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Chemically Fueled Supramolecular Materials

Xiaoyao Chen,[+] Michaela A. Würbser,[+] and Job Boekhoven*

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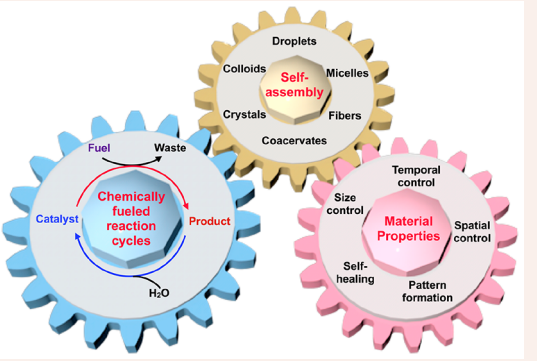
CONSPECTUS: In biology, the function of many molecules is regulated through nonequilibrium chemical reaction cycles. The prototypical example is the phosphorylation of an amino acid in an enzyme which induces a functional change, e.g., it folds or unfolds, assembles or disassembles, or binds a substrate. Such phosphorylation does not occur spontaneously but requires a phosphorylating agent with high chemical potential (for example, adenosine triphosphate (ATP)) to be converted into a molecule with lower chemical potential (adenosine diphosphate (ADP)). When this energy is used to regulate an assembly, we speak of chemically fueled assemblies; i.e., the molecule with high potential, the fuel, is used to regulate a self-assembly process. For example, the binding of guanosine triphosphate (GTP) to tubulin induces self-assembly. The bound GTP is hydrolyzed to guanosine diphosphate (GDP) upon assembly, which induces tubulin disassembly. The result is a dynamic assembly endowed with unique characteristics, such as time-dependent behavior and the ability to self-heal. These intriguing, unique properties have inspired supramolecular chemists to create similar chemically fueled molecular assemblies from the bottom up. While examples have been designed, they remain scarce partly because chemically fueled reaction cycles are rare and often complex. Thus, we recently developed a carbodiimide-driven reaction cycle that is versatile and easy to use, quantitatively understood, and does not suffer from side reactions. In the reaction cycle, a carboxylate precursor reacts with a carbodiimide to form an activated species like an anhydride or ester. The activated state reacts with water and thereby reverts to its precursor state; i.e., the activated state is deactivated. Effectively, the precursor catalyzes carbodiimides' conversion into waste and forms a transient activated state.



We designed building blocks to regulate a range of assemblies and supramolecular materials at the expense of carbodiimide fuel. The simplicity and versatility of the reaction cycles have democratized and popularized the field of chemically fueled assemblies. In this Account, we describe what we have "learned" on our way. We introduce the field exemplified by biological nonequilibrium self-assembly. We describe the design of the carbodiimide-driven reaction cycle. Using examples from our group and others, we offer design rules for the building block's structure and strategies to create the desired morphology or supramolecular materials. The discussed morphologies include fibers, colloids, crystals, and oil- and coacervate-based droplets. We then demonstrate how these assemblies form supramolecular materials with unique material properties like the ability to self-heal. Besides, we discuss the concept of reciprocal coupling in which the assembly exerts feedback on its reaction cycle and we also offer examples of such feedback mechanisms. Finally, we close the Account with a discussion and an outlook on this field. This Account aims to provide our fundamental understanding and facilitate further progress toward conceptually new supramolecular materials.

1. INTRODUCTION

In chemically fueled supramolecular materials, molecules assemble to form functional nanostructures.[1−3] Unlike conventional supramolecular materials, chemically fueled assembly does not occur in equilibrium but requires chemical energy to take place and sustain. That energy is harvested from fuels through a chemically fueled reaction cycle. These materials are inspired by biological architectures that are often regulated through energy-consuming processes. Prototypical examples of such biologically chemically fueled architectures are the intermediate filaments and microtubules,[4−7] driven by reaction cycles that hydrolyze adenosine triphosphate (ATP) and guanosine triphosphate (GTP), respectively. These cycles use the free energy liberated upon hydrolysis to regulate the assembly process, for example, by transiently activating proteins for self-assembly. Thus, self-assembly can only take place when fuel is present. In this context, we use the term fuel for a reagent with high chemical potential that can be liberated by a chemical reaction cycle. Because the material consumes

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the fuel it needs to form, a finite amount of fuel results in a material with a finite lifetime (Figure 1); thus, temporary

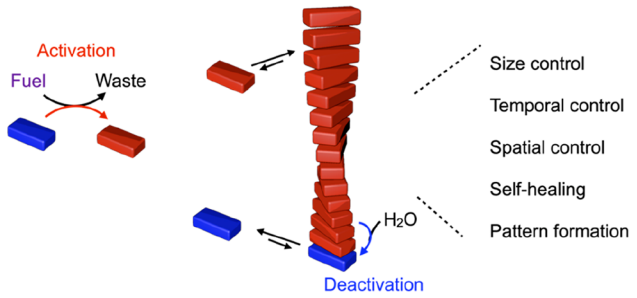

Figure 1. Chemically fueled self-assembly (left) leads to materials with unique material properties (right).

materials can be created, of which the lifetime depends on kinetic parameters, like the amount of fuel added and reaction rates. Moreover, where fuel is applied matters for where the material is formed.

In other words, spatial and temporal control over material properties are some of the unique material properties of this class of materials.[8−11] Due to the dynamics of the assembly and disassembly process, these materials can be endowed with the ability to self-heal.[12,13] Moreover, the short-lived nature of the building blocks implies that their residence time in the assemblies is regulated by the kinetics of the reaction cycle, meaning that the size and morphology of these assemblies can be controlled.[14]

A 2010 paper described the first example of chemically fueled self-assembly where fiber formation by a small molecule was coupled to the hydrolysis of methyl iodide as fuel.[15] A carboxylate on the small molecule reacted with the methyl iodide. The esterification changed the properties of the molecule such that it could assemble into fibers. Hydrolysis restored the small molecule leading to its disassembly. The simultaneous activation and deactivation gave rise to its unique material properties like self-healing behavior and temporal control.[16]

Recognizing the field's potential, we identified a major challenge: the lack of a fuel-driven chemical reaction cycle that can be broadly applied—we missed a reaction cycle that was versatile and easy to use, was quantitatively understood, and did not suffer from side reactions. Thus, in 2017, we set out to design and develop the carbodiimide-fueled reaction cycle.[17] The reaction cycle is widely used to drive the formation of supramolecular materials, including transient hydrogels, colloids, and temporary emulsions that deliver drugs.[18−28]

This Account offers our experience developing the reaction cycle and coupling it to chemically fueled supramolecular materials. We start by describing what constitutes a chemically fueled reaction cycle and how this can be coupled to the assembly of molecules. We offer our fundamental understanding and what we have learned in pursuing these materials. Finally, we aim to facilitate the further progress of the field by outlining our "lessons learned."

