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Self-Organization

Dissipative Self-Assembly of a Molecular Gelator by Using a Chemical Fuel**

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The construction of energy-dissipating self-assembling systems,[1] which, like self-assembled structures found in nature are formed transiently, far from equilibrium, and under the constant influx of chemical energy, still represents a frontier in nanoscale assembly.

The self-assembly of small molecules, polymers, proteins, nanoparticles, colloids, and particles with sizes that approach the mesoscale under thermodynamic equilibrium conditions has been a powerful approach for the construction of a variety of structures of nano- to micrometer dimensions, like vesicles, capsules, and nanotubules.[2] The reversible nature of self-assembly processes has been exploited in switchable,[3] adaptive,[4] and autopoietic[5] self-assembling systems, which lead to novel responsive materials and artificial systems that are capable of self-replication and compartmentalization. Recently, there has also been a strongly growing interest in self-assembled materials obtained under non-equilibrium conditions. For instance, the formation of hierarchically structured membranes in a reaction-diffusion field,[6] and the orthogonal self-assembly of molecular gels with surfactants,[7] liquid crystals,[8] or other components[9] can be controlled through the processing conditions, thus leading to a much richer structural diversity compared to equilibrium-processed materials. These self-assembled structures offer new and intriguing opportunities for functional materials and biomimetic cellular structures. Nevertheless, in all these cases, the final self-assembling systems reside in a (local) thermodynamic minimum state.

Despite these advances, the permanent nature of these synthetic self-assembled structures does not compare well to the complex spatiotemporally confined self-assembly processes seen in natural systems, which for instance allow the dynamic compartmentalization of incompatible processes, responsiveness, and self-healing. Natural self-assembled structures such as the cytoskeleton[10] and phospholipid membranes[11] are formed by dissipative self-assembly (DSA).[12] In general, DSA systems consist of non-assembling entities which, through activation by an energy source, assemble into ordered structures. Energy dissipation causes deactivation of the building blocks, hence leading to a collapse of the formed structures. A typical example is microtubule assembly that uses guanosine-5′-triphosphate (GTP) as an energy source, which in turn catalyzes the hydrolysis of GTP and therefore its own collapse.[13] The microtubule assembly process is controlled by feedback loops that lead to self-organization, including oscillatory behavior and nonlinear responses of microtubule formation, which are essential for rapid morphogenic alterations, self-healing, and self-replication.

These fascinating properties of natural DSA systems have motivated research on their artificial counterparts. Several artificial DSA systems based on natural building blocks have been reported.[14] Examples of fully artificial DSA systems are most commonly found in the top-down engineered mesoscopic regime[15] with hard inorganic or polymeric objects. The few examples that concern soft matter are mostly fueled by light,[16] whereas the dissipation of "chemical fuels" has been used to drive mechanical motion.[17] It remains a challenge to develop a DSA system that is chemically fueled.

A first step towards the development of a self-organizing self-assembly system is the construction of a simple DSA system without feedback control loops. Such a simple system typically follows a sequence of processes.[18] Firstly, an energy source activates the precursor building blocks so that self-assembly is favored. Upon self-assembly, the activated building block can dissipate its energy, thus resulting in the formation of the initial building block and disassembly of the architecture. A requirement is that the rate of energy dissipation (P_d) should be lower than the consumption of fuel (P_c) to allow the formation of self-assembled architectures (Figure 1).

Herein we present a synthetic DSA fibrous network that uses chemical fuel as an energy source. A gelator precursor is converted into a gelator by reaction with a chemical fuel, thus leading to self-assembly. Hydrolysis of the gelator, which is labile under ambient conditions, leads to energy dissipation and disassembly of the formed structures.

