycle 1 and 2:

  \[
  \text{R}_2\text{O} \quad + \quad \text{N}^\text{+} \text{C} = \text{N} \quad \rightarrow \quad \text{R}_2\text{O} \quad \text{N}^\text{+} \text{C} = \text{N}.
  \]

  For reaction cycle 3 and 4:

  \[
  \text{R}_1\text{OH} \quad + \quad \text{N}^\text{+} \text{C} = \text{N} \quad \rightarrow \quad \text{R}_1\text{OH} \quad \text{N}^\text{+} \text{C} = \text{N}.
  \]

- **Reaction 2**: Reaction of the O-isoacylurea with a second carboxylic to form the anhydride or NHS ester (rate constant \( k_2 \), second order, or pseudo-first order reaction):

  For reaction cycle 1 and 2:

  \[
  \text{R}_1\text{OH} \quad + \quad \text{N}^\text{+} \text{C} = \text{N} \quad \rightarrow \quad \text{R}_1\text{OH} \quad \text{N}^\text{+} \text{C} = \text{N}.
  \]

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² Tena-Solsona, M.; et al., J. Self-Selection of Dissipative Assemblies Driven by Primitive Chemical Reaction Networks. *Nature Communications* **2018**, *9* (1), 2044
³ Rieß, B.; et al., J. Dissipative Assemblies That Inhibit Their Deactivation. *Soft Matter* **2018**, *14* (23), 4852–4859
For reaction cycle 3:

\[
\begin{align*}
\text{For reaction cycle 4:} \\
\end{align*}
\]

- Reaction 3: Hydrolysis of O-isoacylurea (rate constant \(k_3\), first order chemical reaction):

\[
\begin{align*}
\text{For reaction cycle 1 and 2:} \\
\end{align*}
\]

- Reaction 4: Hydrolysis of the product, i.e., anhydride or NHS-ester (deactivation rate constant \(k_d\), first order chemical reaction):

\[
\begin{align*}
\text{For reaction cycle 3:} \\
\end{align*}
\]

\[
\begin{align*}
\text{For reaction cycle 4:} \\
\end{align*}
\]
**Determination of the rate constants for the model.** The rate constant for the direct hydrolysis of carbodiimide $k_d$ with a first order rate constant of 1.3$x$$10^{-5}$ s$^{-1}$ was determined in earlier work by HPLC.$^1$ The second order rate constant for the formation of the O-acyl urea by reaction with and EDC $k_F$ was determined for each precursor by monitoring the EDC consumption with HPLC (see Figure S2 for representative kinetic traces). The formation of anhydride or NHS-ester with a second order rate constant $k_2$ could not be determined because the O-acyl urea was never observed. It was therefore set to be 1 times the rate constant of $k_F$. As a result, the O-acyl urea never reached concentrations over 1 $\mu$M in the kinetic model. For the same reason, the first order rate constant for the direct hydrolysis of the O-acyl urea $k_s$ could not be determined empirically. The ratio of $k_2$ and $k_s$ was thus varied to fit the HPLC data for several concentrations of initial EDC concentrations for each precursor. The zero-order reaction rate for the hydrolysis of anhydride, called effective hydrolysis rate $r_{\text{eff}}$, was determined from the slope of the linear decay of the anhydride concentration (measured by HPLC) over time in a regime where: 1) all EDC had been consumed and 2) droplets were present (see Figure S2 for exemplary traces). The effective deactivation rates are listed in Table S2. In order to calculate the deactivation rate constant $k_d$, the effective deactivation rate $r_{\text{eff}}$ was divided by the solubility $c_{\text{out}}^{(0)}$ that we determined empirically for each product (vide infra). In other words, the zero-order hydrolysis rate constant was found by the relationship $r_{\text{eff}} = k_d c_{\text{out}}^{(0)}$.

**Determining the product solubilities and effective deactivation rates.** The solubilities of the droplet forming products were determined empirically by comparing UV/Vis traces with HPLC traces. The formation of the product upon fuel consumption was monitored initially by turbidity measurements at 600 nm. We established an arbitrary threshold of absorbance of 0.01 above which the solutions are considered “turbid” and droplets were present. At the time the absorbance value fell below the defined threshold value, the concentration of the anhydride was determined by a separate HPLC measurement which allowed us to determine the effective deactivation rates (see above). These experiments were carried out for each droplet forming molecules and in triplicate (n=3) (see Table S2).

**Measuring the interfacial surface tension.** The interfacial surface tension between water and the droplet forming product was determined using the video-based optical contact angle measuring system OCA 25 from DataPhysics, equipped with the software SCA 22-pendant drop (Table S2). 1 mL Braun disposable syringes and regular needles of 0.8 mm radii were used for C$_6$SA and C$_8$SA anhydride. 1 mL Hamilton syringe and upwards bent needles of 0.26 mm radii were used for C$_5$, C$_6$, C$_{10}$SA and isoC$_{10}$ acid. To measure the interfacial surface tension of the isoC$_{10}$ ester was not possible, instead the value for the isoC$_{10}$ acid was determined and assumed to be similar compared to the isoC$_{10}$-NHS ester.

Droplets of the commercially available anhydrides or isoC$_{10}$ acid were dispensed in a buffer solution of 200 or 500 mM MES at pH 6 (depending on the sample preparation conditions already described) at a different dosing rates (from 0.1 to 3 $\mu$L/s). The measurements were repeated 10 times (n=10).

The interfacial tension value for C$_{10}$SA could not be determined because its density is too close to the density of buffered aqueous solution (i.e., droplets neither float nor sink during the time scales of droplet growth). The corresponding interfacial tension value for C$_{10}$SA was estimated considering the correlation between carbon number and interfacial tension values for C$_6$SA, C$_8$SA and C$_{10}$SA (see Figure S1).

Finally, the surface tension of tBuPA could not be determined. When in situ activated by the EDC-driven reaction cycle, tBuPA forms droplets and is a liquid, but as a bulk material it is a solid. We therefore mixed the anhydride of tBuPA with the anhydride of C$_8$SA in various ratios and measured the interfacial surface tension of the mixtures. Then, we linearly extrapolated to 0% C$_8$SA to yield the interfacial surface tension of the anhydride of tBuPA.
**Calculations of the Ostwald ripening rate.** In order to compare the accelerated average volume growth rate to classical Ostwald ripening, we have considered Eq. (12) (see section “Theoretical Model” below). The parameters we used are the molecular volume, solubility and interfacial surface tension, which have all been determined empirically or calculated (Table S2). The diffusion constant was estimated to be similar for all compounds and equal to 500 \( \mu m^2/s \). The calculated values for the Ostwald ripening rates are given in Table S3.
### 3. Supporting Tables and Figures

#### Table S1: Chemical assessment of all products by HPLC and ESI-MS.

| Name                  | Structure | HPLC retention time (min) | HPLC calibration value (meas.) | Mass calculated (g/mol) | Mass found (g/mol) |
|-----------------------|-----------|---------------------------|-------------------------------|-------------------------|--------------------|
| tBuPA anhydride       | ![Structure](image1) | 11.82 (method 2)          | 2.74 (1 µL injection)          | Mw = 204.2             | Mw = 205.1 [Mw+H]^+|
| C6SA anhydride        | ![Structure](image2) | 7.78 (method 1)           | 5.19 (25 µL injection)         | Mw = 182.1             | 183.1 [Mw+H]^+    |
| C8SA anhydride        | ![Structure](image3) | 12.84 (method 2)          | 5.04 (25 µL injection)         | Mw = 210.1             | 211.2 [Mw+H]^+    |
| C10SA anhydride       | ![Structure](image4) | 13.39 (method 2)          | 5.48 (25 µL injection)         | Mw = 238.2             | 239.2 [Mw+H]^+    |
| C5 anhydride          | ![Structure](image5) | 14.0 (method 3)           | 2.48 (25 µL injection)         | Mw = 186.2             | 209.2 [Mw+Na]^+   |
| C6 anhydride          | ![Structure](image6) | 14.8 (method 3)           | 2.62 (25 µL injection)         | Mw = 214.3             | 237.2 [Mw+Na]^+   |
| isoC10-NHS-ester      | ![Structure](image7) | 13.46 (method 2)          | 5.20 (20 µL injection)         | Mw = 172               | N/A                |

