Dissipative Self-Assembly Driven by the Consumption of Chemical Fuels

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Dissipative self-assembly leads to structures and materials that exist away from equilibrium by continuously exchanging energy and materials with the external environment. Although this mode of self-assembly is ubiquitous in nature, where it gives rise to functions such as signal processing, motility, self-healing, self-replication, and ultimately life, examples of dissipative self-assembly processes in man-made systems are few and far between. Herein, recent progress in developing diverse synthetic dissipative self-assembly systems is discussed. The systems reported thus far can be categorized into three classes, in which: i) the fuel chemically modifies the building blocks, thus triggering their self-assembly, ii) the fuel acts as a template interacting with the building blocks noncovalently, and iii) transient states are induced by the addition of two mutually exclusive stimuli. These early studies give rise to materials that would be difficult to obtain otherwise, including hydrogels with programmable lifetimes, vesicular nanoreactors, and membranes exhibiting transient conductivity.

1. Introduction

The reversible formation of actin filaments\(^1\) is essential for intracellular transport,\(^2\) cell motility,\(^3\) and other processes critical to life. Actin filaments (or F-actin) are formed by supramolecular polymerization of globular actin (G-actin) upon the binding of a high-energy molecule, adenosine triphosphate (ATP). Upon polymerization, the G-actin–ATP complex undergoes a conformational change that activates it toward the hydrolysis of ATP to orthophosphate (\(P_i\)) and adenosine diphosphate (ADP). This ATPase activity of F-actin is as much as 40 000 higher than of its monomeric form.\(^4\) The resulting F-actin–ADP is unstable and it spontaneously depolymerizes to G-actin–ADP. The subsequent replacement of ADP with ATP completes the cycle. Therefore, each assembly-disassembly cycle is associated with the consumption of a chemical fuel (or a net chemical reaction, \(ATP + H_2O \rightarrow ADP + P_i\)). In other words, the reversible formation of actin filaments is an example of dissipative self-assembly (DSA).

DSA processes are ubiquitous in nature, where entities on length scales spanning several orders of magnitude—from actin filaments to living organisms—require continuous consumption of energy to remain in the assembled state. However, DSA is a mode of self-assembly that stands in sharp contrast\(^5\) to most examples of synthetic self-assembled structures and materials,\(^6\) which are stable in time and reside in a global or local minimum in the free-energy landscape. Although energetically unfavorable, DSA provides natural systems with temporal control over assembled structures and serves as an inspiration for designing novel synthetic materials. In fact, recent years have witnessed an explosion of interest in synthetic DSA systems, which are the focus here.

2. Recent Examples of Dissipative Self-Assembly

The pioneering example of a synthetic chemically fueled DSA system was developed by van Esch et al.\(^15\) and it can be schematically presented as sequence 1 in Figure 1. Initially, the system exists in a non-assembled state (i.e., building blocks, shown in light blue in Figure 1, are solvated). The building blocks can be activated toward self-assembly using a “fuel” (shown in red; step 1a). The resulting “activated building blocks” (dark blue) are involved in two competing processes. First, they undergo a “deactivation” reaction (typically hydrolysis) in which the original (non-assembling) building blocks are regenerated (Figure 1, case 1b). Alternatively, they can self-assemble into larger structures—but only if their concentration is higher than the critical aggregation concentration (CAC). Within these assemblies, the activated building blocks undergo the same “deactivation” reaction (1b in Figure 1). The process in which the activated building blocks—either free or assembled—are hydrolyzed is typically described as “energy dissipation”.

The formation and stability of the self-assembled structures is governed by the relative rates of fuel consumption (\(k_{1a}\)) and energy dissipation (\(k_{1b}\)). If \(k_{1a} > k_{1b}\), transient assemblies will be observed; in the opposite case, energy will be dissipated without self-assembly taking place. Overall, the system is perturbed (pushed out of equilibrium) by the addition of a fuel whose decomposition brings the system back to equilibrium, where the building blocks are not assembled. It is important to point out that the fuel can also decompose/hydrolyze directly; successful
realization of this mode of DSA requires that the rate of direct decomposition be low compared with \( k_{1a} \). In other words, the building blocks should act as efficient catalysts for the decomposition of the fuel, where the intermediate in the catalytic cycle has a propensity to self-assemble into larger structures.