2. THE DESIGN OF CHEMICALLY FUELED SUPRAMOLECULAR MATERIALS

2.1. The Chemically Fueled Reaction Cycle

The chemical reaction cycle comprises a minimum of two reactions, i.e., a building block activation and a deactivation reaction. The activation reaction is the exergonic reaction between a precursor and a molecule with high chemical potential, which releases a waste with lower chemical potential and the activated building block, i.e., a fuel, that yields an activated building block. The deactivation reaction returns the activated building block to the building block through another pathway than the activation. Typically, deactivation is a reaction between the activated building block and a highly abundant species like the solvent such that the reaction occurs spontaneously. Again, this reaction should be strongly favored toward the original building block, which is an irreversible reaction. Thus, adding fuel converts a pool of building blocks into a non-equilibrium state, with building blocks temporarily activated for self-assembly.

We clarify the design of the reaction cycle with an example from biology, i.e., a reaction cycle that uses the energy released by hydrolyzing ATP to drive an ion pump.[29,30] The chemical fuel ATP does not hydrolyze easily. Still, its hydrolysis to adenosine diphosphate (ADP) is strongly favored when a catalyst exists, which makes ATP a remarkable fuel; i.e., the hydrolysis of ATP to ADP releases a high amount of energy, but ATP is relatively stable. We will return to that stability in the design considerations for chemically fueled self-assembly. A catalyst, like the enzyme Ca^{2+} ATPase, can accelerate the liberation of that energy and use it for a function, in this case, to pump Ca^{2+} across a membrane. This energy transduction is done in a complex cascade of processes but can be reduced to two critical steps. In the first step, a nucleophile in the active center of the ATPase is phosphorylated, which converts ATP to ADP, i.e., the activation. This activation changes the conformation of the enzyme and pushes two Ca^{2+} ions across the membrane. In the second step, the phosphorylated nucleophile is hydrolyzed, liberating the original enzyme and inorganic phosphate, which returns the enzyme to its initial state, i.e., deactivation. In this cycle, ATP hydrolyzes to ADP, and the enzyme is temporarily phosphorylated and changes its function so that calcium can be transported from one side to the other side of a membrane, thereby creating a transmembrane calcium gradient. Crucially, the enzyme ATPase in this reaction cycle is not altered and can thus undergo thousands of cycles.

The example above taught us two important design considerations: (1) fuels have a high chemical potential that is not easily liberated, and (2) the building block in the chemical reaction cycle is effectively a catalyst that liberates the chemical energy of the fuel. In the process of activation and deactivation, the building block (catalyst) performs its function.

In 2017, we recognized that chemically fueled reaction cycles that follow the above design considerations were scarce. Moreover, reaction cycles that were versatile and easy to use and did not suffer from side reactions did not exist. Therefore, we introduced the carbodiimide-fueled reaction cycles.[17] Noteworthy, the Hartley group published a carbodiimide-driven reaction cycle practically at the same time as when we introduced it.[31] Like ATP, carbodiimides are incredible fuels due to the high amount of energy released in their hydration to their corresponding ureas. Moreover, they are relatively unreactive toward nucleophilic attack by water, i.e., they are relatively stable. Specifically, EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) has a half-life of 37 h in water at pH 7.[32] Like the hydrolysis of ATP, a catalyst is required to facilitate the hydrolysis of the fuels. That catalyst is

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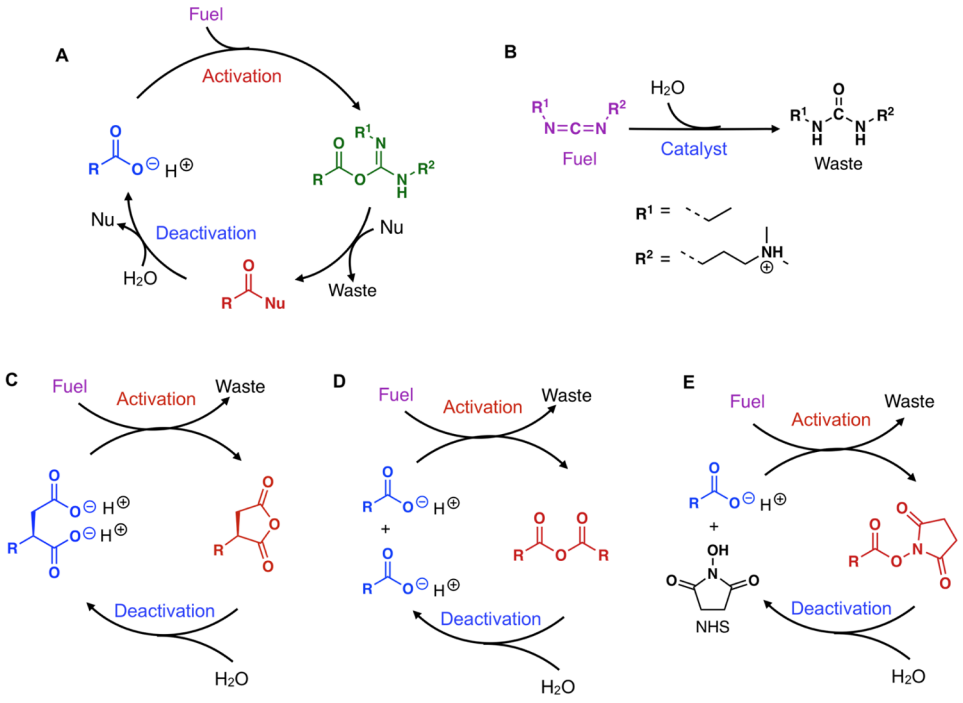
Scheme 1. Carbodiimide-Driven Reaction Cycles[a]



[a](A) Schematic representation of a carbodiimide-driven reaction cycle. (B) The net reaction in the cycle: the hydration of the carbodiimide which is EDC here. (C) Intramolecular anhydrides are formed when building blocks (catalysts) bear two carboxylic groups and experience an intramolecular attack. (D) Intermolecular anhydrides are formed when catalysts only bear one carboxylic group and undergo an intermolecular attack from a second carboxylic acid. (E) NHS-esters are formed when catalysts only bear one carboxylic group and undergo an intermolecular attack from an NHS.

a carboxylic acid in our reaction cycle. In the activation reaction, the building block reacts with the carbodiimide to form an intermediate *O*-acylurea (depicted in green in Scheme 1A), followed by an attack of a second nucleophile which yields the activated building block and urea as waste. In the deactivation reaction, this activated building block hydrolyzes back to its carboxylic acid by reacting with water (Scheme 1A). Thus, the net reaction is the hydration of a carbodiimide. We, therefore, refer to the carboxylate as the catalyst for the hydration of the carbodiimide. It accelerated the hydration of carbodiimide by offering an alternative reaction pathway and did not get converted itself in the overall process. However, the catalyst transiently changed its chemical nature which can be used for a function (Scheme 1B). In the cycle, the hydration occurs in several steps, which lower the highest energy barrier of the direct hydration, and thus, the overall hydration occurs orders of magnitude faster compared to without carboxylic acid as a catalyst. A difference between the carbodiimides and ATP as fuels is that, in the ATP-fueled cycles, part of the fuel is transiently transferred. In contrast, carbodiimides function as an energy supply but are not part of the activated building block.[17,22,31]

