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Adaptive Polymeric Assemblies for Applications in Biomimicry and Nanomedicine

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ABSTRACT: Dynamic and adaptive self-assembly systems are able to sense an external or internal (energy or matter) input and respond via chemical or physical property changes. Nanomaterials that show such transient behavior have received increasing interest in the field of nanomedicine due to improved spatiotemporal control of the nanocarrier function. In this regard, much can be learned from the field of systems chemistry and bottom-up synthetic biology, in which complex and intelligent networks of nanomaterials are designed that show transient behavior and function to advance our understanding of the complexity of living systems. In this Perspective, we highlight the recent advancements in adaptive nanomaterials used for nanomedicine and trends in transient responsive self-assembly systems to envisage how these fields can be integrated for the formation of next-generation adaptive stimuli-responsive nanocarriers in nanomedicine.

1. INTRODUCTION

The past decades have witnessed substantial progress in the field of self-assembly, which is regarded as one of the most promising approaches for the construction of dynamic and adaptive systems. For example, great contributions have been made to the development of tailor-made stimuli-responsive (nano)materials that can undergo chemical or physical property changes as a response to external or internal signals. The construction of such adaptive nanoparticles is particularly relevant for the field of nanomedicine. The traditional advantage of using nanocarriers for the transport of drugs in the body is their ability to overcome the pharmacokinetic limitations associated with conventional drugs. The nanocarriers protect the drugs from undesired interactions with the body, they provide a reservoir function for the slow release of the therapeutic compounds and, more specifically, the size regime allows more effective uptake in certain tissues, such as facilitated by the enhanced permeation and retention (EPR) effect observed in a number of tumors. However, features such as long circulation time and effective cell uptake are often not to be united in one and the same particle with a defined shape, size, and surface charge. It is, therefore, important to design particles that can adapt their features based on the environment they are in, exploiting local changes in, for example, pH or oxidative potential. Research into adaptive nanoparticles for nanomedicine applications has, therefore, in recent years, experienced a strong surge in activity.

The current adaptive nanoparticles used in the field of nanomedicine can, thus, undergo reversible responsive processes, for which an outside trigger is always needed to switch the system back to its initial state. This is different from natural dissipative out-of-equilibrium systems, such as those found within living cells, which are governed by the rules of physics and developed through Darwinian evolution. Mimicking the complexity of living systems via the construction of synthetic analogues, thereby ultimately creating life de novo is still standing as one of the grand challenges for scientists. Although synthetic out-of-equilibrium systems are primarily developed from a curiosity-driven point of view to attain molecular assemblies with life-like features, they can also be employed for the construction of more intricate adaptive materials. This field of science has made much progress recently. The first pioneering research used to be mostly directed to transient self-assembly of molecules into fibers and gels; however, nowadays, besides self-regulated structural control, the first examples have emerged of systems with transient function. Such systems have the ability to form architectures that are more diversified in structure and function, allowing them to be employed in applications where dynamics and functional adjustment based on environmental changes are necessary. They are internally regulated and have a built-in mechanism that allows them to switch back to the ground state when the stimulus is removed. Although this so-called autonomous regulation or self-adaptive behavior seems to be still a distant prospect for most adaptive nanoparticles, the first concepts that have been published...
demonstrate the feasibility of this approach, which could lead to a next generation of vehicles employed in nanomedicine.

In this Perspective, we highlight the development of adaptive self-assembled systems for applications in biomimicry and nanomedicine. The design, synthesis, and utilization of such dynamic and adaptive features provides us on the one hand with a deeper understanding into the complexity of cellular life. On the other hand, these systems and concepts will be of great added value for the field of nanomedicine, in which transient behavior can further improve the spatiotemporal control of nanocarrier function. We will start with highlighting some specific examples of the current state of adaptive nanoparticles in nanomedicine and then follow up with recent trends in transient adaptive systems with life-like features. We will end with a perspective on how these two fields could be merged synergistically for a new generation of adaptive carriers in nanomedicine.