In the original system of van Esch and co-workers, \( N,N' \)-dibenzoyl-\( l \)-cystine (DBC) (Figure 2a), which is readily soluble in water, was activated toward self-assembly using methyl iodide as the fuel[15]. The reaction proceeded efficiently at 35 °C and afforded DBC dimethyl ester, which self-assembled into one-dimensional fibers stabilized by a combination of intermolecular hydrogen bonds and hydrophobic interactions. The reaction of the ester groups with OH\(^-\) regenerated DBC, causing the fibers to disassemble. The system could be “refueled” by adding the second aliquot of MeI. More potent methylating agents, such as dimethyl sulfate, enabled the reversible formation of

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**Figure 1.** Synthetic examples of dissipative self-assembly (DSA). 1: Covalent activation of a precursor towards self-assembly (1a) followed by its deactivation to the initial non-assembling building block. The deactivation within the assembled structures can occur by a reaction with an external agent (indicated in green; 1b) or spontaneously (1c). The deactivation can also occur prior to self-assembly (1b’/1c’). 2: Self-assembly guided by a template that is spontaneously degraded in the system. 3: DSA via a simultaneous addition of an activator (orange; reacts fast)–deactivator (yellow; reacts slowly) pair.

**Figure 2.** DSA by a covalent modification of the precursor. a) Methylation of carboxylates cancels out the repulsive interactions between the building blocks and triggers self-assembly (DBC = \( N,N' \)-dibenzoylcystine). Subsequent hydrolysis of the methyl esters regenerates the initial building blocks. b) Photographs of a solution containing DBC and the dimethyl sulfate fuel at pH 11 at different times following the addition of the fuel. c) A cryogenic transmission electron microscopy (cryo-TEM) image of nanofibers assembled from activated DBC. b,c) Reproduced with permission.[16] Copyright 2015, The American Association for the Advancement of Science. d) Biocatalytic DSA fueled by aspartame.[20] Enzyme = \( \alpha \)-chymotrypsin.
nanofibers—and, consequently, gelation—at room temperature.[16] This follow-up study highlighted two unique features of fuel-driven materials. First, it is possible to control their lifetimes and mechanical properties (e.g., persistent storage modulus) by the amount of fuel introduced to the system. Second, these materials have the ability to “self-heal”: once perturbed, they can reform (if the fuel is available). Remarkably, fluorescence microscopy analysis of this active material revealed coexisting populations of growing and shrinking fibers, reminiscent of the dynamic instability of microtubules.[16] This dynamic instability in a synthetic system is driven by Me₂SO₄ hydrolysis.

Transient methylation of carboxylates using a chemical fuel was also employed to control the clustering of colloidal polystyrene (PS) particles, ≈700 nm in diameter, grafted with a block copolymer brush comprising poly(acrylic acid).[17] At a slightly basic pH of 9, these COOH groups were mostly deprotonated and no aggregation was observed due to the repulsive electrostatic interactions between the like-charged particles. Addition of Me₂SO₄ induced methylation of the COO⁻ groups and, consequently, clustering of the PS particles. Two assembly–disassembly cycles could be realized; the addition of the third aliquot of Me₂SO₄ resulted in clusters that remained stable as a result of the decreasing concentration of OH⁻[17]. This concept could in principle be easily extended to particles of other sizes, including nanoparticles.

Boekhoven and co-workers devised an alternative way to temporarily remove the charges on the COO⁻ groups and thus form transient materials.[18] They converted Fmoc-protected aspartic acid (D) and glutamic acid (E) to the corresponding charge-neutral anhydrides using carbodiimides (mostly EDC) as the fuels. These anhydrides spontaneously assembled into colloidal particles; when short peptides containing D and E (such as Fmoc-AVD and Fmoc-AAE) were used instead, the addition of EDC resulted in the self-assembly of anisotropic fibers and the formation of hydrogels. Both the particles and the gels were transient and they gradually disassembled as the anhydrides hydrolyzed. The study by Boekhoven and co-workers highlights an important feature of many DSA systems: namely, that the aggregation of the activated building blocks can alter the rate of energy dissipation. Whereas Fmoc-E anhydride that formed at a low concentration (below the CAC) hydrolyzed rapidly, colloids assembled above the CAC persisted significantly longer. Within these colloids, the anhydrides were effectively shielded from the environment (i.e., water); in other words, the colloids exerted a negative feedback on anhydride hydrolysis.[18]