Various activated carboxylates are obtained through this cycle depending on the second nucleophile. The most common ones are intramolecular anhydrides, intermolecular anhydrides, and NHS-esters. Intramolecular anhydrides (Scheme 1C) are obtained if the building block bears two carboxylic groups, such as succinic acid derivatives.[17] In contrast, an intermolecular anhydride (Scheme 1D) or an NHS-ester (Scheme 1E) is formed if the building block only has one carboxylic group in the absence or presence of NHS, respectively.[11,22] However, there are advantages and disadvantages for each specific cycle. In the intramolecular anhydride-forming cycle, the transient products have short half-lives of less than 2 min[17,33,34]—our record holder is the tripeptide Fmoc-AVD at 18 s.[17] A short half-life can be advantageous to achieve dynamic supramolecular materials. The intramolecular anhydride-forming cycle also uses its fuel effectively; i.e., because of the neighboring carboxylate, almost all *O*-acylurea is converted into anhydride instead of hydrolyzing. A significant disadvantage is that the scope of the precursor is limited to succinic or glutaric acid derivatives.

In contrast, the NHS-ester-forming cycle and intermolecular anhydride-forming cycle both have a broad scope of precursors and yield products with longer half-lives. For example, NHS-esters have half-lives in a range of 200−300 min,[35] while intermolecular anhydrides have intermediate half-lives among these three reaction cycles.[22,31] However, the main downside of the intermolecular anhydride-forming cycle is the hydrolysis of the *O*-acylurea or its rearrangement into the *N*-acylurea, which decreases the efficiency of the fuels.

Side products like *N*-acylurea are a severe issue of reaction cycles. The building block acts as a catalyst, so it has to undergo hundreds of thousands of turnovers. If a side reaction yields a stable product, the building block can no longer act as a catalyst for the reaction cycle. For example, a 1% side reaction would remove 63% of the building block after as little as 100 turnovers. In the carbodiimide-driven reaction cycle, a well-known side reaction is the *N*-acylurea formation when the *O*-acylurea intermediate undergoes irreversible N−O arrangement. This first-order side reaction can be suppressed by ensuring *O*-acylurea is as short-lived as possible, i.e., having a

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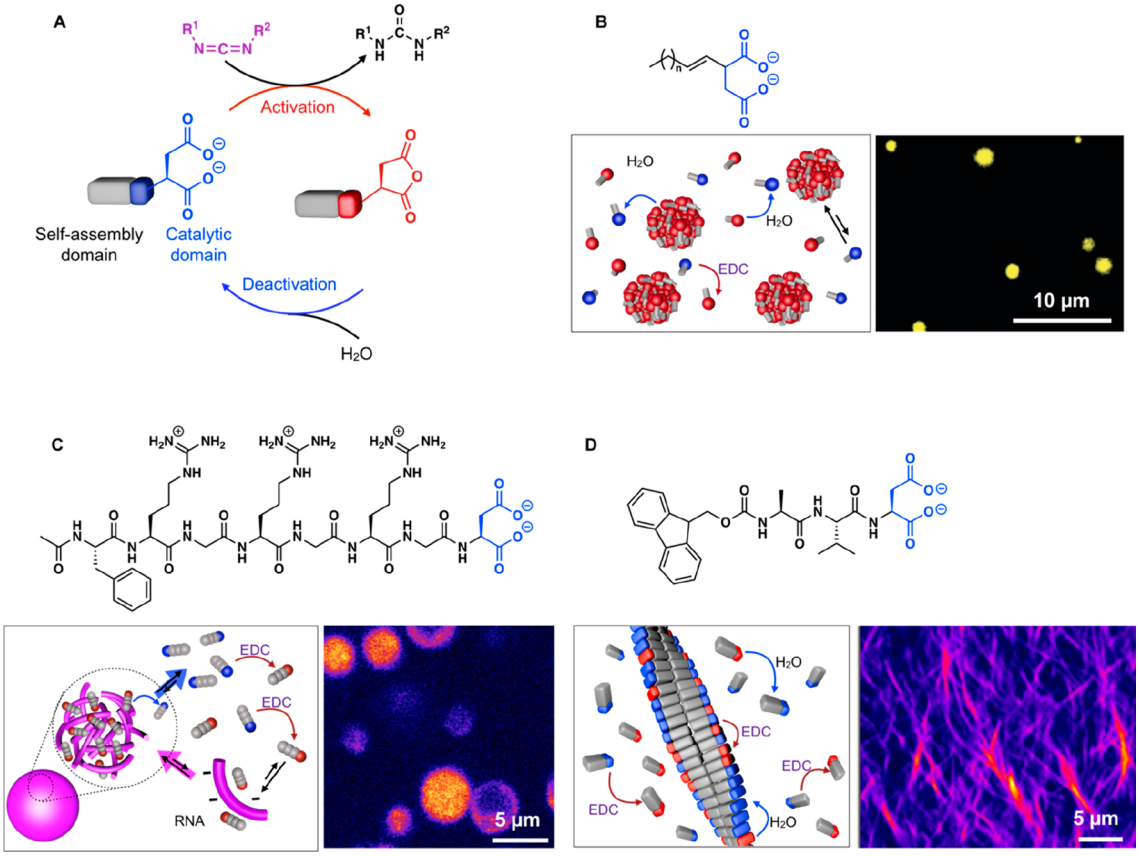

Figure 2. Building blocks for chemically fueled self-assembly. (A) A simple molecular design for building blocks. Two functional domains are required: a self-assembly domain (depicted as a gray block) that directs the assembly after activation and a catalytic domain (depicted as a blue block) that is the catalyst for fuel conversion in the reaction cycle. Dicarboxylates, as the catalytic domain, ensure that the building blocks repel each other sufficiently before activation. (B) Building blocks with a hydrophobic tail for oil droplets or colloids. Micrograph of (B) reproduced with permission from ref 18. Copyright 2020 The Royal Society of Chemistry. (C) Building blocks with cationic motifs interacting with a polyanion for complex coacervate-based droplets, Ac-F$(RG)_3$D-OH as an example here. Micrograph of (C) reproduced with permission from ref 21. Copyright 2020 Springer Nature Ltd. (D) Building blocks with a β-sheet-forming peptide for fibrillar assemblies, Fmoc-AVD-OH, as an example. Micrograph of (D) reproduced with permission from ref 34. Copyright 2020 The American Chemical Society.