2. ADAPTIVE SELF-ASSEMBLED SYSTEMS FOR NANOMEDICINE APPLICATIONS

2.1. Self-Assemblies with Adaptive Size. Contrary to conventional “stealth nanoparticles”, adaptive nanocarriers possess the ability to maintain a larger initial size and nearly neutral surface charge during blood circulation, but once they have reached the desired tissue, their adaptive behavior allows them to achieve a tailored morphology transformation, surface charge reversal and disruption of the nanocarriers to improve the accumulation and retention of the therapeutic compounds.21,35–38 This is especially relevant for the targeting of tumor tissues.34,39,40 Indeed, it is well-known that the vasculature pH and redox state of the tumor microenvironment are considerably different from healthy tissue.41,42 Many research groups have developed strategies to build adaptive transformable nanocarriers (by responding to pH, light, temperature, etc.) to enhance the therapeutic efficacy of their carrier systems.33,44 The chemistry of such adaptive nanocarriers is programmed to achieve bond cleavage, protonation, or conformational changes in the diseased region.43,45 A range of different triggers is pursued for selective and active responsiveness, such as spatially controlled external stimuli, tumor-associated abundant enzymes, the acidic pH and redox state of the tumor microenvironment to construct multistage delivery systems that combine the opposing features that promote both long circulation time and deep penetration into tumor tissue by adapting to the local microenvironment once they reach the desired target site.46–48

External triggers such as ultrasound, magnetic field, light, and heat can be applied to induce the adaptive behavior of nanoparticles in vivo.49,50 For instance, nanoparticles can be spatiotemporally shrunken in size by employing external stimuli in the targeted diseased region, which enables more effective tissue penetration and on-demand delivery. In this respect, NIR laser-induced targeted cancer thermo-chemotherapy represents a novel anticancer strategy with facile control and practical efficacy.49,51 It does not require any targeting moieties, as the control is achieved by the point source of the laser employed. A recent study by Ge and coworkers describes an interesting application of this methodology for the treatment of hypoxic tumors.46 They constructed ROS cleavable polymeric vesicles that were loaded with photosensitizer-modified dendrimer clusters and hydrogen peroxide. Upon irradiation with a 660 nm laser, the singlet oxygen produced by the photosensitizer cleaved the copolymer of the vesicle carrier system. This resulted in disruption of the polymeric vesicles and release of the dendrimers, which enabled deeper penetration of the targeted tissue. Furthermore, the carriers also provided the vesicle with self-supplied oxygen produced by the thermal degradation of hydrogen peroxide upon irradiation with an 808 nm laser, which contributed to the total ablation of hypoxic hypopermeable pancreatic tumors through photodynamic damage.

The tumor microenvironment is characterized by its acidic pH due to the altered glucose metabolism known as the Warburg effect, which is considered as an appropriate internal trigger to achieve adaptive size behavior.49,52 Based on this principle, Wang et al. developed ultra-pH-sensitive cluster nanobombs (SCNs) by rational self-assembly of poly(ethylene glycol)-b-poly(2-azepane ethyl methacrylate)-modified PAMAM dendrimers, which were conjugated with a platinum prodrug.52 The designed self-assembled nanoparticle complexes attained a relatively large size around 80 nm at neutral pH. Upon accumulation/retention in the slightly acidic tumor microenvironment (pH ~ 6.5), the poly(2-azepane ethyl methacrylate) blocks became hydrophilic due to their pH responsiveness and rapid protonation, which led to the instantaneous dissociation into small dendrimer building blocks, resulting in a sharp size transition to less than 10 nm. Upon disassembly, the covalently conjugated Pt prodrug was reduced by intracellular abundant glutathione (GSH) to achieve a therapeutic effect. This rapid size-switching feature not only facilitated nanoparticle extravasation and accumulation via the enhanced permeability and retention effect but also allowed faster nanoparticle diffusion and more efficient tumor penetration, thereby contributing to more pronounced drug penetration and therapeutic efficacy in vivo with poorly permeable BxPC-3 pancreatic tumor models (Figure 1).