Ulijn and co-workers designed a DSA system based on biocatalytic peptide bond formation and hydrolysis.[19,20] In the example shown in Figure 2d, the assembly–disassembly cycle involving phenylalanine amide (F-NH₂) as the precursor was fueled by the hydrolysis of DF methyl ester (DF-OMe; aspartame). Specifically, the α-chymotrypsin-catalyzed transacylation reaction afforded hydrogelator DFF-NH₂, which rapidly self-assembled into one-dimensional nanofibers. The same enzyme catalyzed the hydrolysis of DFF-NH₂ into DF and the initial building block (F-NH₂), and the gel dissolved within ≈24 hr.[20] The lifetime of the gel could be controlled by the choice of the building block: for example, tripeptide DFY-NH₂ formed and self-assembled as fast as DFF-NH₂; however, the resulting gel persisted for only ≈4 hr. Interestingly, other precursors (e.g., W-NH₂ and V-NH₂) treated with the aspartame fuel did not form a gel, indicating that the tripeptide either did not form (i.e., direct hydrolysis of the fuel took place) or that it hydrolyzed rapidly (to DF and W-NH₂/V-NH₂). Similar to the examples described above, Ulijn and co-workers’ system exhibited limited reversibility due to the build-up of waste.[20] Only three assembly–disassembly cycles could be completed, with the yield of the transient tripeptide corresponding to 60% and 30% in the second and third cycles, respectively, compared with that of the first cycle.

In fact, waste accumulation is a notorious open problem in most chemically fueled systems and new strategies for efficient waste removal represent an important direction in the development of DSA systems. In one elegant example, Hermans and co-workers confined their self-assembled structures within a membrane reactor whose walls were permeable to small-molecule fuel and waste, but not to the assemblies.[21] A perylenediimide bi-functionalized with two identical oligopeptide sequences was used as the building block for the self-assembled structures. Each of these oligopeptides contained two arginine residues, resulting in an overall +4 electric charge on each building block. At the same time, both oligopeptide sequences comprised a serine residue prone to kinase-catalyzed phosphorylation in the presence of ATP. Installing two phosphate groups rendered the building blocks electroneutral, thus triggering their self-assembly into a supramolecular polymer. Phosphatase—the second enzyme present in the system—catalyzed the reverse reaction, i.e., phosphate hydrolysis and consequently, disassembly (Figure 1, step 1b). Non-equilibrium steady states comprising the desired levels of the activated building block (and therefore of the supramolecular polymer) could be maintained by supplying the system with a specific influx rate of the ATP fuel. Once the supply of ATP was discontinued and the ATP present in the system was consumed, the phosphatase-catalyzed hydrolysis deactivated the building blocks, resulting in depolymerization.[21]

The activation of the building block (step 1a in Figure 1) can rely on a reaction as simple as protonation. Angulo-Pachón and Miravet developed a sucrose-fueled system in which long-chain carboxylates could be transiently protonated and activated toward self-assembly by acidifying the solution with CO₂.[22] When an aqueous solution containing a carboxylate precursor and baker’s yeast was treated with sucrose, CO₂ production by the yeast induced the protonation of the precursor and its self-assembly into fibrillar networks. Gel formation was observed ≈2 h after the addition of sucrose; the consumption of sucrose and the elimination of the CO₂ gas from the system restored the original pH and regenerated the initial solution of non-gelating carboxylates. Continuous feeding with sucrose was required to maintain the self-assembled fibrillar network.

However, fuel does not need to undergo a chemical reaction with the building blocks in order to activate them toward self-assembly; it can also interact with them noncovalently, acting as a template. This mode of DSA is schematically presented as 2 in Figure 1 and is exemplified by ATP-templated vesicles developed by Prins and co-workers.[21,24] In the presence of ATP, a surfactant comprising a long alkyl chain and a cationic head group (“building block” in Figure 3a) had a high tendency to assemble into vesicles, ≈70 nm in diameter.[23] Upon the addition of ATP to a solution containing both the surfactant and potato apyrase—an enzyme catalyzing ATP hydrolysis—the vesicles that were initially formed gradually disassembled. The
lifetime of the vesicles could be controlled by the rate of ATP hydrolysis, which depended on the concentration of the enzyme in the solution. An interesting question concerns the kinetics of the hydrolysis of free ($k_{ATP}$) vs vesicle-bound ATP ($k_{ATP-vesicle}$). If $k_{ATP} > k_{ATP-vesicle}$, vesicle formation would have an inhibitory effect on the hydrolysis reaction. In the opposite case, the vesicle would activate ATP toward hydrolysis, similarly to the ATPase action of F-actin (vide infra). In an interesting application of this system, the vesicles were employed as transient nanoreactors: a model addition reaction involving two apolar substrates was facilitated in the presence of the vesicles, which interacted with both substrates, thereby increasing their effective molarity.[23]