high concentration of a second nucleophile, or using a building block with a nucleophile in the proximity, like aspartic acid, or adding catalytic amounts of additional reagents, e.g., pyridine or DMAP.[36]

Because carbodiimides are popular peptide coupling reagents, the diversity of our fuels is high. It ranges from polar, water-soluble carbodiimides like EDC to more apolar carbodiimides like DIC (*N*,*N*′-diisopropylcarbodiimide) and DCC (*N*,*N*′-dicyclohexylcarbodiimide). The latter can be applied as fuels in organic solvents or mixtures of organic solvents. We found optimal conditions for the chemically fueled reaction cycle to be 200 mM MES buffer with EDC as a fuel at a pH range between 3.5 and 7.4.[37] A lower pH decreases the reactivity of the carboxylic acids, and no activated building block is found. A pH higher decreases the electrophilicity of the carbodiimides. Hardly any product is found beyond pH 7.4. Notably, anhydride hydrolysis, i.e., the deactivation reaction, is practically insensitive to pH at this range. MES is a perfectly suitable buffer within this range as it has sufficient capacity at these pH values. Moreover, it is non-nucleophilic, unlike more common buffers like phosphate, carbonate, or TRIS. Nucleophilic buffers react with the fuel leading to poor performance of the cycle.

The kinetics of the reaction cycle is analyzed using techniques that give quantitative concentrations with reasonable time intervals, like HPLC and ^{1}H NMR. We recently found that short-lived anhydrides hydrolyze on the acidic HPLC column, and we thus developed a method to quench all chemical reactions in the cycle immediately that uses benzylamine.[38] The activation is inhibited because EDC loses its reactivity at a high pH caused by benzylamine. In contrast, the deactivation is stopped by converting the *O*-acylurea and anhydride to its corresponding, stable benzylamide.

2.2. How to Make the Building Block Assemble?

We explored two strategies to assemble the activated building block, i.e., the charge abolishment and linker strategy. In the first, the building blocks consist of two functional domains—a domain that directs the assembly after activation (depicted in gray in Figure 2A) and a domain that is the catalyst for fuel conversion (depicted in blue). The catalytic domain comprises two carboxylates, and thus, the building blocks repel each other sufficiently before activation, thereby solubilizing the building block (Figure 2A). Finally, activation converts the catalytic domain to its corresponding cyclic anhydride making it lose two negative charges and thus resulting in a decrease in electrostatic repulsion, thereby inducing self-assembly.

For example, when the self-assembly domain is a hydrophobic tail, and the catalytic domain is succinic acid (Figure

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2B), a soluble building block is activated into an oil-droplet forming molecule.[18,39] We tested a small library of building blocks with increasing hydrophobicity, e.g., 2-buten-1-ylsuccinic acid (C_4), 2-hexen-1-ylsuccinic acid (C_6), 2-octen-1-ylsuccinic acid (C_8), and 2-decen-1-ylsuccinc acid (C_{10}). The longer the aliphatic chain, the lower the solubility of the activated building block and, thus, the greater its propensity to form an emulsion of oil droplets.[18] Because the aliphatic tail has only isotropic interactions with itself, there is no molecular order in the assemblies, which keeps the assembly liquid. In contrast, when hydrophobic assembling domains are used that can undergo anisotropic interactions like pi-pi interaction, we can expect more order in the assemblies leading to colloids. Indeed, a suspension of transient colloids is formed when the hydrophobic group is a 9-fluorenylmethoxy-carbonyl (Fmoc) group with strong pi-pi stacking interactions. For example, the anhydride of Fmoc-E-OH self-assembles into uniform colloids.[17,20] Moreover, these emulsions of oil droplets and suspensions of colloids all display a linear-decay profile; i.e., they decay with zero-order kinetics. This surprising behavior results from a self-protecting mechanism which we discuss in the section on feedback mechanisms.

Emulsions are also formed if the self-assembly segment has cationic motifs. In this case, the activated building block interacts with a polyanion, like RNA, to form a complex coacervate-based droplet. In the design, we used a zwitterionic peptide with the sequence of Ac-F(RG)$_n$D-OH, where the cationic arginines (R) are separated by noncharged glycines (G). Aspartic acid (D) is used as the catalytic domain. For example, Ac-F(RG)$_3$D-OH has an overall charge of the peptide +1 before activation (Figure 2C). Upon activation, the two anionic carboxylates are converted into their anhydride, which abolishes two negative charges; i.e., the building block is overall +3 in the activated state. Therefore, the cationic-activated building blocks can induce complex coacervation with anionic polymers such as RNA (poly-U) or poly(styrenesulfonate) (PSS).[21,40−42] Unlike the oil droplets discussed above, these are based on ionic interactions and remain hydrated. Their high-water content makes them especially appealing for the microencapsulation of hydrophilic substances, e.g., proteins, polynucleotides, or ionic drugs. It also makes them a great model for membraneless organelles or protocells.[43] Compared to complex coacervates comprising a mixture of oppositely charged polyelectrolytes, hydrophobic interactions of amphiphilic molecules or polymers can initiate micelle formation. For instance, a block copolymer consisting of a hydrophilic poly(ethylene glycol) and a second hydrophilic poly(styrene-*alt*-maleic acid) block can form transient micelles, considering an amphiphilic switch and an increase in the hydrophobicity of the poly(styrene-*alt*-maleic acid) building block. We induced this switch by applying EDC as fuel, leading to a conversion of acid to anhydride building blocks, thus to an increase of hydrophobicity and, last, to transient micelle formation.[44]

Anisotropic interactions in the self-assembling domain can form one-dimensional assemblies.[33] For example, β-sheet-forming peptides in the self-assembling domain result in fibrillar assemblies formed upon activation (Figure 2D). These fibers entangle to form a network that converts a liquid into a hydrogel. Increasing the β-sheet-forming propensity increases gel strength. For instance, gels from the anhydrides of Fmoc-AVD-OH are stiffer than those by Fmoc-AAD-OH because valine (V) has a greater β-sheet-forming propensity than alanine (A).[17,34]

The chemical reaction cycle that forms NHS-esters (Scheme 1E) also benefits from the charge abolishment strategy to drive self-assembly, which was used to obtain transient clusters of nanoparticles (NPs) by using NPs functionalized with carboxylic acids on their surface. Activated by a carbodiimide, the carboxylic acid on the surface is converted to an NHS-ester. Thereby, the activated NPs self-assemble into clusters. This cycle works well to induce different clusters of NPs, including gold NPs, silicon NPs, and iron NPs.[11,45]