Reactive gels have been previously reported[19] and the hydrolysis of ester functions has been exploited to achieve an enzymatically controlled gel–sol phase transition.[20] The design of the dissipative self-assembling system presented here is based on dibenzoyl-(L)-cystine (DBC; Bz = benzoyl), a well-known pH-responsive hydrogelator .[21] Above their pK_a value (ca. 4.5), intermolecular repulsion occurs between the anionic carboxylic acid groups of DBC, and therefore DBC

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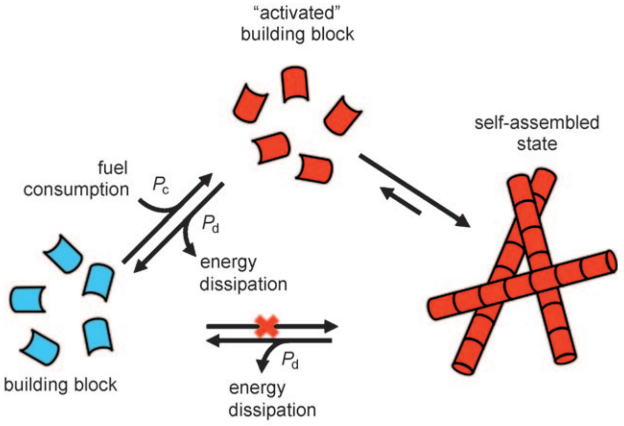


Figure 1. Dissipative self-assembly: a monomeric building block (blue) is activated by fuel consumption and is able to assemble (forming red fibers). In the assembled state it can dissipate its energy and revert to its monomeric state (blue).Energy is both consumed and dissipated in one cycle; self-assembly can occur only if sufficient energy is available.

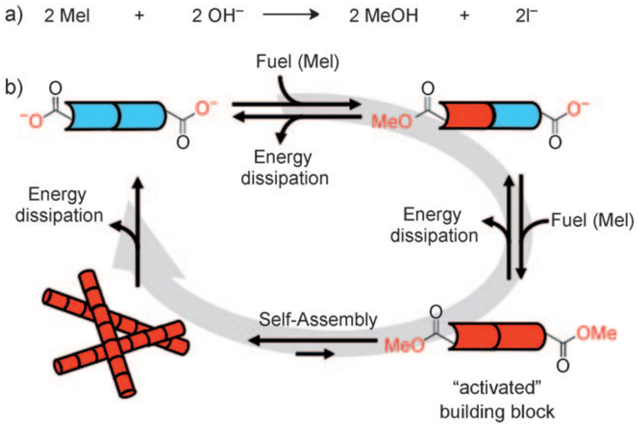
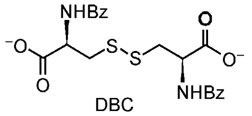


Figure 2. a) The net reaction of one DSA cycle. b) Reaction cycle of the dissipative system. DBC dicarboxylate (top left) can react with MeI (fuel) to give monoester DBC-OMe. DBC-OMe can dissipate its energy through hydrolysis to form DBC or react again with the fuel to form diester DBC-OMe$_2$. The diester can assemble into fibers, which will be in equilibrium between formation and hydrolysis of the diester until all the MeI has reacted.



DBC

remains in the isotropic solution. However, reduction of the pH value to below the pK_a value leads to protonation and hence neutralization of the carboxylate groups. As a result, the molecules self-assemble into elongated fibers, which are stabilized by intermolecular hydrogen bonds and hydrophobic interactions. The assembly of DBC diesters, which do not contain carboxylate groups, is independent of the pH value.[22] This observation implies that above the pK_a value, esterification of nongelating DBC should lead to the formation of fibrous aggregates.

Carboxylate groups can be esterified using alkylating agents.[23] In this particular case, alkylation by methyl iodide (MeI) in water under ambient conditions was successful, albeit rather slow. To increase the rate of alkylation, the reaction was performed at 35 °C. Under these conditions, the hydrolysis reaction of MeI was found to be negligible.[24] On the contrary, the formed ester is prone to hydrolysis, which reforms the initial nongelator DBC. The rate of hydrolysis can be controlled by the pH value, which, combined with temperature control of the esterification rate, makes the system highly tunable. We found that at pH 7 and 35 °C, the formation of ester was faster than its hydrolysis, which is a requirement for a DSA system. In the alkylation of DBC to form the diester, two molecules of MeI are consumed, and two iodide ions are released. The subsequent hydrolysis to DBC consumes two hydroxide ions and produces two molecules of MeOH (Figure 2 a). This net reaction implies a decrease of the pH value. To counter this decrease, the reaction was carried out in a pH-stat setup, which provides the additional advantage that hydroxide ion uptake (and therefore the rate of ester hydrolysis) can be monitored (see the Supporting Information).