In certain acidic tumor microenvironments, pH gradients can even reach values as low as 5.0–6.0 in the subcellular organelles.53 Additionally, the rapid growth and proliferation of tumors also lead to hypoxia and production of abundant reactive oxygen species (ROS).53–55 Taking advantage of these unique characteristics of the tumor tissue, Tan et al. recently introduced a hydrophobic poly ferrocene block conjugated to a DNA aptamer sequence which initially self-assembled into 80 nm micelles assisted by the hydrophobic interaction of the ferrocene moieties.56 In the presence of oxidants under acidic conditions, the ferrocene moieties underwent the Fenton reaction and were easily oxidized into hydrophilic ferrocenium salts, changing the hydrophilic/hydrophobic ratio. It led to a size decrease of the assemblies as they were rearranged into micelles of less than 10 nm in size, stabilized by the π−π stacking interaction of ferrocene with G-quadruplexes; this thereby improved the tumor penetration ability of the DNA polymer hybrid assembly. These nucleic acid assemblies could also release highly toxic hydroxyl radicals into the tumor microenvironment, achieving in vivo catalytic therapy for efficient cancer treatment. These strategies in the design of size adaptive nanocarriers successfully allow to combine longer blood circulation, enhanced accumulation/retention, deeper tumor penetration as well as efficient cancer cell internalization into one single nanoplatform.

2.2. Self-Assemblies with Adaptive Shape. Biological structures, varying from molecules, viruses, and bacteria to cells have evolved into precise shapes to mediate communication, interaction, and function.57–59 As an example, viruses are to be found in a range of different geometries, varying from
Since object shape is crucial in many natural processes, it is logical to expect that this also accounts for the interaction of biological systems with synthetic particles. Indeed, apart from the strong correlation of nanoparticle size with their biomedical performance, the shape of nanomedicine particles has also proven to play a pivotal role in a number of interactive processes between nanoparticles and cells. For instance, compared to particles with spherical morphology, high-aspect-ratio polymeric assemblies displayed improved cellular internalization via distinct uptake mechanisms, which were similar to the uptake of rod-like bacteria by nonphagocytotic cells. Worm-like particles showed advantageous fluid dynamics over other morphologies including spherical, rod-shaped, and fingerprint-like morphologies.

The shape of polymeric nanoparticles has also proven to play a vital role in ligand–receptor binding interactions with cells. In comparison to their spherical counterparts, rod-like polystyrene nanoparticles that were equipped with a trastuzumab antibody as targeting ligand demonstrated a greater ability to boost binding as well as avidity with their targets, displaying an enhanced specific uptake and decreased nonspecific internalization. Because of this improved binding and internalization, rods modified with antibodies showed a much more enhanced killing effect toward BT-474 breast cancer cells in comparison to the antibody itself. Mathematical modeling suggests that this is caused by enhanced polyvalent interactions of the nanorods with the cell surface, which contribute to increased avidity and specificity, and which are modulated by the balance of favored adhesion and entropic losses, as well as shear-induced detachment that reduces binding. This improved targeting behavior of rod-like polystyrene particles was also observed in in vivo experiments in mice under physiological conditions.

In the above examples, shape is still a static feature, and the particles were preset in their specific morphology. In this respect, it is of great interest that researchers have become skilled in the production of self-assembled structures via inter/intramolecular driving forces to construct a wide range of discrete nanoscopic architectures with unique morphological features. Their self-assembled nature introduces a dynamic character in these particles, which can be employed to develop systems with adaptive shapes by subtle changes in the local microenvironment. Although not as far developed as size-switchable particles, the first examples of this type of adaptive particles have now been reported, which will be discussed in this section.

Nanoparticles based on smart polymer self-assemblies can be morphologically switched in response to a change in the local environment. For instance, subtle changes in the balance between polymer viscosity and interfacial tension allowed polymeric nanoparticles to undergo a stimulus-responsive shape switch in real-time, which could be precisely controlled over minutes and days. It was shown that such processes can be modulated by external triggers such as chemical agents, pH, and temperature. Shape switching behavior was further utilized in selective manipulation of the macrophage phagocytosis process of disc-shaped polymeric nanoparticles composed of poly(lactic-co-glycolic acid) (PLGA), as reported by Mitragotri et al. In their work, PLGA elliptical discs were opsonized with mouse IgG and incubated with mouse peritoneal macrophages. The discs switched their shape to spheres in due time, after which they were internalized by the macrophages. As a control, discs that were designed not to undergo a shape switch process were not phagocytosed because of their large aspect ratio.