The chemical reaction cycle that forms intermolecular anhydride (Scheme 1D) is the second strategy we used to induce self-assembly. In this strategy, two nonassembling molecules are linked together to form an assembling building block, i.e., the linker strategy. The building blocks again have a self-assembly domain and a catalytic domain. The difference is that the nonassembling building blocks are coupled upon activation to yield an intermolecular anhydride, a less-soluble building block for self-assembly. This linker strategy formed droplets by dimerizing fatty acids like valeric or caproic acid.[22] Also, fibrillar substructure in the irregular micron-sized assemblies could be observed by oligomerization of isophthalic acid to polyanhydrides upon activation.[46]

Despite following the strategies in the section above, we observed cases where disassembly does not occur after deactivation; i.e., the building block remains kinetically trapped in the assembled state after deactivation (Figure 3). On the

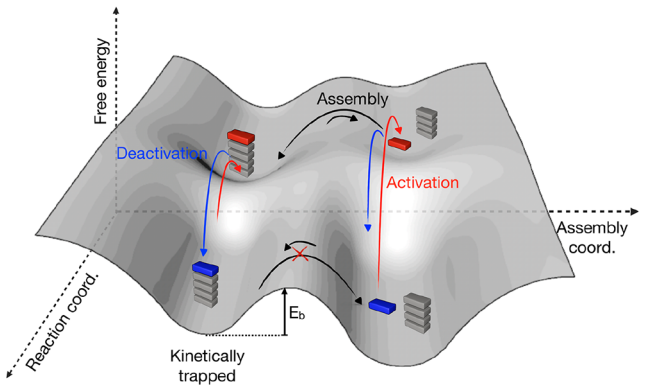

Figure 3. Schematic representation of the free energy landscape of chemically fueled assembly.

one hand, the kinetic trapping of building blocks causes unwelcome delays in disassembly. On the other hand, it provides an exciting opportunity to engineer dynamic instabilities, i.e., rapid disassembly of kinetically trapped deactivated building blocks. For example, the dynamic instabilities of microtubules benefit from the kinetic trapping of building blocks.[6,7] In detail, microtubule building blocks do not disassemble immediately despite being rapidly deactivated, whereas microtubules collapse and rapidly disassemble the trapped building blocks only when the tubule's end-cap becomes unstable due to faster deactivation than assembly.

We can clarify the phenomenon of kinetic trapping with two energy landscapes: one for the activated- and one for the deactivated building block. Importantly, the reaction coordinate diagram describes the free energy landscape of the catalyst, not the fuel and the waste (Figure 3). A non-assembled, nonactivated state represents the minimum. Upon activation, the building block transitions along the reaction coordinate to the energy landscape of the activated building

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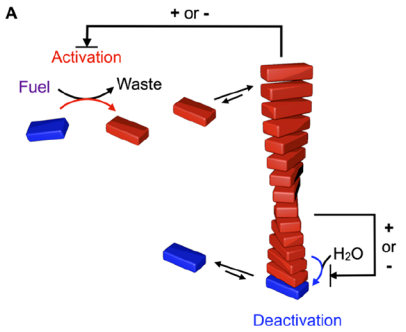

B

Number	Feedback	On	Name	Observed in
1	+	Activation	Self-amplification	Fibers
2	-	Activation	Self-inhibition	Not observed yet
3	+	Deactivation	Self-destruction	Droplets/Fibers
4	-	Deactivation	Self-protection	Colloids/Droplets/ crystals

Figure 4. Feedback mechanism. (A) + (B): The four possible positive or negative feedback mechanisms of the assembly on activation and deactivation of its chemical reaction cycle. The blunt arrow of the feedback-related ones specifies negative feedback (inhibition), and the sharp arrow presents positive feedback (stimulation).