#### Table S2: Physical-chemical characterization of all products.

| Name                  | Structure of product | Mw (g/mol) | Density (g/mL) | Molecular Volume (m³/mol) | Effective deactivation rate $r_{eff}$ (µM/min) | Solubility (mM) (n=3) | Interfacial tension (mN/m) (n=10) |
|-----------------------|----------------------|------------|----------------|---------------------------|-----------------------------------------------|-----------------------|-----------------------------------|
| tBuPA anhydride (cycle 1) | ![Structure](image8) | 204        | 1.53           | 1.45x10⁻⁴                 | 294.0 ± 0.6                                  | 0.6 ± 0.6             | ~3*                               |
| C6SA anhydride (cycle 2)  | ![Structure](image9) | 182        | 1.07           | 1.70x10⁻⁴                 | 546.0 ±0.05                                  | 2.88±0.05             | 10.5 ± 0.3                        |
| C8SA anhydride (cycle 2)  | ![Structure](image10) | 210        | 1.02           | 2.06x10⁻⁴                 | 51.5 ±0.04                                   | 0.15±0.04             | 6.3 ± 0.7                         |
| C10SA anhydride (cycle 2)| ![Structure](image11) | 238        | 1.00           | 2.38x10⁻⁴                 | 3.4 ±0.02                                    | 0.02±0.02             | 4.2 ± 0.4                         |
| C5 anhydride (cycle 3)   | ![Structure](image12) | 186        | 0.944          | 1.97x10⁻⁴                 | 59.4 ±0.2                                    | 0.9±0.2               | 18.2 ± 0.2                        |
| C6 anhydride (cycle 3)   | ![Structure](image13) | 214        | 0.928          | 2.31x10⁻⁴                 | 3.3 ±0.08                                    | 0.10±0.08             | 17.0 ± 0.9                        |
| isoC10-NHS-ester (cycle 4) | ![Structure](image14) | 172        | 0.908          | 1.89x10⁻⁴                 | 0.44 ±0.02                                   | 0.15±0.02             | 7.69 ± 0.04                       |
**Table S3: List of hydrolysis and growth rates for all droplet forming molecules in batch- and continuous fuel experiments.**

| Name                          | Calculated Ostwald ripening rate (µm³/min) | Average volume growth rate batch-fueled experiments (µm³/min) (n=3) | Average volume growth rate continuous-fueled experiments (µm³/min) (n=3) | Rescaled deactivation rate S (s⁻¹) | Degree of acceleration (η) |
|-------------------------------|--------------------------------------------|---------------------------------------------------------------------|-----------------------------------------------------------------------|---------------------------------|-----------------------------|
| tBuPA anhydride (cycle 1)    | 0.0032                                     | 0.305 ± 0.03 (95 x accelerated)                                      | N/A                                                                  | 90                              | N/A                         |
| CsSA anhydride (cycle 2)     | 0.045                                      | 0.15 ± 0.03 (3.4 x accelerated)                                      | 0.58 ± 0.2 (15 x accelerated)                                        | 7.10                            | 3.84                        |
| CsSA anhydride (cycle 2)     | 0.0021                                     | 0.038 ± 0.009 (19 x accelerated)                                     | 0.11± 0.02 (52 x accelerated)                                        | 28                              | 2.89                        |
| CsSA anhydride (cycle 2)     | 0.00024                                    | 0.0034 ± 0.001 (14 x accelerated)                                     | 0.015±0.007 (63 x accelerated)                                        | 20                              | 4.29                        |
| Cs anhydride (cycle 3)       | 0.044                                      | 0.15 ± 0.03 (3.4 x accelerated)                                      | N/A                                                                  | 2                               | N/A                         |
| Cs anhydride (cycle 3)       | 0.0046                                     | 0.017 ± 0.01 (3.7 x accelerated)                                      | N/A                                                                  | 1.1                             | N/A                         |
| isoC₁₀-NHS-ester (cycle 4)   | 0.0021                                     | 0.0022 ± 0.0005 (1.1 x accelerated)                                   | 0.0031 ± 0.003 (1.5 x accelerated)                                    | 0.21                            | 1.22                        |
**Table S4: List of rate constants used in kinetic model.**

| Name                        | Product structure | $k_F$ (M·sec$^{-1}$) | $k_2$ (M·sec$^{-1}$) | $k_3$ (M·sec$^{-1}$) | $k_d$ (s$^{-1}$) | Solubility (mM) |
|-----------------------------|-------------------|-----------------------|----------------------|----------------------|------------------|-----------------|
| tBuPA anhydride (cycle 1)  | ![Structure](image) | 0.50                  | 1.0 x $k_F$          | 0.0 x $k_F$          | 1.0 x 10^{-2}   | 0.6             |
| C$_6$SA anhydride (cycle 2)| ![Structure](image) | 0.14                  | 1.0 x $k_F$          | 0.6 x $k_F$          | 2.0 x 10^{-3}   | 2.88            |
| C$_8$SA anhydride (cycle 2)| ![Structure](image) | 0.16                  | 1.0 x $k_F$          | 0.2 x $k_F$          | 5.72 x 10^{-4}  | 0.15            |
| C$_{10}$SA anhydride (cycle 2)| ![Structure](image) | 0.16                  | 1.0 x $k_F$          | 0.35 x $k_F$         | 2.8 x 10^{-3}   | 0.02            |
| C$_5$ anhydride (cycle 3)  | ![Structure](image) | 0.023                 | 1.0 x $k_F$          | 0.8 x $k_F$          | 1.1 x 10^{-3}   | 1.2             |
| C$_6$ anhydride (cycle 3)  | ![Structure](image) | 0.023                 | 1.0 x $k_F$          | 2.2 x $k_F$          | 6.5 x 10^{-4}   | 0.08            |
| isoC$_{10}$-NHS-ester (cycle 4) | ![Structure](image) | 0.035                 | 1.0 x $k_F$          | 0.5 x $k_F$          | 4.9 x 10^{-5}   | 0.15            |

**Figure S1: Surface tension determination for C$_{10}$SA.** Linear correlation between carbon number and interfacial tension value. Black markers are acquired data. The red marker is an interpolated data point for C$_{10}$SA based on a polynomial fit. The error bars refer to the standard deviation (n=10).
Figure S2. Kinetics of seven precursor-product combinations in response to a batch of fuel. Concentration profile of product and EDC over time for a) 10 mM of tBuPa and 10 mM EDC, b) 10 mM of C6SA and 25 mM of EDC, c) 10 mM of C8SA and 10 mM of EDC, d) 7.5 mM of C10SA and 1 mM of EDC, e) 300 mM of C5 and 10 mM of EDC, f) 100 mM of C6 and 2 mM of EDC and h) 5 mM of isoC10, 5 mM of NHS and 1 mM of EDC. Markers represent HPLC data points while black lines represent the calculated concentrations using the reaction-diffusion model (see section “Theoretical Model” below). The dashed red lines stand for the solubility values of the corresponding products.
Figure S3. Mean droplet volume growth and number of droplets against time observed in batch-fueled mode. The mean droplet volume and droplet number against time observed for the seven precursor-product combinations described in Figure S2 after EDC addition. The markers represent the data as measured by image analysis software in three experiments (n=3), the black line represent the average of those three experiments. The dashed red line represents results calculated from the reaction-diffusion model (see section “Theoretical Model” below). The blue line represent the calculated classical Ostwald ripening. Please note that droplet nucleation is fast on the time scale of experimental imaging. For an estimate of the typical nucleation time, see section “Nucleation of droplets” below.
Figure S4. Kinetics of four precursor-product combinations in response to a continuous addition of fuel. Concentration profile of the steady state as measured by HPLC. The steady state was induced by addition of an initial batch of fuel to a solution of precursor followed by a continuously fueling. a) 50 mM of EDC was initially added to a 10 mM C₆SA solution followed by a continuous flow of 55 mM/h. b) 10 mM of EDC was initially added to a 10 mM C₈SA solution followed by a continuous flow of 4 mM/h. c) 10 mM of EDC was initially added to a 7.5 mM C₁₀SA solution followed by a continuous flow of 0.6 mM/h. Markers represent HPLC data points while black lines represent the calculated concentrations from the theoretical reaction-diffusion model (see section “Theoretical Model” below). The error bar represents the standard deviation between three experiments (n=3).
Figure S5. Mean droplet volume growth against time observed in steady state. Mean droplet volume, number of droplet counts, and size distribution histograms over time for the four precursor-product combinations described in Figure S4. The markers represent the data as measured by image analysis software in three experiments (n=3), the black line represent the average of those three experiments. The dashed red line represents results calculated from the reaction-diffusion model (see section “Theoretical Model” below). The blue line represent the calculated classical Ostwald ripening.
**Figure S6.** Selection of images obtained via confocal microscopy for the continuous fueling experiments at different time points and for three different precursors.