Nanomedicine particles that are able to undergo adaptive behavior by responding to the microenvironment of diseased tissue could facilitate the development of disease-selective therapeutics and targeted drug delivery. For instance, in a recent example reported by Wang et al., an “on-site transformation” strategy was demonstrated, based on self-assembled chitosan–peptide conjugates (CPCs) with a labile PEG corona that were sensitive to enzymes present in the targeted area; the morphology changing property was employed for designing high-performance antibacterial therapeutics. Upon arrival of the particles at the infectious microenvironment, the presence of gelatinase resulted in cleavage of the enzyme-susceptible peptides, which led to the release of the outer PEG layer. As a result, the hydrophobic/hydrophilic ratio of the molecular building blocks changed, upon which the spherical self-assemblies spontaneously reorganized, via chitosan chain–chain interactions, into fibrous structures. After that, the remaining exposed peptide sequence on the particle surface adopted an α-helical conformation that led to multivalent cooperative electrostatic interactions with bacteria and the subsequent disruption of their cell membranes. Further in vivo tests proved that the morphology-adaptive nanoparticles displayed higher accumulation and longer retention time compared to their morphology-stable counterparts, which led to improved antibacterial performance both in vitro and in vivo.

![Figure 1](image-url)
Another interesting feature of rod-like nanostructures is their ability to be transported through tumor pores more rapidly; they can furthermore undergo alignment with the blood flow, which increases the probability of convective delivery.\(^7,8,89\) For example, in comparison with spherical gold nanoparticles of the same hydrodynamic diameter, nanorods displayed 4× more enhanced penetration in orthotopic mammary tumors after administration in mice.\(^88\) The construction of adaptive self-assembled particles that can change their shapes to facilitate tumor penetration is however underexplored, due to difficulties in building block design. In a recent example reported by Gao et al. (Figure 3), orally administered semielastic nanoparticles composed of a core of PLGA, which was covered by a lipid shell, could efficiently overcome multiple intestinal barriers and display enhanced diffusivity through the mucus compared with unresponsive particles.\(^90\) The semielastic spherical NPs possessed the ability to adapt to ellipsoids within the complex mucosal hydrogel mesh structure, which ultimately induced rotation-facilitated fast diffusion and tumor penetration, revealed by molecular dynamics simulations and super-resolution microscopy imaging. The faster diffusion and enhanced tumor penetration resulted in increased bioavailability of doxorubicin (Dox; up to 8-fold) loaded in the particles, compared to a free Dox solution. In comparison, rigid NPs displayed poor adaptive ability and could not deform, and overly soft NPs were impeded by interactions with the hydrogel network, thus, displaying poor tumor penetration. These shape adaptive nanoparticles indicate that the strategy of modifying nanoparticle rigidity and adapting particle shape can be utilized to overcome multiple biological barriers that are traditionally encountered during drug delivery.

3. SELF-ASSEMBLED SYSTEMS WITH ADAPTIVE BEHAVIOR

As described in the previous section, adaptive behavior can facilitate nanoparticle efficacy in nanomedicine applications. Still, the number of synthetic nanoparticles that are adaptive with respect to size or shape are limited. This is in sharp contrast to biology, where adaptivity is a ubiquitous phenomenon; dynamic control over shape and size is an essential element in various biological processes.\(^2,5\) As mentioned in the Introduction, adaptive behavior of biological systems often shows a transient behavior, which allows them to autonomously turn back to their resting state.\(^24,91,92\) If nanoparticles could be developed that regulate, in a biomimetic fashion, their activity based on environmental cues, this would open up tremendous new opportunities for the field of nanomedicine.\(^1,93\) More specifically, concepts can be exploited that are developed by the field of science interested in bottom-up synthetic biology. In this discipline, biomimetic assemblies are made which are dynamic and show out-of-equilibrium behavior. Adaptation of these concepts...
would allow the construction of nanocarriers that display stimulus-regulated control over their features.

In most cases, naturally occurring systems are associated with an energy consumption process to obtain spatial and temporal control over the biological complexity and functionality. Most frequently, this spatiotemporal control is manifested in the form of energy dissipation, concentration diffusion, autonomous dynamics, and feedback loops. Scientists call this energy-driven life-like behavior. Translating this behavior to functional self-assemblies is an exciting way to create complex, biomimetic systems. In this regard, fuel-driven structural and functional processes under temporal control are thought to play a key role in providing man-made materials with biological features in terms of natural organization and function.