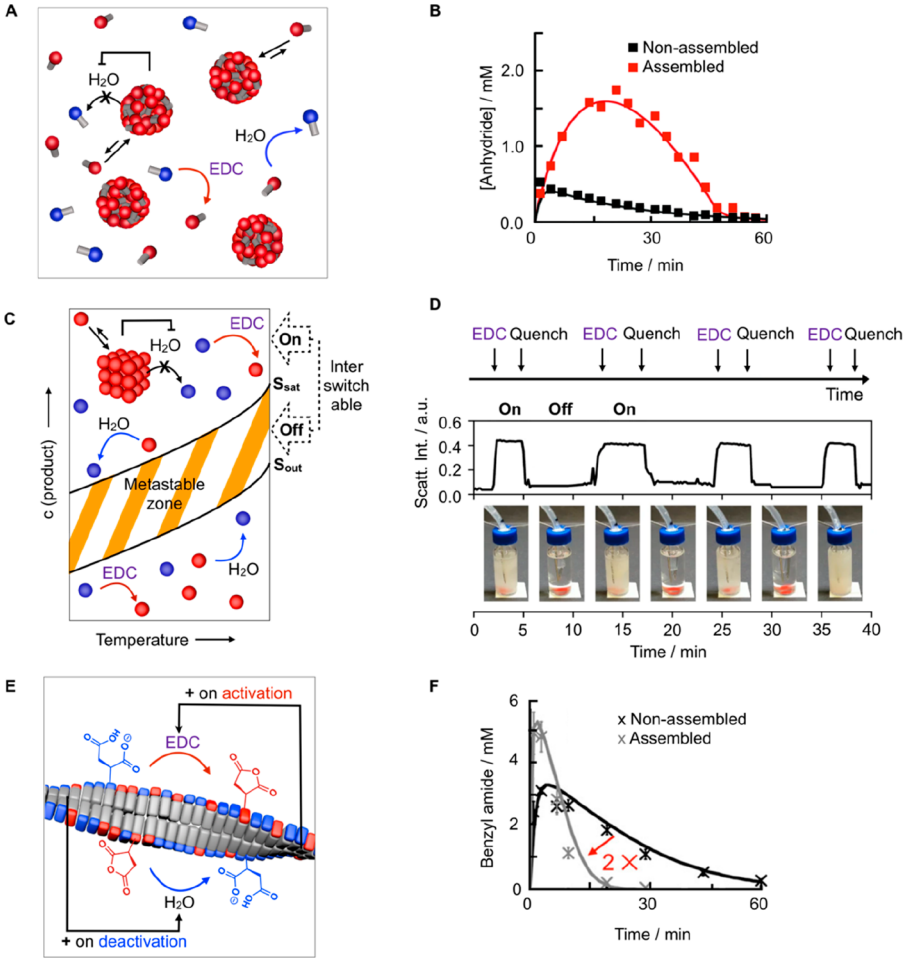

Figure 5. Examples of feedback reciprocal coupling in chemically fueled assembly. (A) Schematic representation of self-protection (negative) feedback mechanism of droplet assemblies on deactivation by hydrolysis. (B) Anhydride concentration against time of 10 mM Fmoc-E-OH (red) and 10 mM Cbz-E-OH (black) fueled with 10 mM EDC. The assembled Fmoc-E-OH colloids lead to an inhibition of hydrolysis compared to the nonassembled Cbz-E-OH. Markers represent experimental data measured by HPLC; solid lines represent data estimated by the kinetic model. (B) reproduced with permission from ref 20. Copyright 2018 The Royal Society of Chemistry. (C) Schematic representation of the bistability mechanism in the phase diagram of the activated building block's crystallization. Besides an "on" state with crystals and an "off" state without one, the metastable zone can contain crystals or not, depending on the history of the samples. The crystals exert self-protection on the activated building block. (D) Turbidity (scattering light) over time of switching on (addition EDC) and off (quenching) of the bistable system. (C) and (D) reproduced with permission from ref 53. Copyright 2022 Nature Publishing Group. (E) Schematic representation of positive feedback of assemblies on activation and deactivation due to a change in the microenvironment of the assemblies. (F) Benzylamide concentration (equals anhydride concentration) against time of 10 mM Ac-FAVD-OH (black) and 10 mM Fmoc-AVD-OH (gray) fueled with 100 mM EDC. A 2-fold increase in deactivation was observed in the case of the assembled Fmoc-AVD-OH fibers. Markers represent experimental data measured by HPLC; solid lines represent data estimated by the kinetic model. (E) and (F) reproduced with permission from ref 34. Copyright 2020 The American Chemical Society.

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block. To transition to that state, it converts fuel into waste (not depicted). Here, the minimum is the building block in an assembled state. The building block can transition to the initial energy landscape in the assembled state through deactivation.

Deactivation can occur in the assembled and nonassembled states. In certain building blocks, the energy barrier of the deactivated in the assembly to the disassembled state is higher than the thermal energy available, and the deactivated building block remains kinetically trapped in the assembled state. This kinetic trapping tends to happen due to strong molecular attractive interaction. For example, fibers from Fmoc-AVD-OH or Fmoc-AVE-OH did not fully disassemble after the reaction cycle because of the strong β-sheet formation and hydrophobic interactions.[17] Moreover, the self-assembly into the fibers changes the apparent pK_a of the carboxylic acids compared to nonassembled peptides. That shift in the pK_a implies that the deactivated building blocks remain partly protonated within the fibers, and thus the repulsive interactions between the peptides severely decreased.[47] Clusters of nanoparticles can also get trapped when a large amount of fuel is added. Similarly, the strong molecular interactions prevent disassembly, and increasing pH can bring them back to their thermodynamic minimum.[45] Thus, to avoid kinetical traps, we should have an appropriate molecular design to ensure the activated building blocks are endowed with sufficiently strong molecular attractive interaction for assembly yet weak interactions for disassembly after deactivation.[33]

3. FEEDBACK MECHANISMS

In the following section, we discuss studies in which this concept has a higher degree of complexity. We demonstrate chemically fueled assemblies that exert feedback on their chemical reaction cycle. In other words, the cycle regulates the assemblies, and reciprocally, the assemblies affect the cycle. Such feedback mechanisms play a crucial role in biology and can lead to behavior like bistability in memory storage, kinetic gating for molecular motion, and more.[48−51] As an example, the unassembled, GTP-bound tubulin is relatively stable in the GTP-fueled self-assembly of tubulin but is catalyzed by neighboring tubulin upon assembly.

We identified four possible feedback mechanisms: the assembly accelerates the activation (1) or deactivation (3) (both are positive feedback), or they decelerate the activation (2) or deactivation (4) (both are negative feedback) (see Figure 4A). We refer to the four modes of feedback as (1) self-amplification, i.e., positive feedback on the activation, (2) self-inhibition, i.e., negative feedback on the activation, (3) self-destruction, i.e., positive feedback on the deactivation, or (4) self-protection, i.e., negative feedback on deactivation (Figure 4B). Self-destruction and self-inhibition lead to a decreased amount of activated building blocks. In contrast, the concentration of activated building blocks increases for self-amplification and self-protection.[20] A combination of these mechanisms can produce exciting, complex behavior like oscillations, pattern formation, and bistability.[52,53]

Implementing reciprocal coupling into our chemically fueled assemblies occurred more serendipitously than through design. For example, we discovered a self-protection mechanism in chemically fueled colloids by Fmoc-E-OH.[17,20] As phase separation between water and the assemblies occurs, the reactant required for deactivation, in our case, water, is expelled from the assembly (Figure 5A). Consequently, the deactivation exclusively occurs on the anhydride that remains in the water phase, which equals its solubility concentration, c_{sol}. The hydrolysis rate r is then defined by $r = k_{hyd} \cdot c_{sol}$, where k_{hyd} is the hydrolysis rate constant. Both k_{hyd} and c_{sol} are constants, resulting in a linear decay profile (0th order) of the total anhydride concentration. Hence, the assembled anhydride self-protects itself from hydrolysis—the assembly itself exerts negative feedback on the deactivation. In the case of Fmoc-E-OH-containing colloids, we found a significantly lower anhydride deactivation rate than the nonassembled counterpart, e.g., Cbz-E-OH. (Figure 5B).

This self-protection mechanism frequently occurred in our assemblies, and we identified design rules. These are (1) the deactivation reaction between the activated building block and the solvent or a reagent in the solvent and (2) the assemblies should be prominent in three dimensions and expel the solvent.[20] Indeed, we observed this mechanism for spherical assemblies, e.g., colloids, oil droplets, and crystals with a hydrophobic interior.[20,22,53,39] Interestingly, deactivation occurs through zeroth-order and is thus rate independent of the concentration of the building blocks. This opens the door to drug-delivery platforms, where drug-loaded droplets release a hydrophobic drug with linear kinetics (see section 4).[18,54]

The ability to store memory is a critical property of life.[55] Herein, a key feature is the principle of bistability, a system with two states which can be toggled between in response to a transient signal. In contrast to biology, in which bistable states mostly rely on autocatalytic and chemical reaction processes,[56,57] we designed a system based on small molecules consisting of our EDC-driven reaction cycle coupled to crystallization of *N*-Boc aspartic anhydride.[53] Similar to the previously described oil droplets, the chemically fueled crystals exert negative feedback on the deactivation (self-protection of the activated building block), allowing us to regulate steady-state product levels. In continuously fueled reactors, we found two possible states, an off-state without crystals and an on-state in which crystals are present (Figure 5C). In a metastable zone between these two states, crystals exist or do not, depending on the history of the sample; if the solution reaches the supersaturation concentration S_{sat}, crystals will nucleate and bring the steady state out of the metastable zone because of the feedback. However, the crystals will dissolve when they reach a concentration below the anhydride's solubility (S_{out}). We studied a variety of triggers to switch on (fuel, salts) and off (benzylamine, quench) the crystallization and succeeded in switching back and forth between the two states (Figure 5D). It is a simple yet effective way of creating a memory-storing process, e.g., developing a pixel-based device that can be printed and erased. On a miniaturized level, we envision using this principle in microfluidic droplets or vesicles where crystallization could initiate deformation or self-division of the compartment.