**Figure S7.** Selection of two collision events (a and b) obtained via confocal microscopy during batch mode experiment (C₆SA with 10 mM EDC). The droplets collide, stick together for several frames, and then unstick. No fusion events were ever observed.
Figure S8. Cryo-TEM micrographs of the chemically fueled emulsion in the first minute of formation. a) Chemically fueled droplets formed by C₆SA with 50 mM EDC, b) chemically fueled droplets formed by C₈SA with 5 mM EDC, c) C₁₀SA with 1mM of EDC. The white arrows indicate some of the droplets observed. All scale bars are 100 nm.
4. Theoretical Model for Accelerated Droplet Growth

In the absence of fuel, droplets grow via two mechanisms: droplet fusion and Ostwald ripening. Droplet fusion is set by the frequency of diffusing droplets to encounter each other and the energetic barrier to undergo fusion given such an encounter. Ostwald ripening is driven by different Laplace pressures among droplets. Both processes, fusion and Ostwald ripening, decrease the free energy, in particular, they reduce the total surface of droplets and drive the system to its equilibrium states of a single droplets coexisting with a solvent that is poor in droplet components. Moreover, both processes lead to a linear increase of the average droplet volume, \( \langle V \rangle (t) = mt \) and a decrease of droplet number proportional to \( 1/t \).

However, the contribution of each process to the average droplet volume growth rate \( m \) depends on the considered system (used chemical compounds, composition, etc.).

For chemically fueled systems, where fuel is consumed to drive chemical reactions away from equilibrium, the average volume need not necessarily increase linearly with time. However, if it does (as we have shown experimentally), the average droplet volume growth mechanism can be affected by the properties of the chemical reactions, e.g., reaction rates or fuel. Below, we will derive a theoretical model that accounts for the fuel-driven chemical reactions in the oil-poor phase (outside the oil droplets). By means of this model, we will show how these fuel-driven chemical reactions affect the average volume growth rates of the oil droplets. In particular, we will find that that the growth rate can be significantly accelerated compared to classical Ostwald ripening. For the system of interest, we also show that fusion has a negligible impact on the growth rate.

Model for fuel driven chemical reactions outside of the droplets

Our system is essentially composed of five different type of molecules: non-hydrolyzed fuel \( F \) (shortly referred to as fuel in the following), precursor \( A \), intermediate product composed of fuel and precursor \( AF \), product \( B \), and water as solvent. The fuel \( F \) can undergo two chemical reactions: The fuel \( F \) can get slowly hydrolyzed to waste \( W \) in the presence of water with a rate constant \( k_0 \) (reaction 0, Eq. (1a)), or, the fuel \( F \) drives the transition from the precursor \( A \) to the intermediate product \( AF \) with a chemical flux that is proportional to the fuel concentration \( c_F \) with a rate constant \( k_F \) (reaction 1, second order chemical reaction). This intermediate product can spontaneously react back to the precursor with a rate \( k_3 \), or irreversibly turn over to the product (reactions 1-3, Eq. (1b)). The turn-over to the product (reaction 2) occurs either spontaneously with a rate \( \tilde{k}_2 \) (first-order reaction) or requires precursor and reacts in a second-order reaction with a rate \( k_2 = k_2 c_A \). In the presence of water, this product hydrolyses and thereby turns over to the precursor (Eq. (1c)) which we shortly refer to as deactivation. Considering a constant pH and dilute conditions relative to water, deactivation follows a first order chemical reaction with a deactivation rate constant \( k_d \). All reactions 0-4 can be summarized by the following reaction schemes:

\[
\text{Reaction 0: } \quad F \xrightarrow{k_0} W, \quad (1a)
\]

\[
\text{Reactions 1-3: } \quad A \xrightarrow{k_F c_F/k_3} AF, \quad \tilde{k}_2 \text{ or } k_2 = k_2 c_A \rightarrow B, \quad (1b)
\]

\[
\text{Reaction 4: } \quad B \xrightarrow{k_d} A. \quad (1c)
\]

At large enough concentration of product \( B \), droplets can form. Since fuel \( F \), precursor \( A \) and intermediate product \( AF \) are well soluble in water the droplets dominantly contain the rather hydrophobic product \( B \). These product-rich droplets effectively represent oil droplets that are phase separated from the water-rich solvent. The equilibrium concentration of \( B \) inside the droplet, \( c^{(0)}_{\text{in}} \), exceeds the equilibrium concentration outside \( c^{(0)}_{\text{out}} \) (often called solubility) by three to four orders of magnitude. The absence of fuel \( F \) and water
inside the droplets restricts the chemical reaction kinetics (Eq. (1)) to the outside of the droplets. Thus, droplets function as storages for product components $B$ which can grow and shrink due to influx or efflux of $B$ through the droplet interfaces. Most importantly, these fluxes are fully determined by diffusion and chemical reactions of the components outside the droplets.

We can thus expand the non-linear diffusive fluxes outside the droplets up to the first order and account for weak spatial inhomogeneities.\(^1\) For dilute concentrations of the fuel $F$ and the components $A, AF$ and $B$ outside of the droplets relative to the solvent water, the chemical reaction kinetics can be accounted for by the lowest leading order corresponding here to chemical reactions of first or second order. Specifically, depending on the type of precursor component, activation occurs with a rate $k_{FC_A}$ due to the reaction between the fuel and one precursor $A$ or two precursors (consuming twice the amount of precursors), respectively.

We find the following reaction-diffusion equations outside the droplets:

\begin{align}
\partial_t c_F &= -k_F c_F c_A + D_F \nabla^2 c_F - k_0 c_F + j_F , \tag{2a} \\
\partial_t c_A &= -k_F c_F c_A + k_3 c_A F + k_d c_B + D_A \nabla^2 c_A , \tag{2b} \\
\partial_t c_{AF} &= k_F c_F c_A - (k_3 + \tilde{k}_2) c_{AF} + D_{AF} \nabla^2 c_A , \tag{2c} \\
\partial_t c_B &= k_2 c_{AF} - k_4 c_B + D_B \nabla^2 c_B - J , \tag{2d}
\end{align}

where $D_k (k = F, AF, A, B)$ are the diffusion constants and $j_F$ is the fuelling flux. Droplets are considered to be only composed of product $B$ in our model. Growth and shrinkage of droplets affects the concentration of product outside and is captured by the point sinks and sources assuming that droplets are small compared to the typical inter-droplet distance:

\begin{equation}
J(\vec{x}, t) = c^{(0)}_A \partial_t \sum_i N(t) \delta(3) (\vec{x} - \vec{x}_i) \frac{4\pi}{3} R_i(t)^3 , \tag{2e}
\end{equation}

where $N(t)$ denotes the droplet number and $R_i$ the radius of droplet $i$. These sinks and sources account for the change in the total droplet volume

\begin{equation}
V_d(t) = \frac{4\pi}{3} \sum_i N(t) R_i(t)^3 \tag{3}
\end{equation}

as a function of time $t$. In the following we will determine how the chemical reactions and diffusion of all the components outside the droplets affect the growth or shrinkage of droplets of radius $R$.