We found that DBC can be converted into its gelating diester by reaction with MeI (fuel). Hydrolysis of the diester back to its nongelating carboxylate derivative (DBC) is the energy-dissipating step. The addition of sufficient fuel to the system will result in a state in which the diester is continuously formed and hydrolyzed. When the diester is present in a sufficiently high concentration, fibrous aggregates or gels can be formed. However, as soon as the source of energy is consumed, the system will hydrolyze to the diacid and return to its thermodynamically stable non-aggregated state (Figure 2 b).

The DSA system was tested by the addition of MeI (1.0 mmol, resulting in an initial concentration of 100 mM) to an aqueous solution of DBC (10 mL,100 mM) at pH 7. The course of the reaction was followed by HPLC–MS, dynamic light scattering, and the consumption of hydroxide ions. HPLC–MS showed that the monoester DBC-OMe was at a concentration of 0.25 mM after 3 hours (Figure 3 a). During the course of the reaction, the concentration of DBC-OMe increased further until the concentration leveled off around 10 mM after 100 hours, thus suggesting that a steady state between the rate of formation of the monoester and its hydrolysis had been reached.

The increased DBC-OMe concentration should result in an increased rate of formation of the diester DBC-OMe$_2$, and after 72 hours DBC-OMe$_2$ was indeed detected by HPLC–MS. Analogously to DBC-OMe, the concentration of DBC-OMe$_2$ reached a plateau around 1.8 mM after 100 hours of reaction time. Interestingly, this increase was accompanied by an increase in turbidity, hence indicating the formation of large aggregates. Light scattering studies confirmed the increase in the formation of large structures (Figure 3 b) and bright field microscopy revealed the presence of fibers in the system (Figure 4 a). The fibers were separated from the solution by two centrifugation and washing steps and HPLC–MS showed that the fibers consisted of 96 % DBC-OMe$_2$ and

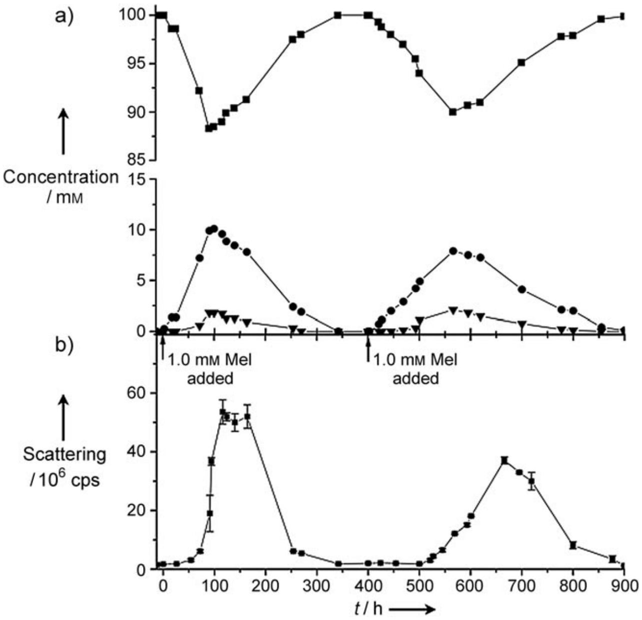


Figure 3. a) Concentrations of DBC (■), DBC-OMe (●), and DBC-OMe$_2$ (▼) as determined by HPLC–MS over time. After 3 h the formation of DBC-OMe was observed. After 100 h the maximum concentrations of DBC-OMe and DBC-OMe$_2$ were reached and the hydrolysis rate is higher than the methylation rate. b) Quantitative measurement of aggregation by dynamic light scattering (DLS; $\lambda = 633$ nm) in counts per second. Aggregation is observed after 70 h.