Assemblies are known to be able to catalyze reactions.[58] We found a nanofiber-forming peptide in which the assembly accelerated both the activation and deactivation by changing the microenvironment of the building block and its activated state (Figure 5E).[34] We studied a library of nanofiber-forming peptides (Fmoc-AVD-OH and others), compared it to the nonassembling peptide Ac-FAVD-OH, and found that the higher the organization within the assembled structure, the greater the acceleration of both activation and deactivation. The change in the building block's microenvironment induces a decrease in the pK_a of the carboxylates, in line with pK_a shifts in proteins and other peptide-based self-assembly.[59] Thus, the

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coassembled building blocks showed partial protonation of the carboxylates, changing the activation mechanism from a stepwise proton transfer to a concerted one, thereby increasing the activation rate 5-fold. Moreover, we observed a 2-fold acceleration of the deactivation presumably caused by the β-sheet, hydrogen-bonding interactions in the Fmoc-AVD-OH fiber assemblies.

In a recent study, we combined the knowledge of emerging positive feedback on deactivation with the feedback regulation of droplets to create a self-immolating material.[19] Here, feedback operates in a self-destructive manner supported by the *in situ* formation of micelles by the surfactant building blocks.

4. CHEMICALLY FUELED SUPRAMOLECULAR MATERIALS

We explored chemically fueled supramolecular materials with temporal control for the sustained and controlled release of pharmaceutical drugs.[18,54] We used the building blocks with an aliphatic tail and simple aliphatic acids like heptanoic acid that form oil droplets upon activation. Given its fuel dependence, the emulsion is transient and decays to its original homogeneous solution state after all fuel is depleted and all anhydride hydrolyzed. These emulsions benefit from the self-protection mechanism discussed in section 3. Thus, the anhydride hydrolyzes with linear kinetics until all droplets are gone. We anticipated that such linear decay could lead to a linear release of hydrophobic contents. Indeed, when loaded with hydrophobic drug molecules, around 15% of the drug was released within the first hour (burst release). Afterward, the platform showed a linear release profile for the remaining drug amount with a drug delivery half-life (t_{50}) of 17 h (Figure 6A). That release rate could be tuned by varying the initial anhydride concentration while keeping the initial drug concentration. For both anhydrides (C_{10} and C_7C_7), we determined a linearly increasing t_{50} with an increasing initial oil concentration (Figure 6B). We found a release period window ranging from around 1.5 to 23 h. Ultimately, we envision our platform to be applied for coating medical devices or implants.

We found that by applying EDC to a solution of Fmoc-D-OH, the conversion of acid into anhydride leads to an increase in turbidity that lasted until all fuel was depleted, showing the potential application as a self-erasing medium, i.e., self-erasing ink.[17] Therefore, we embedded Fmoc-D-OH into a polyacrylamide hydrogel. Then, using a stencil with the desired message, the fuel EDC was spray-coated onto the gel, which immediately led to a change in turbidity where the fuel covered the gel surface (Figure 6C). Then, as time passed, the message self-erased, showing a tunable lifetime dependent on the provided fuel amount.

Furthermore, we showed that carboxylic acid-containing block copolymer micelles could be applied as temporally controlled nanoreactors.[44] Herein, the micelles were fuel-driven and of transient nature depending on the added fuel concentration. We used the micelles' ability to form hydrophobic domains to locally up-concentrate Diels–Alder reactants (Figure 6D). To our excitement, product yields of the Diels–Alder reaction of *N*-benzyl maleimide and ethyl sorbate could be significantly improved and regulated by the amount of provided fuel (Figure 6E). The transient character of the system is especially appealing in terms of excluding product inhibition which might be a drawback for conventional micellar systems.

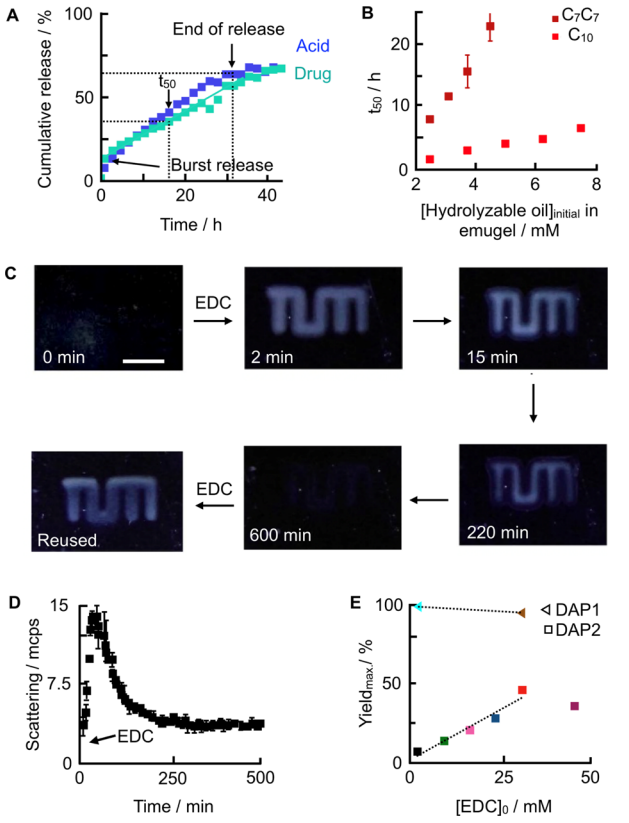

Figure 6. Temporally controlled supramolecular materials. (A) Cumulative release of 25 μM drug Nitrendipine loaded in an emugel of 3.75 mM C_7C_7 in 1% agar gel over 45 h (PBS buffer, 37 °C). Around 15% of the drug was released in the first hour (burst), and the remaining drug was released linearly. (B) t_{50} depending on the initial concentration of anhydride C_7C_7 (dark red) and C_{10} (light red) in the emugel. t_{50} increases linearly with an increasing oil concentration. (A) and (B) reproduced with permission from ref 54. Copyright 2021 Elsevier. (C) Photographs of a self-erasing ink platform over time fueling 10 mM Fmoc-D-OH embedded in a polyacrylamide gel with 1000 mM EDC. After 600 min, the ink was entirely erased. The gel was rinsed for reuse. The scale bar represents 1 cm. (C) reproduced with permission from ref 17. Copyright 2017 Springer Nature. (D) Scattering rate as a function of time fueling 5 mM of block copolymer PEG_{114}-*b*-$PSMA_{40}$ with 5 mM EDC. The substantial increase in the scattering rate represented the transient micelle formation at the expense of EDC. (E) Maximum yield of Diels–Alder product 1 (DAP1, triangle) from *N*-benzyl maleimide and sorbic alcohol and Diels–Alder product 2 (DAP2, square) from *N*-benzyl maleimide and ethyl sorbate in dependence on the initial EDC concentration. Markers represent HPLC data. (D) and (E) reproduced with permission from ref 44. Copyright 2021 John Wiley and Sons.