The first examples of self-assembled adaptive systems were mostly focused on chemically fueled transient molecular self-assembly into fibers and gels with self-regulated structural control. Following up on these studies, transient functional systems were composed that showed dynamic adaptive changes depending on the environmental conditions. The integration of enzymatic reaction networks with self-assembled systems turned them catalytically active in an attempt to more closely mimic the complexity of living systems. Internal regulation in these systems allowed them to detect and produce a response to external stimuli and return to the ground state when the stimuli were removed. More recently, the first examples of adaptive colloidal systems have been reported, which however still lack true autonomously regulated self-adaptive behavior. Yet, the initial studies with vesicular and colloidal particles are highly promising and indicate the potential of these transient structures for the development of next-generation vehicles that can potentially be used in nanomedicine. In this section, these three stages of development of transient, adaptive systems will be discussed.

3.1. Transient Adaptive Systems Featuring Fibers and Gels. Systems that exploit sol–gel transitions are one of the simplest, yet elegant designs of dissipative self-assembly networks. Boekhoven and co-workers developed a series of active fibers able to mimic the transient behavior of microtubules. Their strategy was based on a pH-responsive gelator, dibenzoyl-L-cystine (DBC), the pK_a of which is around 4.5. When the pH was below the pK_a, the carboxylic groups were protonated, which led to neutralization and consequent self-assembly of DBC in long fibers through intermolecular hydrogen-bonding. By introducing the DBC-diesters, which can self-assemble at all pH ranges, a dissipative self-assembly system was created. The addition of methyl iodide (MeI) to DBC under basic conditions resulted in the transient formation of DBC-(OMe)_2 fibers, which were disassembled over a period of time. This was caused by the spontaneous hydrolysis of the formed esters, which restores the system to its original state. To reduce the long lifetimes of the system, a follow-up study was recently performed by the same group. They showed that the concentration of the chemical fuel can be used to control the mechanical properties of the gel. Short-lived weak gels were obtained in the presence of lower concentrations of MeI, while the addition of higher concentrations of MeI caused the formation of long-lived stiff gels.

More recently, a peptide hydrogel system was reported by the Walther group that contained a biocatalytic pH-regulated feedback-induced transient state. At high pH, the peptide employed was in a molecularly dissolved state. Upon addition of an acidic buffer and the concomitant fast pH decrease, peptide assembly was induced. This effect was counteracted by the enzyme urease, which was present in the reaction medium and which converted urea into basic ammonia, restoring the original pH levels. Urea was added

![Figure 4](image-url)

Figure 4. (a) Dissipative self-assembly process of a molecular gelator to form fibrillar networks from an activated building block, which is obtained from a synthetic gelator in a dissipative self-assembly process that is fueled by an alkylating agent. The system reverts to its thermodynamic equilibrium, when the available energy is depleted. (b) Biocatalytic temporal control of a self-regulating transient pH-state by kinetic balancing of two antagonistic feedback-controlled enzymatic pH reactions, in which a biocatalytic reaction network is used for internal pH modulation; the enzyme urease converts urea to the basic ammonia, a process that is amplified by autocatalytic positive feedback, while the antagonistic esterase transforms an ester to acid under negative feedback control. c) Schematic of the transient alkaline pH-profile (red) with an initial lag time as produced by the biocatalytic reaction network in conjunction with the coupled self-assembly response (blue). Initially, the system is dormant and then becomes activated by simultaneous injection of the urea and the ester. Addition of an acidic buffer elongates the quasi-dormant period and implements an initial lag time to the system. (a) Reproduced from ref 100 with permission from John Wiley and Sons. Copyright 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (b, c) Reproduced with permission from ref 101. Copyright 2017 American Chemical Society.
to the medium together with the acidic buffer. The lifetimes of the transient acidic profile were adjusted by varying the urease concentration, triggering the self-regulated pH reversal. This feedback-induced pH regulation enabled access to time-programmed hydrogels, with lifetimes being regulated from a few minutes to several hours. Those spontaneously regulated hydrogels were employed to temporally block microfluidic channels and reroute fluid flow in a simplistic vascular network model in a time-preprogrammed fashion.

Chemically fueled dissipative out-of-equilibrium systems can suffer from waste products and can fail after a few cycles of operation. Several systems are developed to exploit waste-free strategies that include light, magnetism, and ultrasound. Another stimulus to control the self-assembly of nanostructures without producing waste products is ultrasound, which is extensively used to overcome the kinetic barriers in the processes of dissolution and gelation.