**Homogeneous model to estimate chemical reaction rates**

Here we will consider the homogeneous case in the absence of droplets ($J = 0$). Without droplets we can also neglect the diffusion terms and arrive at a set of coupled homogeneous, ordinary chemical reaction equations:

\begin{align}
\partial_t c_F &= -k_F c_F c_A - k_0 c_F + j_F , \tag{4a} \\
\partial_t c_A &= -k_F c_F c_A + k_3 c_A F + k_d c_B , \tag{4b} \\
\partial_t c_{AF} &= k_F c_F c_A - (k_3 + \tilde{k}_2) c_{AF} , \tag{4c} \\
\partial_t c_B &= \tilde{k}_2 c_{AF} - k_4 c_B . \tag{4d}
\end{align}

\(^1\)Weber, C. A. and Zwicker, D. and Jülicher, F. and Lee, C. F., Physics of active emulsions, *Reports on Progress in Physics*, 82, 064601, (2019).
These coupled equations are used to estimate the rate constants of the chemical reactions of \( k_0, k_F, k_3, \tilde{k}_2 \) and \( k_d \). These parameters could be estimated by fitting the numerically calculated time traces of the product concentration \( c_B(t) \) and fuel concentration \( c_F(t) \) to the experimental measurements. However, this strategy turn out to be tricky for some reaction cycles as concentration levels may fall below the detection limit of HPLC. Thus, we consider the case of droplets which approximately enforces the product concentration outside to the equilibrium concentration \( c_B \approx c_{B(0)}^{(out)} \). The corresponding numerical solutions fit well the experimental measurements of fuel and total concentrations of products and allow us to extract kinetic parameters. For the parameters obtained from fitting, see Table S4. However, these equations are not suited to calculate the average droplet volume which requires considering the sinks and sources of product \( B \) described by \( J \) (Eq. (2e)).

**Model simplifications for experimentally relevant conditions**

In this section we simplify our model Eqs. (2) in order to describe the growth kinetics of droplets for two experimentally considered protocols of how fuel is added to the system. In particular, we consider the batch-fueled protocol at late times when most of the fuel has been depleted and the continuously fueled protocol where the total concentration of products in the system is approximately constant in time. Focussing on these experimentally relevant cases, we will be able to derive explicit mathematical expressions for the growth of the average droplet volume \( \langle V \rangle(t) \) with time \( t \) that agree well with the experimental measurements. Below we discuss the considered approximations.

**Fuel stability in water.** First of all, the hydrolysis of fuel due to the reaction with water (reaction 0) typically occurs with the smallest rate compared to all the other reaction steps. The corresponding half life is in the order of a day while experiments are conducted within a few hours. Thus, we neglect the degradation of fuel via the slow hydrolysis of fuel in the presence of water in our equations.

**Short-lived intermediate product.** As a second approximation, we consider the intermediate product composed of fuel and precursor, \( AF \), to be short-lived reacting quickly to the precursor and the product. We can thus use a quasi-static approximation for the \( AF \) concentration, \( \partial_t c_{AF} \approx 0 \). Moreover, we consider the \( AF \) concentration field to be homogeneous. The quasi-static concentration of the intermediate product \( c_{AF} \approx \frac{k_F c_F c_A}{(k_3 + \tilde{k}_2)} \) can be substituted in Eq. (2). This step leads to a reduced reaction scheme compared to Eq. (1) where precursor gets activated by fuel and product gets deactivated via hydrolysis:

\[
\text{precursor } A \xrightarrow{\frac{\lambda k_F c_F}{k_3}} \text{fuel } F.
\]

Here, the quasi-static approximation of the intermediate product gives a yield factor \( \lambda = \frac{\tilde{k}_2}{(\tilde{k}_2 + k_3)} \). This factor ranges between zero \((k_3 \gg \tilde{k}_2)\) and one \((k_3 = 0)\). It accounts for the reduced production of product \( B \) due to the metastability of \( AF \) complex and can be estimated for different chemical systems. Note that depending on the reaction pathway the rate \( \tilde{k}_2 \) is either constant or varies with precursor, i.e., \( k_2 = \tilde{k}_2 c_A \) (reaction 3). After using this quasi-static approximation the system is only composed of three types of molecules, namely fuel \( F \), precursor \( A \) and product \( B \).

**Fast diffusion of precursor.** As a further approximation we neglect diffusion of precursor because of its high diffusion constant in the order of \( 500 \text{ } \mu \text{m}^2 \text{s}^{-1} \). However, product-rich droplets act as sinks and sources for the \( B \) material. Thus, we keep the diffusion term for the \( B \) component because it determines the concentration gradients relevant for inter-droplet diffusion and thereby the droplet growth.

Using the three approximations described above, Eqs. (2) yield a set of reduced reaction-diffusion equations...
for the (hydrolyzed) fuel $F$, the precursor $A$ and the product $B$:

\[
\begin{align*}
\partial_t c_F &= -k_F c_F c_A + j_F , \\
\partial_t c_A &= -\lambda k_F c_F c_A + k_d c_B , \\
\partial_t c_B &= \lambda k_F c_F c_A - k_d c_B + D_B \nabla^2 c_B - J .
\end{align*}
\]

(6a) \hspace{1cm} (6b) \hspace{1cm} (6c)

**Batch-fueled protocol**

Eqs. (6a)-(6c) can be simplified further by specifying the experimental fuel supply protocol. In the case of the batch-fueled protocol, which was carried out by addition of a single batch of fuel, we can consider the regime where fuel is already depleted, $c_F \approx 0$, but droplets are still present. In this regime we can study the following decoupled equation for the product $B$ outside the droplets:

\[
\partial_t c_B = -k_d c_B + D_B \nabla^2 c_B - J .
\]

(7)

This equation defines the reaction length scale $\ell_{\text{deact}} = \sqrt{D_B/k_d}$. For inter-droplet distances below the reaction length scale, diffusion between droplets is fast compared to deactivation. For inter-droplet distances above the reaction length scale $\ell_{\text{deact}}$ and for large time scales, droplets maintain the outside concentration far away from the droplets $c_B(x,t) \approx c^{(0)}_{\text{out}}$. This gives after using Eq. (7), $J \approx -k_d c^{(0)}_{\text{out}}$. After integration over the system volume $V_{\text{sys}}$, we find that

\[
\partial_t V_d \approx -V_{\text{sys}} r_{\text{eff}}/c^{(0)}_{\text{in}} ,
\]

(8)

where $V_d(t)$ is the total droplet volume (Eq. (3)) and we have defined the effective deactivation rate

\[
r_{\text{eff}} = k_d c^{(0)}_{\text{out}} .
\]

(9)

**Decay of the total droplet volume.** For the batch-fueled protocol and for small supersaturation we obtain for the total droplet volume

\[
V_d(t) \approx \frac{V_{\text{sys}}}{c^{(0)}_{\text{in}}} \left[ \left( \lambda c_F(t = 0) - c^{(0)}_{\text{out}} \right) - r_{\text{eff}} (t - t_{\text{max}}) \right] ,
\]

(10)

for $t > t_{\text{max}}$, where $t_{\text{max}}$ denotes the time when the total $B$ concentration reaches a maximum indicating that most fuel had been depleted. Moreover, $\lambda c_F(t = 0)$ corresponds to the amount of precursor $B$ that has been produced by the initial amount of fuel $c_F(t = 0)$.

**Growth law of average droplet volume in batch-fueled systems.** The time-dependent behavior of precursor, product and total droplet volume makes it difficult to calculate the average volume from the time-dependent behavior of the droplet size distribution. However, for the single batch-fueled systems, we can use our estimate of the total droplet volume Eq. (10) and calculate the average droplet volume via

\[
\langle V \rangle(t) = \frac{V_d(t)}{N(t)} ,
\]

(11)

where the droplet number $N(t)$ decays with time $t$ corresponding to droplet dissolution. Dissolution of droplets may occur on different time scales: For classical Ostwald ripening droplets dissolve on a time scale $\tau_{\text{clas}}$ while in the presence of deactivation of products and activation of precursors droplets dissolve...
on a time scale $\tau_d$. In the following we will estimate $\tau_d$ which will allow us to find an equation for the average droplet volume $\langle V \rangle (t)$ as a function of time.

For classical Ostwald ripening, the droplet number $N(t) = \beta (\tau_{\text{class}}/t)$, where the droplet dissolution time scale is $\tau_{\text{class}} = V_0(t)/(3\alpha \Gamma)$ with the numerical coefficients $\alpha = 4/27$ and $\beta \approx 0.211$. Here, the volume growth rate $\Gamma = D_B \ell_c (0)/c_{\text{out}}^{(0)}$ with the capillary length $\ell_c \approx 2\gamma/(k_BTc_{\text{in}}^{(0)})$ and $\gamma$ is the surface tension. For classical Ostwald ripening the average volume grows linearly with time:

$$\langle V \rangle(t) \bigg|_{\text{class}} = \frac{8}{9\beta} \frac{D_B \gamma c_{\text{out}}^{(0)}}{k_BTc_{\text{in}}^{(0)}} t =: m_{\text{class}} t.$$  \hfill (12)

where $m_{\text{class}}$ denotes the ripening rate due to classical Ostwald ripening. When fuel is depleted in our system, the turn-over from product to precursor gives rise to an additional efflux of products out of the droplet. This efflux occurs at a specific time scale, namely the time to dissolve a droplet by the deactivation of products outside the droplets. Remember that deactivation of products via hydrolysis requires water which is at negligible concentration inside the product-rich oily droplets.