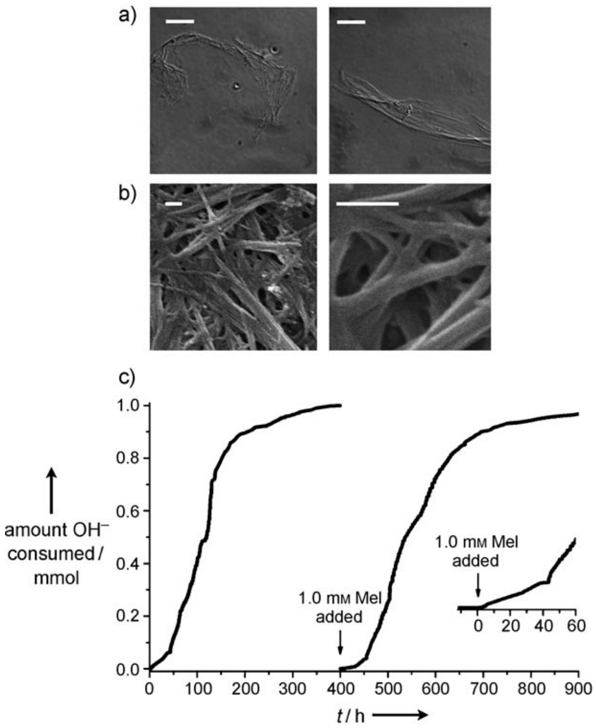


Figure 4. a) Bright field micrographs of fibers after 100 h. Scale bars = 20 μm. b) SEM images of fine structure of separated fibers after 100 h. Scale bars = 0.5 μm. c) Hydroxide ion uptake by the system over time. The inset shows a magnification of the first 60 h. At $t = 0$, 1 mmol MeI was added and the hydroxide uptake started to grow exponentially. After 350 h both the esters had been hydrolyzed and the hydroxide ion uptake leveled off at 1 mmol. After 400 h, a further 1 mmol of MeI was injected and the process was repeated.

4% DBC-OMe (see the Supporting Information). The centrifuged pellet was also examined by scanning electron microscopy (SEM), which revealed the fine structure of the fibers (Figure 4b).

After 120 hours, the concentrations of both esters clearly started to decrease, which indicated that the rate of hydrolysis was higher than the rate of ester formation most likely as a result of the decreasing fuel concentration. After 350 hours, HPLC–MS showed that both esters had been hydrolyzed to DBC, and light scattering confirmed that all the fibers had disassembled.

The ester hydrolysis was also followed by monitoring hydroxide ion uptake. By adding fuel to the system, the hydroxide ion uptake started to grow exponentially, which confirmed the hydrolysis of the ester (Figure 4c). Moreover, the exponential growth confirmed that the ester was accumulating. After 350 hours, the uptake leveled off, thus indicating that hydrolysis had stopped. The total amount of hydroxide taken up by the system was in excellent agreement with the initial amount of fuel added.

The concurrent gelator conversion, formation, and disassembly of fibers, and consumption of fuel shows that the transient self-assembly is driven by energy dissipation. At the end of the process, when all fuel is consumed, the system reverts back to its thermodynamic minimum; that is, an isotropic solution.

Encouraged by the above results, we anticipated that the addition of a second batch of fuel would again induce self-assembly. Indeed, after a second addition of MeI (1 mmol) the formation of large aggregates was observed, accompanied by the formation and hydrolysis of the DBC ester. The same concentrations of ester were obtained as in the first cycle. Bright field microscopy revealed the presence of fibers, however, the scattering intensity did not reach the previous level, which might indicate that the fuel consumption products (MeOH and I$^-$) disturb fiber formation to some extent.

We have shown how the dissipative self-assembly of a synthetic gelator can be brought about by using a chemical fuel. A nongelating diacid is activated to self-assemble through a double alkylation by methyl iodide, the chemical fuel. After a certain period, the formation of the diester is observed, which leads to the assembly of fibrous aggregates. These diesters are prone to hydrolysis, thus resulting in energy dissipation. When all the MeI has reacted, hydrolysis of the gelator molecules results in the disassembly of the fibrous aggregates. This system meets all requirements for dissipative self-assembly; reaction of a building block with a fuel leads to activation, which favors self-assembly. This activated building block can be deactivated by energy dissipation. Activation occurs at higher rates than deactivation, hence leading to a steady self-assembled state, which is continuously being formed and degraded as long as energy in the form of chemical fuel is available. To the best of our knowledge, the approach described here is the first chemically fueled synthetic DSA system, which is a new approach towards complex self-assembled structures and smart materials. However, this system is not comparable with the complexity of natural DSA systems. The next steps towards this goal would include improved kinetics (e.g., by the use of enzyme responsive gelators[4b,c]) to allow faster switching, and the incorporation of autocatalytic feedback mechanisms that lead to oscillating behavior through self-organization.[25] Such systems can evolve into DSA architectures that are intrinsically capable of self-healing by continuous regeneration.

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