In a related example, Guo and co-workers induced the electrostatic co-assembly of DNA and a tetraphenylethylene (TPE) derivative appended with four positively charged quaternary ammonium groups into pseudospherical particles, ≈20 nm in diameter.[25] By taking advantage of the aggregation-induced emission (AIE) property of TPE derivatives, these researchers were able to follow the assembly and disassembly of the NPs using fluorescence spectroscopy, whose low detection limit allowed them to work at low ($\approx10 \times 10^{-6}$ M) concentrations of the TPE building block. Indeed, the addition of DNA to a solution of the TPE derivative and deoxyribonuclease I (DNase I)—a DNA-cleaving enzyme—resulted in the rapid appearance of bright blue fluorescence.[25] DNase I present in the system gradually hydrolyzed DNA into short oligonucleotides, which had no appreciable affinity to the TPE derivative. Consequently, the fluorescence decreased, with a rate proportional to the amount of the enzyme in the solution. Interestingly, the system could be refueled with DNA for as many as ten cycles;[25] this high reversibility of the process is probably due to the relatively low interference of the waste product (oligonucleotides) with the self-assembly process.

The third approach to chemically fueled DSA is schematically shown as 3 in Figure 1 and is exemplified by concept of a dormant deactivator from Walther and co-workers.[26–28] In this case, the system is brought out of equilibrium by the simultaneous addition of a pair of antagonistic signals. One of these signals, a fast promoter (orange in Figure 1), induces a self-assembly process; the other, a dormant deactivator, triggers disassembly, but not until it is activated to do so. This activation occurs spontaneously with a time delay, which can be programmed (Figure 4a). In the example shown in Figure 4b, the self-assembly of a block copolymer comprising a poly(ethylene glycol) (PEG) block and a poly(diethylammonium)ethyl methacrylate) (DEAEMAH+) block into micelles was induced by the addition of a strong base (NaOH), which rapidly (rate $v_d$)
deprotonated the DEAEMAH\textsuperscript{+} moieties. An ester introduced at the same time acted as a dormant deactivator; its hydrolysis generated an acid (with a rate $v_4$), which induced reprotonation of the DEAEMA blocks and consequently, disassembly of the micelles. Although the rate of protonation ($v_2$ in Figure 4b) is very fast ($v_1 \approx v_2$), the slow generation of the acid ($v_5 \ll v_2$) prevented the direct annihilation of the promoter and the deactivator by separating their existence in time, thus making the transient existence of the micelles possible. Importantly, the duration of the transient pH-state (the blue background in Figure 4a) could be controlled by the choice of the dormant deactivator.$^{[26,29]}$ Injecting a mixture of NaOH and $\delta$-gluconolactone ($\delta$-GL) increased the pH from $\approx$5.5 to $\approx$8.7; the relatively fast hydrolysis of $\delta$-GL resulted in a rapid decrease of pH, which dropped halfway (i.e., to pH $\approx$ 7.1) within less than 10 min. When the less reactive $\varepsilon$-capronolactone was used, however, it took more than 1 day for the system to reach pH $\approx$ 7.1. These vastly different kinetics of deactivation were translated into the lifetimes of the transient micelles, which could be programmed to exist for periods spanning more than four orders of magnitude.$^{[26]}$ Of course, this strategy is also applicable to acid/dormant-base combinations as well as to other activator/deactivator combinations.$^{[27]}$ For example, the Nitschke group demonstrated an elegant example of fuel-controlled, transient disassembly of a metal–organic cage using a pair of a reducing agent and an oxidizing agent.$^{[30]}$ 