For a single, small droplet of volume $V_s$ in a subsystem of volume $V_{\text{sub}}$, the growth kinetics is governed by

$$\frac{dV_s}{dt} = -k_d V_{\text{sub}} \frac{c_{\text{out}}^{(0)}}{c_{\text{in}}^{(0)}},$$  \hfill (13)

where products of concentration $c_{\text{out}}^{(0)}$ are deactivated outside the droplet with the deactivation rate constant $k_d$. The solution to the above equation describes droplet dissolution by deactivation on a time scale

$$\tau_d = \frac{c_{\text{in}}^{(0)}}{r_{\text{eff}}},$$  \hfill (14)

where $\phi = V_{s,0}/V_{\text{sub}} \ll 1$ is the droplet volume fraction in our simple one droplet system and $V_{s,0}$ corresponds to the typical droplet volume in the order of a few $\mu m^3$. In experiments, e.g., for $C_8\text{SA}$, $\phi \approx 2 \cdot 10^{-3}$. We can estimate this value by, for example, considering a time point in the early stage of the droplet kinetics. To this end, we use $V_s(t = 0) \approx 2\mu m^3$ and an inter-droplet distance of $10\mu m$. This value of the inter-droplet distance also leads to a subsystem size of $V_{\text{sub}} = 10^3 \mu m^3$. Alternatively, the droplet volume fraction can be estimated assuming that most of the precursors have turned over to products. In this case the droplet volume fraction is determined by the total concentration $c_{\text{tot}}$, i.e., $\phi \approx (c_{\text{tot}} - c_{\text{out}}^{(0)})/(c_{\text{in}}^{(0)} - c_{\text{out}}^{(0)}) \approx c_{\text{tot}}/c_{\text{in}}^{(0)} \approx 2 \cdot 10^{-3}$ (with $c_{\text{out}}^{(0)} \ll c_{\text{in}}^{(0)}$), for $c_{\text{tot}} = 10\text{mM}$ and $c_{\text{in}}^{(0)} = 5 \cdot 10^3 \text{mM}$ for the case of $C_8\text{SA}$.

Eq. (14) suggests that increasing the deactivation rate $r_{\text{eff}}$ also increases the speed of droplet dissolution, i.e., decreases the dissolution time $\tau_d$. Considering typical parameters for experimentally considered precursors, we find that the dissolution of droplets via classical Ostwald ripening is much slower than via deactivation, $\tau_{\text{class}} \gg \tau_d$ (see Table S5). Thus, the efflux from dissolving droplets may be dominated by deactivation rather than by the inter-droplet gradients due to Laplace pressure differences (Ostwald ripening). However, whether a droplet dissolves or not, is set by the droplet Laplace pressure. So smaller droplets will shrink and dissolve first, while bigger droplets hardly grow. Please note that these statements require that diffusion between droplets is fast compared to the chemical reaction, i.e., the distance between droplets is smaller compared to $\ell_{\text{deact}} = \sqrt{D_B/k_d}$ which is well-fulfilled in the experimental studies (C$_8$SA):

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2Lifshitz, I. and Slyozov, V., The kinetics of precipitation from supersaturated solid solutions, *Journal of Physics and Chemistry of Solids*, 19, 35, (1961).
In particular, using Eq. (6a) a constant fuel implies and the total concentration of products is constant, i.e., activation and deactivation of products is balanced. For the continuously fueled protocol, we can consider the experimental regime where the fuel concentration once the fuel has been completely depleted, i.e., $c_B(t > t_{\text{max}})$, gives:

$$N(t) = \tilde{\beta} \left( \tau_d / t \right) ,$$

where $\tilde{\beta}$ represents a numerical prefactor that could be calculated from the droplet size distribution. For all chemical precursors (except of $C_B$) we have found a common value of $\tilde{\beta} = 12.5$ (see Table S5), which is used to compare the theoretical results with the experimental data as a function of time.

Using Eqs. (11), (10) and (15), neglecting the contributions of Ostwald ripening on the average volume growth and restricting ourselves to the regime $t > t_{\text{max}}$, gives:

$$\langle V \rangle(t) = \langle V \rangle(t_{\text{max}}) + \frac{V_{\text{sys}}}{\tilde{\beta}} \phi \left( \frac{\left( \lambda c_F(t = 0) - c_{\text{out}}^{(0)} \right) r_{\text{eff}}(t - t_{\text{max}}) - (r_{\text{eff}})^2 (t - t_{\text{max}})^2}{c_{\text{in}}^{(0)}} \right) .$$

The droplet volume fraction $\phi$ can be estimated by the product material available for droplets at the time $t_{\text{max}}$, which corresponds to the maximum concentration of $B$. In the batch-fueled system this corresponds to the maximum concentration of $B$ once the fuel has been completely depleted, i.e., $\phi \approx \left( \lambda c_F(t = 0) - c_{\text{out}}^{(0)} \right) / c_{\text{in}}^{(0)}$ (with $c_{\text{out}}^{(0)} \ll c_{\text{in}}^{(0)}$). We can thus simplify Eq. (16) and obtain

$$\langle V \rangle(t) = \langle V \rangle(t_{\text{max}}) + \frac{V_{\text{sys}}}{\tilde{\beta}} \frac{r_{\text{eff}}(t - t_{\text{max}})}{c_{\text{in}}^{(0)}} \left[ 1 - \frac{r_{\text{eff}}(t - t_{\text{max}})}{\lambda c_F(t = 0) - c_{\text{out}}^{(0)}} \right] .$$

This result suggests an initial linear scaling with time which agrees with the experimental observations. The corresponding slope is proportional to the effective deactivation rate $r_{\text{eff}}$ which agrees well with the experimental measurements (see main text, Figure 3f). Moreover, the time when all droplets shrink depends on the initial fuel concentration $c_F(t = 0)$ and can be estimated by $t_x = t_{\text{max}} + (\lambda c_F(t = 0) - c_{\text{out}}^{(0)}) / r_{\text{eff}}$. Using experimental parameters, this equation gives results that are in a good agreement with the experiment, e.g. for $C_8$SA: $t_x \approx 2h$. Table S5 displays the other parameters used to obtain the fits. Experimentally, it is very difficult to estimate the total volume of the system $V_{\text{sys}}$. In the experiments, droplet numbers were determined considering an area of size $(246 \mu m \times 246 \mu m)$. Since, droplets are approximately on a distance of $10 \mu m$ from each other we decide to make a rough estimate of the total system volume: $V_{\text{sys}} = (246)^2 \cdot 10 \mu m^3 \approx 6 \cdot 10^3 \mu m^3$.

**Continuously-fueled protocol**

For the continuously fueled protocol, we can consider the experimental regime where the fuel concentration and the total concentration of products is constant, i.e., activation and deactivation of products is balanced. In particular, using Eq. (6a) a constant fuel implies $j_F = k_F c_F c_A$ and from Eq. (6c), the constant total concentration $c_{B,\text{tot}} = c_B + \frac{c_{\text{in}}^{(0)}}{V_{\text{sys}}} V_d$, leads to a quasi-static equation for the products

$$0 \approx \lambda j_F - k_d c_B + D_B \nabla^2 c_B ,$$

where growth and shrinkage of droplets is quasi-statically balanced by the change of concentration far away from the droplets $c_{B,\infty}$. For weak spatial heterogeneities, i.e., $c_B(\vec{x}, t) \approx c_\infty(t)$, with the far field
Table S5: Fitting parameters for the batch-fueled protocol. The time scales of droplet dissolution due to classical Ostwald ripening $\tau_{\text{class}} = V_d(t)/(3\alpha \Gamma)$ and via deactivation in the batch-fueled system $\tau_d$ (Eq. (14)) are shown for comparison. Both time scales are evaluated at the early stage of the droplet kinetics. From the experimental time traces of the total $B$ concentration we extract the time $t_{\text{max}}$ corresponding to the maximum in the total $B$ concentration and the corresponding average volume at this time point. Initial fuel concentrations $c_F(t = 0)$, $r_{\text{eff}} = k_d c_{\text{out}}^{(0)}$, the equilibrium concentration inside $c_{\text{in}}^{(0)}$ and outside $c_{\text{out}}^{(0)}$, and the yield factors $\lambda$ can be calculated from the rates shown in Table S4.