The Ulijn group recently reported transient reconfiguration of aromatic dipeptide amphiphile nanostructures by using ultrasound. Fmoc-phenylalanyl-leucine (Fmoc-FL) and Fmoc-tyrosyl-leucine (Fmoc-YL) dipeptides formed tapes and straight fibers, respectively, in aqueous solutions. When they were exposed to ultrasound irradiation for 5 min, Fmoc-FL-dipeptide tapes adopted twisted fibrillar structures and Fmoc-YL-dipeptide fibers transformed into spherical aggregates. When the ultrasound was switched off, both architectures reconfigured into the initial structures. The same transient reconfiguration was observed in iterative ultrasound on–off cycles. Thus, the work represents a rational use of a noninvasive stimulus and shows competing H-bonding/hydrophobic interactions can be modulated temporarily.

Replicators that are formed and destroyed simultaneously are one of the promising systems that may help us to understand the mechanisms behind life. The Fletcher group developed a system composed of a hydrophobic alkene A and a hydrophilic alkene B that were conjugated to an amphiphilic building block C in the presence of a Grubbs second generation catalyst (Figure 5a,b). The building block C, which self-assembles into instable micelles, could also be destroyed by the same catalyst, thereby yielding a thermodynamically stable product D. The formation of C is an autocatalytic process where an initial lag phase was followed by an exponential increase which moved the system toward out of equilibrium. The kinetic profile of the reaction showed a maximum concentration of C, followed by depletion as the reactant A and self-assembled C ran out. When the fuel was supplied in batches, the replicator was formed and depleted again. It is noteworthy to mention that D did not form at the beginning of the reaction but only when C reached a significant concentration. This implies that destruction occurs only when C is self-assembled into micelles.

The Otto group applies a dynamic combinatorial chemistry approach to explore the emergent behavior of synthetic self-replicating peptides. A short peptide chain composed of hydrophilic and hydrophobic amino acids was functionalized with a dithiol group to allow a reversible exchange reaction to study the spontaneous emergence of replicators from dynamic combinatorial libraries (DCLs). The continuous exchange drives the system to equilibrium, but irreversible processes such as self-replication drive the library out of equilibrium. Recently published inspirational work by Sadowink et al. shows the diversification of self-replicating molecules in DCLs (Figure 5c). Addition of a second building block, which was structurally similar to the first one, led to the emergence of a set of replicators, where one was the descendant of the other. These mutants (constitutional isomers) were formed simultaneously via auto- and cross-catalysis, they competed for common building blocks. Modest differences in selectivity resulted in complementary sets that occupied different niches. The behavior observed in this system marks an important step toward creating Darwinian evolution at the molecular level using fully synthetic molecules.
3.2. Adaptive Systems Featuring Enzymatic Reaction Networks. Implementing the principle of out-of-equilibrium in synthetic systems has recently gathered considerable attention since it enables access to active materials with unprecedented properties. However, most of the existing reports are limited to molecule-level systems; nanoscale out-of-equilibrium systems have received little attention. An improved understanding of assembled nanosystems with a more interactive, autonomous and life-like state is however highly important as an intermediate step to the complexity of living systems. Recently, numerous examples of nucleic acid- and DNA-based systems have been developed to tackle this challenge.\textsuperscript{113−115} It is noteworthy that the difficulty of achieving and studying out-of-equilibrium nanosystems lies in developing a delicate balance between energy input and response output to allow the formation of a transient state of the system. Additionally, it is more difficult to couple chemical or enzymatic reaction networks to an intricate nanosystem, for example based on polymer self-assembly, to generate precisely controlled output.\textsuperscript{116−118} Despite the presence of many existing enzymatic reactions that direct the regulatory pathways of assemblies in living organisms, translating them into synthetic systems is still in its infancy because of the limited availability of purified enzymes and their narrow window of operation. Here we will discuss some recent examples on how enzymatic networks can be employed for creating transient behavior.