Reversible self-assembly can also be accomplished by coupling$^{[31]}$ self-assembling species to chemical oscillators—systems exhibiting rhythmic fluctuations in the concentration of selected chemical reagents. Provided that these reagents can activate an external species toward self-assembly, reversible self-assembly over multiple cycles can, in principle, be achieved. A pioneering system of this type was reported by Grzybowski and co-workers, who coupled gold and silver NPs functionalized with 3-fluoro-4-hydroxythiophenol (FHTP) to the methylene glycol/sulfite/gluconolactone (MGSG) oscillator. The MGSG oscillator features a reversible pH change ranging between 6.8 and 9.3. Importantly, the pK\textsubscript{a} value of FHTP ($\approx$8.3) falls within the pH range of the oscillator; hence, reversible protonation of the ligands, and therefore self-assembly of NPs, could be achieved by integrating FHTP-functionalized NPs with this oscillator.$^{[32]}$ In a related study, Au NPs capped with mercapto-dodecanoic acid (pK\textsubscript{a} = 5) were coupled with the BrO\textsubscript{3}\textsuperscript{-}/SO\textsubscript{3}\textsuperscript{2-}/ HS\textsuperscript{2-} oscillator (pH range 4–7); the extended oscillation period of this system ($\approx$30 min compared with $\approx$2 min for the MGSG oscillator) allowed the authors to gain interesting insights into the dynamics of reversible NP self-assembly.$^{[33]}$ Very recently, the same strategy was used to induce rhythmic self-assembly of polymeric vesicles.$^{[34]}$ At a high pH, a PEG–poly(acrylic acid) (PAA) block copolymer was readily soluble in water. Decreasing the pH (by means of the BrO\textsubscript{3}\textsuperscript{-}/SO\textsubscript{3}\textsuperscript{2-}/[Fe(CN)\textsubscript{6}]\textsuperscript{4-} oscillator, whose pH fluctuates between 6.5 and 3.5) induced protonation of the PAA block and consequently, self-assembly into vesicles.$^{[34]}$ Beyond controlling self-assembly by pH changes, Zhang and co-workers demonstrated the possibility of reversibly aggregating a PEG–poly(lysine·HCl) copolymer by coupling it to yet another oscillator, the IO\textsubscript{3}\textsuperscript{-}/NH\textsubscript{3}OH\textsuperscript{-}/OH\textsuperscript{-} system, which exhibits a time-varying concentration of iodine.$^{[35]}$ I\textsubscript{2} can reversibly bind to the ethylene glycol moieties, rendering the PEG block effectively hydrophobic, thus inducing self-assembly of the copolymer into spherical aggregates. Unfortunately, a drawback of many chemical oscillators is that they usually comprise complex mixtures of chemicals, often making them highly sensitive to and incompatible with additional species, including building blocks of higher-order structures.

3. Conclusions and Outlook

In all of the examples discussed above, a chemical stimulus induced the self-assembly of a precursor into a higher-order architecture. However, the resulting structures are stable; it is the presence of an external reagent (such as OH\textsuperscript{-} or an enzyme) that established the dissipative conditions.$^{[16]}$ In a

Figure 5. a) The dissipative, thioester-fueled self-assembly of a membrane transport system. b) Changes in the current (top), potential (center), and conductance (bottom) through a lipid bilayer transiently incorporating a channel-forming activated building block. Reproduced with permission.$^{[37]}$ Copyright 2014, Royal Society of Chemistry.
unique synthetic example whereby the activated building block was inherently unstable (pathway \textit{1e}/\textit{1c} in Figure 1), Fyles and co-workers treated a long-chain mercaptan ("precursor" in Figure 5a) with a thioester ("fuel", Figure 5a).[37] Unlike the precursor, the product of the reaction—a thioester appended with polar groups on both ends ("activated building block" in Figure 5a)—had a propensity to form conducting channels within bilayer membranes, whose transport activity was probed by means of voltage-clamp experiments. At the same time, the channel-forming thioester was unstable: the fuel was designed by means of voltage-clamp experiments. At the same time, the arrows indicate the addition of the thioester fuel, which is followed by temporarily enhanced conductivity.

The design of such intrinsically unstable self-assembled systems is challenging and represents one of the frontiers of the field. An even more ambitious goal involves the design of systems, which exist in the assembled state only when exposed to an external energy source (including chemical fuels), whereby the self-assembled structures—and not the individual building blocks—oscillate with an external energy.[38] Realizing these and related goals will bring us closer to the rich and varied functionality of the materials found in nature and will enable the development of novel life-like materials.

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Conflict of Interest
The authors declare no conflict of interest.

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