concentration $c_{\text{in}}(t) \simeq c_{\text{out}}^{(0)}$ in the limit of small supersaturation levels. In this limit, Eq. (18) gives the condition for the fueling flux $j_F$ to ensure a balance between chemical activation and deactivation of products:

$$j_F \simeq \frac{r_{\text{eff}}}{\lambda}.$$  \hspace{1cm} (19)

Growth law of average droplet volume in continuously fueled systems. To calculate the average droplet volume in Eq. (11), we follow a similar procedure as used in the case of batch-fueled systems which was outlined in the previous section. The difference is that products are produced with the effective flux $\lambda j_F \simeq r_{\text{eff}}$ (Eq. (19)). Here, we consider a simplified case where big droplets of volume $V_b$ grow due to an influx of activated precursors and small droplets of volume $V_s$ shrink due to an efflux of $B$ replacing deactivated products outside the droplets. For both types of droplets the growth equations are

$$\frac{dV_s}{dt} = -k_d V_{\text{sub}} \frac{c_{\text{out}}^{(0)}}{c_{\text{in}}}.$$  \hspace{1cm} (20a)

$$\frac{dV_B}{dt} = k_d V_{\text{sub}} \frac{c_{\text{out}}^{(0)}}{c_{\text{in}}}.$$  \hspace{1cm} (20b)

These equations yield two time scales, namely the time of droplet dissolution via deactivation $\tau_d$ (Eq. (14)) and the time of droplet dissolution via activation of products $\tau_a$, which are given by

$$\tau_a = \frac{V_{b,0}}{V_{\text{sub}}} \frac{c_{\text{in}}^{(0)}}{r_{\text{eff}}} = \tau_d \frac{V_{b,0}}{V_{s,0}}.$$  \hspace{1cm} (21)

Here, $V_{s,0}$ and $V_{b,0}$ are the volumes of the small and the big droplets at time $t = 0$. The time scale for the change in average droplet volume is essentially dominated by the dissolution time scale $\tau_d$ (Eq. (14)), but due to the additional activation of products the corresponding time scale is slightly shorter with a factor $\xi > 1$, where $\xi$ characterizes the relative increase of the slope for continuously fueled compared to the batch-fueled systems; see next section for the estimate of $\xi$. In other words, average droplet volume growth is faster for continuously fueled protocol than the growth in the case of a single batch-fueled protocol. Using mass conservation of $A$ and $B$ in the limit of small supersaturation, $c_{\text{tot}} = c_A + c_{\text{out}}^{(0)} + V_d c_{\text{in}}^{(0)}/V_{\text{sys}}$, and $N(t) \simeq \beta_1 (\tau_d/t)$, we find for the average volume in the continuously fueled supply mode:

$$\langle V \rangle(t) = V_{\text{sys}} \frac{\xi}{\beta} \frac{r_{\text{eff}}}{\langle c_{\text{in}}^{(0)} \rangle^2} \left( c_{\text{tot}} - c_{\text{out}}^{(0)} b \right) t,$$  \hspace{1cm} (22)
where the concentration ratio \( b = (c_{out}^{(0)} + c_A)/c_{out}^{(0)} = 1 + \frac{k_A}{k_f r_{f,p,e}} \) depends on the stationary fuel concentration \( c_{F,0} \). In particular \( b = 2.7 \) for C₆SA, 3.14 for C₇SA and 22.7 for C₁₀SA. The droplet volume fraction \( \phi \) can be estimated using Eq. (24) and Eq. (25), we can estimate \( \phi \approx \phi_{out} \). The droplet volume fraction thus reads \( \phi \approx \left( \phi_{out} - b \right) / c_{in}^{(0)} \) (with \( c_{out}^{(0)} / c_{in}^{(0)} \)). The final expression for the average volume in the continuously fueled supply mode becomes:

\[
\langle V \rangle(t) = \frac{V_{sys}}{\beta} \frac{r_{eff}}{c_{in}^{(0)}} t .
\]

(23)

According to Eq. (23) the average volume scales linearly with time. The corresponding linear slope is proportional to \( r_{eff} = k_d c_{out}^{(0)} \) (see main text, Figure 4f). Both results are in good agreement with the experimental observations.

**Difference in growth rates between batch-fueled and continuously fueled protocol**

The non-dimensional factor \( \xi > 1 \) characterizes the degree of acceleration of average droplet volume growth in the continuously fueled compared to the batch-fueled mode and is relevant in the condition for the accelerated Ostwald ripening (see next section). To estimate \( \xi \), we consider again a system of two droplets and calculate the change of average volume between \( t = 0 \) and \( t = \tau_d \) (Eq. (14)), i.e., \( \Delta \langle V \rangle(t = \tau_d) \) as \( \langle V \rangle(t = \tau_d) = \langle V \rangle(t = 0) + \langle V \rangle(t = \tau_s) \). In the continuously fueled protocol, activation and deactivation are balanced, leading to \( \langle V \rangle(t = \tau_s) = V_{s,0} + V_{b,0} \). Thus, the rate of change of the average droplet volume for the continuously fueled protocol reads

\[
\frac{\Delta \langle V \rangle(t = \tau_s)}{\tau_s} \bigg|_{\text{cont}} = \frac{V_{s,0} + V_{b,0}}{2 \tau_s} .
\]

(24)

For the case of single batch-fueled system, this change in the average volume is different. Considering for simplicity that small droplets shrink while big drops do not change their volume, \( \langle V \rangle(t = \tau_s) = V_{b,0} \), the rate of change of the average droplet volume for the batch-fueled mode reads

\[
\frac{\Delta \langle V \rangle(t = \tau_s)}{\tau_s} \bigg|_{\text{init}} = \frac{V_{b,0} - V_{s,0}}{2 \tau_s} .
\]

(25)

Using Eq. (24) and Eq. (25), we can estimate \( \xi \) which characterizes the degree of acceleration of average droplet volume growth in the continuously fueled supply protocol compared to the batch-fueled mode:

\[
\xi = \frac{\Delta \langle V \rangle(t = \tau_s)}{\tau_s} \bigg|_{\text{cont}} / \Delta \langle V \rangle(t = \tau_s) \bigg|_{\text{init}} = \frac{\langle V \rangle}{\sigma} ,
\]

where we have expressed the volume of the small and big drop by \( V_s = \langle V \rangle - \sigma \) and \( V_b = \langle V \rangle + \sigma \), with \( \sigma \) \((0 < \sigma < \langle V \rangle)\) denoting the standard deviation of the volume distribution. The knowledge of \( \sigma \) requires the knowledge of the distribution of droplet volumes which could be obtained from an analysis of the transport equation of the size distribution. Here, we restrict ourselves to a rough estimation of the coefficient \( \xi \) considering that the distribution is broad. A broad size distribution is expected for droplet growth because droplets grow and shrink and eventually dissolve. For such a broad size distribution, \( \sigma \ll \langle V \rangle \), and thereby \( \xi \) should be a number larger than one but in the order of \( O(1) \). This is consistent with the experimental observations.

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3 Yao, J. H. and Elder, K. R. and Guo, H. and Grant, M., Ostwald ripening in two and three dimensions, Physical Review B, 45, 8173, (1992).
4 Yao, J. H. and Elder, K. R. and Guo, H. and Grant, M., Theory and simulation of Ostwald ripening, Physical Review B, 47, 14110, (1993).
5 Sagui, C. and Grant, M., Theory of nucleation and growth during phase separation, Physical Review E, 59, 4175, (1999).
observations of a 2 to 5 times accelerated average droplet volume growth in the continuously fueled supply mode compared to the batch-fueled mode (see Table S3). In our model the reason for this increase of the growth rate between both protocols relies on the additional activation of products feeding the growth of bigger droplets. Consistently, a significant growth of bigger droplets is only observed in the case of the continuously fueled supply protocol. Future theoretical approaches will aim to calculate the coefficient $\xi$ based on the full droplet size distribution and calculate the droplet number and the average volume from this droplet size distribution.