Another unique property of chemically fueled supramolecular materials is their ability to self-heal.[12] This behavior sounds intuitive—provided sufficient nonactivated building blocks and fuel remains present, the fuel can reactivate building blocks, and a damaged assembly can be recovered. Nevertheless, examples of this behavior are underexplored,[16] and the exact mechanisms remain elusive. We investigated the healing potential of a fiber-forming peptide in a chemically fueled pseudo steady state.[13] We found that 10 mM Fmoc-AAD-OH fueled with 500 mM EDC led to a dense fiber network that could self-heal after damage.

When the fiber network on a microscopy slide was scratched with a needle, the damaged gap healed over several minutes

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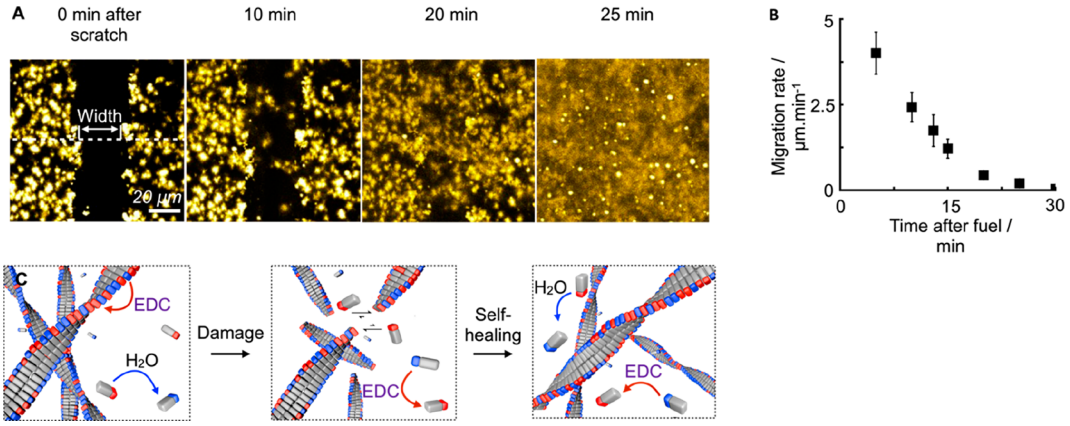

Figure 7. Self-healing character of chemically fueled Fmoc-AAD-OH fibers in a pseudo steady state. (A) Confocal micrographs of a Fmoc-AAD-OH gel damaged 5 min after fuel addition. The decreasing scratch width was analyzed over a migration time of 25 min. Each scratch was performed in triplicate. (B) Fibrillar assemblies' migration rate (healing rate) over damage time after fuel addition. Error bars represent an average (n = 3) of experiments. (C) Proposed self-healing mechanism of chemically fueled Fmoc-AAD-OH in a pseudosteady state. The damage to the fibers leads to increased end-caps for the molecular glue to attach to. (A), (B), and (C) reproduced with permission from ref 13. Copyright 2022 The Royal Society of Chemistry.

(Figure 7A). The healing resulted from a sudden increase in the assembly dynamics around that damaged site which depended strongly on the time in the reaction cycle. If the damage was applied early during the cycle, e.g., after 5 min, a complete network recovery was established compared to only partial recovery when damaged 30 min after fuel addition (Figure 7B). We explained this behavior by the accumulated waste concentration during the cycle hampering the healing effect. We hypothesized that the healing is based on the concept of a chemically fueled "molecular glue" (Figure 7C). Most of the peptide is located within the assemblies. Though deactivated, the fibers will not disassemble due to their kinetically trapped state. However, a small concentration of peptide in solution undergoes a continuous activation and deactivation, but without assembling in fibers. As the fiber's damage significantly increases the number of end-caps, the activated peptide in the solution can attach, leading to a regrowth of the fibrous structure. Herein, the glue concentration equals the peptide's critical aggregation concentration (CAC). We found that our concept was generalizable for other peptides and that the CAC is a significant indicator of a network's self-healing ability.

6. CONCLUSIONS AND OUTLOOK

Chemically fueled self-assembly is a versatile method for creating materials with life-like properties like the ability to self-heal, store information, and be controlled spatiotemporally. The carbodiimide-driven reaction cycle is simple and versatile and can be predicted by kinetic models. Carbodiimides are an attractive fuel as they are kinetically stable but require a catalyst, such as carboxylic acids, to release their energy. Design rules and strategies for regulating the assembled structures have been established, including droplets, colloids, coacervates, micelles, crystals, and fibers. The carbodiimide cycle also includes the concept of reciprocal coupling, where the reaction regulates self-assembly and *vice versa*.

Some unique materials have been formed using this cycle, but it is still far from the complexity seen in biological materials. We still need to gain a fundamental understanding of life-like features, e.g., self-replication, molecular motion, memory storage, or self-healing. The focus has been on soft matter, but the gap between soft and hard materials must be narrowed to advance established principles. For instance, self-healing hydrogels can inspire self-healing actuators or soft robotics, increasing device lifespan and reducing waste and costs.

AUTHOR INFORMATION

Corresponding Author

Job Boekhoven − *Department of Chemistry, School of Natural Sciences, Technical University of Munich, 85748 Garching bei München, Germany;* orcid.org/0000-0002-9126-2430; Email: job.boekhoven@tum.de

Authors

Xiaoyao Chen − *Department of Chemistry, School of Natural Sciences, Technical University of Munich, 85748 Garching bei München, Germany;* orcid.org/0000-0002-0545-6395

Michaela A. Würbser − *Department of Chemistry, School of Natural Sciences, Technical University of Munich, 85748 Garching bei München, Germany*

Complete contact information is available at: https://pubs.acs.org/10.1021/accountsmr.2c00244

Author Contributions

[+]X.C. and M.A.W. contributed equally to this work.

Notes

The authors declare no competing financial interest.

Biographies

Xiaoyao Chen received her Master's degree in Polymer Chemistry and Physics in 2019 from the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences. After that, she joined the Technical University of Munich in 2019 for her Ph.D. degree under the guidance of Prof. Dr. Job Boekhoven. Her research interest was developing a new chemically fueled reaction cycle and understanding quantitatively how to suppress side products.

Michaela A. Würbser received her Master's degree with a focus on inorganic and macromolecular chemistry from the Technical University of Munich in 2018 and joined the Boekhovenlab in April 2019 to obtain her Ph.D. degree. Her studies focused on chemically

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fueled supramolecular materials exerting life-like features, e.g., temporal control or the ability to self-heal.

Job Boekhoven is creating tools to regulate molecule self-assembly, like biology. He's best known for his work on chemical reaction cycles that control molecule assembly or phase separation, leading to new properties like self-healing or controllable lifetime. The assemblies show features associated with living cells like emergence, decay, and self-division. He has a Ph.D. from Delft University (2012) and is now an associate professor at TU Munich.

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