**Average droplet volume for chemically fueled emulsion and Ostwald ripening**

For some precursors, the contribution of Ostwald ripening to the change of the average droplet volume may not be negligible. To be able to fit the average volume with time corresponding to precursors that show only weakly accelerated behaviour, we combine both mechanisms, classical Ostwald ripening (Eq. (12)) and ripening through activation and deactivation, and write

\[
\langle V \rangle(t) = \langle V \rangle(t)_{\text{clas}} + \langle V \rangle(t)_{\text{fueled}},
\]

where the contributions due to activation and deactivation reactions to the average volume $\langle V \rangle(t)_{\text{fueled}}$ are given either by Eq. (16) in the case of the batch-fueled protocol or by Eq. (22) for the continuously fueled system. The equation above can be written in the form

\[
\langle V \rangle(t) = mt - \tilde{m} t^2,
\]

where the slope $m = (m_{\text{clas}} + m_{\text{fueled}})$ is composed of contribution from classical Ostwald ripening and chemical activation and deactivation. According to Eq. (12), the average droplet volume growth rate due to classical Ostwald ripening is $m_{\text{clas}} = \frac{8}{9} \frac{D_B \gamma_{\text{ref}}}{k_B T V_{\text{sys}}}$, while the average droplet volume growth rate due to the chemical reaction is

\[
m_{\text{fueled}} = \frac{V_{\text{sys}}}{c_{\text{in}}(0)} \beta \frac{r_{\text{eff}}}{c_{\text{in}}(0) - c_{\text{out}}(0)},
\]

with

\[
\tilde{m} = \frac{V_{\text{sys}}}{c_{\text{in}}(0)} \frac{r_{\text{eff}}^2}{\beta \lambda c_{\text{in}}(t = 0) - c_{\text{out}}(0)}
\]

and $\tilde{m} = 0$ for the batch-fueled protocol and the continuously fueled supply protocol respectively. Note that in the main text, we have introduced $\alpha = 1/\beta$ for one batch mode and $\alpha = \xi/\beta$ for the continuously fueled mode. Moreover, the equilibrium concentration inside $c_{\text{in}}(0)$ can be well approximated for the considered, oil-like product molecules, as $c_{\text{in}}(0) \approx 1/\nu$, where $\nu$ is the molecular volume of the respective product (see Table S2 for the molecular volumes of all compounds). The expression above allows us to characterize the degree of acceleration of average droplet volume growth relative to Ostwald ripening by

\[
\frac{m}{m_{\text{clas}}} = 1 + \frac{m_{\text{fueled}}}{m_{\text{clas}}} = 1 + \frac{9\beta V_{\text{sys}} k_B T r_{\text{eff}} c_{\text{in}}(0)}{8\beta D_B} \frac{c_{\text{in}}(0)}{c_{\text{out}}(0)} \left\{ \frac{1}{\beta} \right\}
\]

In the absence of chemical reactions, $r_{\text{eff}} = 0$, the acceleration of ripening $m/m_{\text{clas}} = 1$. To split up the parameters that are the same and different between the chemical precursors, we write $m/m_{\text{clas}} = 1 + \xi s/s_0$, where all precursors share, $s_0 = \frac{8 \beta}{9 \beta} \frac{D_B \gamma_{\text{ref}}}{V_{\text{sys}} k_B T} \approx 0.804 \text{s}^{-1}$, and differ in the rescaled effective deactivation rate

\[
s = \frac{r_{\text{eff}} c_{\text{in}}(0)}{c_{\text{out}}(0) \gamma_{\text{ref}}},
\]
where $\xi = 1$ in the \textit{batch-fueled} protocol and $\xi > 1$ in the \textit{continuously fueled} protocol. See Figure 5f in the main text for the plot of the acceleration of ripening $m/m_{\text{class}}(s)$ as a function of the rescaled deactivation rate $s$ (see Table S3 for the used values). As reference we have considered the surface tension $\gamma_{\text{ref}}$ of C$_8$SA. To plot the acceleration of ripening in the \textit{continuously fueled} mode we have used one acceleration factor, in particular $\xi = 2.9$ corresponding to C$_8$SA.

\section*{Condition for accelerated average droplet volume growth}

Here we derive the condition in order to observe accelerated average droplet volume growth relative to classical Ostwald ripening. To this end, we again consider a two droplet system for simplicity. For classical Ostwald ripening the total volume is constant due to conservation of droplet material, and growth and shrinkage occur on a much larger time scale $\tau_{\text{class}}$ compared to the case with chemical reactions. The rate of change of the average droplet volume for two droplets reads in the case of classical Ostwald ripening:

$$\frac{\Delta \langle V \rangle(t = \tau_{\text{class}})}{\tau_{\text{class}}} = \frac{V_{b,0} + V_{s,0}}{2\tau_{\text{class}}}.$$  \hfill (33)

Combining this equation with Eq. (25) allows us to give a condition to observe accelerated average droplet volume growth:

$$\frac{\Delta \langle V \rangle(t = \tau_{\text{class}})}{\tau_{\text{class}}} < \frac{\Delta \langle V \rangle(t = \tau_{\text{d}})}{\tau_{\text{d}}},$$  \hfill (34)

which can be written as

$$1 < \frac{k_d V_{\text{sys}}}{3\alpha \xi D_B \ell_y \phi}.$$  \hfill (35a)

To obtain the inequality above, we have used the expression for the deactivation time scale Eq. (14), the droplet volume fraction $\phi = V_{s,0}/V_{\text{sub}}$. For classical ripening the corresponding time scale is $\tau_{\text{class}} = V_{\text{sys}}/(3\alpha \Gamma)$ with $\alpha = 4/27$, $\Gamma = D_B \ell_y c_{\text{out}}^{(0)}/c_{\text{in}}^{(0)}$ and the capillary length $\ell_y \simeq \sqrt{2\gamma/(k_B T c_{\text{in}}^{(0)})}$. Condition Eq. (35a) means that accelerated average droplet volume growth is likely to be observed for small surface tensions $\gamma$ ($\ell_y \propto \gamma$), but also for large deactivation rate $k_d$ and small product diffusion coefficients $D_B$. However, the parameters in Eq. (35a) also have to fulfill another condition, namely that the spatial domain between the droplets $V_{\text{sub}}^{1/3}$ is dominated by diffusion. In mathematical terms, the distance between droplets $V_{\text{sub}}^{1/3}$ is smaller than the reaction length scale, i.e., $V_{\text{sub}}^{1/3} \ll \ell_{\text{deact}} = \sqrt{D_B/k_d}$. Only in this limit a chemical reaction event (activation or deactivation) leads to a quasi-instantaneous buffering through diffusion of products from the droplets. Rewriting this condition gives

$$\frac{k_d V_{\text{sub}}^{1/3}}{D_B} \ll 1.$$  \hfill (35b)

For C$_8$SA, and $V_{\text{sub}}^{1/3} \simeq 10 \mu$m, the left hand side is approximately $3 \cdot 10^{-4}$, thereby well satisfied experimentally. The condition for accelerated Ostwald ripening Eq. (35a) can be fulfilled together with condition Eq. (35b) if the final, combined condition to observe accelerated Ostwald ripening is satisfied, i.e.:  

$$\frac{D_B}{k_d V_{\text{sub}}^{2/3}} \ll \frac{V_{\text{sys}}}{3\alpha \xi \phi \ell_y V_{\text{sub}}^{2/3}}.$$  \hfill (36)

For C$_8$SA, $\ell_y \simeq 10^{-3} \mu$m. The right hand side of Eq. (36) is about $10^8$ and thus much larger than the left hand side, which is consistent with the observation of the accelerated Ostwald ripening in the experiments.
Nucleation of droplets

In the following, we will estimate the nucleation time for the building blocks to form droplets. If the nucleation of droplets is fast compared to the timescales of droplet growth (which is in the order of minutes), this would explain why droplet nucleation appears to be quasi instantaneous on the time-scale corresponding to the resolution of experimental imaging capturing droplet growth (Fig. S3).

A nucleation event requires the formation of a critical cluster. Only after fluctuations have formed a cluster of B molecules that exceeds a critical radius \( R_c \), this cluster can grow to a larger droplet. This critical radius can be expressed by the capillary length \( \ell_c \) and the supersaturation level \( \varepsilon \), i.e., \( R_c = \ell_c / \varepsilon \). The capillary length is defined as \( \ell_c = \frac{2\gamma}{k_B T_c(0)} \) and we can compute the capillary length for the precursor \( C_8SA \) to \( \ell_c \approx 10^{-3} \mu m \). If the concentration of the droplet material is equal to \( 2c_{out}^{(0)} \) (a reasonable assumption as B material is produced via hydrolysis), the critical radius \( R_c \approx 0.001 \mu m \).

The average size of a single B molecule can be estimated from the molecular volumes of the precursors. We know, that \( C_8SA \) has a molecular volume of \( 2.06 \cdot 10^{-4} \text{mol/m}^3 \). Thus the molecule size \( d \approx 0.001 \mu m \). Interestingly, this molecule size is equal to the critical radius indicating that only a few molecules are necessary to trigger growth to a droplet. In summary, we can conclude that the nucleation is fast on the time scale of experimental imaging.

Hydrolysis at the droplet interface

In the following we will quantify the effects of hydrolysis reaction at the droplet interface. We will compare the number of hydrolysis events per time occurring at the interface compared to the subvolume typically corresponding to a single droplet, \( V_{sub} \). To this end, we will estimate the number of B molecules on the droplet interface \( N_{interface} \) relative to the number of B molecules outside the droplets \( N_{out} \) in the subsystem volume \( V_{sub} \).

Number of molecules at interface: A single molecule has an estimated size \( d \approx 1 \text{nm} \) and an area \( A_m = \pi d^2 \approx 0.32 \text{nm}^2 \). Given a droplet of an average size \( R \approx 1 \mu m \), the total number of molecules at the interface is \( N_{interface} = 4\pi R^2 / A_m = 4 \cdot 10^6 \).

Number of molecules in a subvolume corresponding to a droplet: Given that the interdroplet distance is \( 10 \mu m \), a single droplet is embedded in a subvolume \( V_{sub} = (20 \mu m)^3 \). Therefore, the number of B molecules in this subvolume is:

\[
N_{out} = 20^3 \mu m^3 \cdot c_{out}^{(0)} = 8000 \cdot 0.15 \cdot 10^{-3} M \mu m^3 = 8 \cdot 0.15 (\text{mol/m}^3) \mu m^3 \cdot 6.022 \cdot 10^{-23} (1/\text{mol}) \approx 7 \cdot 10^{11}. \tag{37}
\]

Comparing the two numbers \( N_{interface} \) and \( N_{out} \), we see a difference in at least 4 orders of magnitude. Assuming that the hydrolysis rate at the droplet interface is similar to the one in the subvolume, we can estimate the amount of molecules degraded due to hydrolysis. For the case of hydrolysis at the droplet interface, \( N_{interface} \cdot k_d \approx 3 \cdot 10^4 \), while for the case of degradation in the subsystem volume \( V_{sub} \), \( N_{out} \cdot k_d \approx 4 \cdot 10^9 \). Thus, we conclude that the effect of hydrolysis at the droplet interface is negligible and the dominant mass of droplet material is degraded in the bulk solution.
Growth due to fusion events

During a fusion event two droplets merge and become one droplet. Typically, the volume of the new drop is equal to the sum of the volumes of the two fusing drops. However, the surface area is reduced and thereby the corresponding free energy contribution. Although the total droplet volume does not change due to fusion, the number of droplets decreases and thus the average volume of droplets increases.

To estimate the growth of the average droplet volume, we consider for simplicity that the two encountering droplets have the same size, each given by the average radius \( \langle R \rangle \). After fusion the new droplet is of size \( \langle R \rangle + \Delta R \), where \( \Delta R \) denotes the incremental radius change for a single fusion event. The conservation of volume during such a fusion event requires, \( 2 \left( \frac{4\pi}{3} \right) \langle R \rangle^3 = \left( \frac{4\pi}{3} \right) \langle \langle R \rangle + \Delta R \rangle^3 \), leading to

\[
\Delta R = \langle R \rangle \left( 2^{1/3} - 1 \right). \tag{38}
\]

The rate of collision \( \Delta t^{-1} \) of two droplets that will result in a successful fusion can be estimated as \( \Delta t^{-1} = \lambda D_v / \ell_p^2 \), where \( D_v = k_B T / (6\pi\eta\langle R \rangle) \) is the diffusion constant given by the Stokes-Einstein equation for a droplet of radius \( \langle R \rangle \) diffusing in a liquid of viscosity \( \eta \). Droplet encounters not leading to a successful fusion event are accounted for by the parameter \( \lambda \in [0, 1] \). For \( \lambda = 1 \), all droplet encounters lead to fusion, while for \( \lambda = 0 \) no fusion occurs. We estimate the mean free path \( \ell_p \) of a collision using the interdroplet distance, i.e. \( \ell_p \simeq \left( V_{\text{sys}} / N \right)^{1/3} = V_{\text{sub}}^{1/3} \), where \( N \) is the droplet number and \( V_{\text{sys}} \) the volume of the system. Since the total droplet volume \( V_d = N \left( \frac{4\pi}{3} \langle R \rangle^3 \right) \), we can write the rate of change of the average radius \( \langle R \rangle \) due to fusion as:

\[
\frac{1}{\langle R \rangle} \frac{d\langle R \rangle}{dt} \simeq \frac{1}{\langle R \rangle} \frac{\delta \langle R \rangle}{\delta t} = \lambda \left( 2^{1/3} - 1 \right) D_v \ell_p^{-2}. \tag{39}
\]

After integration, the scaling law for the average volume, \( \langle V \rangle = \frac{4\pi}{3} \langle R \rangle^3 \), reads

\[
\langle V \rangle(t) = \lambda \frac{k_B T}{6\pi\eta} \left( \frac{4\pi}{3} \right)^{2/3} \left( 2^{1/3} - 1 \right) \left( \frac{3V_d}{V_{\text{sys}}} \right)^{2/3} t =: m_{\text{fusion}} t \tag{40},
\]

where the droplet growth rate due to fusion is denoted as \( m_{\text{fusion}} \). Table S6 shows fusion rates \( m_{\text{fusion}} / \lambda \) for all precursors in the batch-fueled protocol.

In order to estimate the parameter \( \lambda \), we consider the precursor isoC\(_{10} \) that exhibits the smallest actual growth rate \( m \). We estimate an upper bound for this parameter, denoted as \( \lambda_{\text{max}} \). To this end, we consider the growth rate of isoC\(_{10} \), i.e., \( m_{\text{isoC}_{10}} = 0.0022 \mu m^3/\text{min} \), as the maximal the fusion rate \( m_{\text{fusion}} \) of isoC\(_{10} \):

\[
\lambda_{\text{max}} = \frac{k_B T}{6\pi\eta} \left( \frac{4\pi}{3} \right)^{2/3} \left( 2^{1/3} - 1 \right) \left( \frac{3V_d}{V_{\text{sys}}} \right)^{2/3} \left( \frac{c_B(t_{\text{max}}) - c_{\text{out}}^{(0)}}{c_{\text{in}}^{(0)} - c_{\text{out}}^{(0)}} \right)^{-2/3} \approx 0.08 \tag{41}.
\]

We see that \( \lambda_{\text{max}} \ll 1 \) implying that most collisions do not lead to a fusion event. Using this value of \( \lambda_{\text{max}} \), we rescale all fusion rates \( m_{\text{fusion}} \) of all other precursors and compare them in Figure S9 with the growth rates for ripening due to activation and deactivation \( m_{\text{fueled}} \) and classical Ostwald ripening \( m_{\text{clas}} \). Based on our estimates, we conclude that fusion hardly contributes to the growth of droplets nor it can explain the dependence of the average droplet volume growth rate on the effective deactivation rate \( r_{\text{eff}} \).
Table S6: Fusion rates $m_{\text{fusion}}$ for precursors in the batch-fueled protocol. The lowest rate of average droplet volume growth is observed for tBuPA.

|          | C₆SA | C₈SA | C₁₀SA | C₅  | C₆  | isoC₁₀ | tBuPA |
|----------|------|------|-------|-----|-----|--------|-------|
| $m_{\text{fusion}} / \lambda \ [\mu m^3/\text{min}]$ | 0.09 | 0.19 | 0.22  | 0.13| 0.04| 0.03   | 0.01  |

Figure S9: Comparison of the average volume growth rates for three precursors in the continuously-fueled protocol. Fusion rates $m_{\text{fusion}}$ are rescaled using $\lambda = \lambda_{\text{max}}$. 