One of the important capabilities of living systems is the adaptation to the environment. Adaptation is a dynamic process which allows the system to sense a change in the environment (input), create a transient response (output), and return to the initial state. Helwig et al. constructed an adaptive enzymatic reaction network based on a fluorogenic compound Z-Phe-Arg-AMC, which was either cleaved by trypsin (Tr) or chymotrypsin (Cr; Figure 6a).\textsuperscript{119} With the help of a computer model, they developed a network that can be optimized and tuned precisely with respect to sensitivity, that is, initial response to the input, and relaxation, that is, the response of the network to return to its initial state. When the fluorogenic substrate was cleaved by Tr, it produced a fluorescent compound, 7-amino-4-methylcoumarin (AMC) and a short peptide chain. At a comparable rate, Tr also activated Cg to the enzyme chymotrypsin (Cr) which could also cleave the fluorogenic substrate, but at a different site. Cleavage with Cr yielded a nonfluorescent compound and AMC was not formed by Tr. To show the adaptive behavior, the network was assembled in a continuously stirred-tank reactor, where the components Tr, Cg, and Z-Phe-Arg-AMC were infused on one side and a portion of the solution was withdrawn non-selectively on the other side. When all the reaction rates within the network were balanced, AMC was formed directly by the constant input of Tr. As the Cr concentration increased, it started to compete with Tr, and the AMC concentration was depleted up to the point that steady-state conditions for AMC were established.

The Hermans lab employed enzymes to develop non-equilibrium steady states in supramolecular polymers. In one of their recent examples, Sorrenti et al. designed a stimuli-responsive system based on the symmetric 3,4,9,10-perylene-dimide-LRRASL peptide hybrid (PDI), where both the serine residues could be phosphorylated by protein kinase A (PKA) in the presence of ATP (Figure 6b).\textsuperscript{120} This resulted first in the formation of the monophosphorylated product of p-PDI and, consequently, the diphosphorylated product, p$_2$-PDI. In a similar manner, p$_2$-PDI could be covalently cleaved with λ-protein phosphatase (λPP), first to p-PDI and then to PDI. Phosphorylation induced an alteration in the supramolecular chirality and resulted in opposite-handed self-assembled structures for PDI and p$_2$-PDI. Self-sorting experiments showed that they could not form mixed assemblies. When ATP was added to a solution that contained PDI, PKA, and λPP, the system showed transient behavior resulting in the change of supramolecular structure and the chirality of the polymer. To achieve nonequilibrium steady states, ATP was continuously added to the same system and waste products were removed selectively in a continuous flow device. Dissipative out-of-equilibrium systems that undergo transient

![Figure 6](image-url)
self-assembly open an avenue for the construction of life-like assemblies that display a high level of adaptability and ultimately could perform complex functions as found in natural systems.

3.3. Adaptive Systems Featuring Colloidal and Vesicular Structures. Although most biomimetic out-of-equilibrium assemblies are based on fibers and gels, there are a number of noteworthy exceptions. Based on a similar principle to Boekhoven’s work, van Ravensteijn et al. developed a colloidal system that can undergo fuel-mediated transient, reversible self-assembly. The particles were functionalized with anionic carboxylic acids which stabilized the colloidal assembly by electrostatic repulsions in aqueous solution buffered to pH 8–9. When a strong methylating agent, dimethyl sulfate, was added to the system as fuel, carboxylic acids were converted into uncharged methyl esters within 2 h. Removal of the charges thus increased the hydrophobic character of the particles, which induced their aggregation. However, the methyl esters were hydrolyzed slowly back to charged carboxylic acids, which redispersed the particles due to electrostatic repulsions. The transesterification reactions in the buffered solution thus ensured reversibility to the system. Refueling the system allowed the initiation of this cycle again. Two additional consecutive cycles confirmed the transient switching between aggregated and disassembled states. Prins et al. reported a different strategy for the transient stabilization of vesicular structures based on a surfactant containing a cationic 1,4,7-triazacyclononane (TACN)-Zn(II) headgroup. The presence of ATP led to the formation of vesicular structures owing to the stabilizing interactions between ATP and the oppositely charged head groups. The introduction of apyrase, which can hydrolyze ATP into AMP + 2Pi, kept the system in the out-of-equilibrium state since the rate of vesicle formation triggered by ATP was more rapid than the consumption rate of ATP. The process of transient vesicle formation was coupled to a chemical reaction to create ATP-mediated temporal control of vesicular nanoreactors.

One of the most remarkable examples of complex, far from equilibrium, behavior emerging from self-assembled nanoparticle systems was achieved by subjecting polystyrene beads to ultrafast laser pulses. In this waste-free system, the colloidal assembly stable beads were manipulated to form different aggregate domains by spatiotemporal temperature gradients mediated by the laser pulses that induced Marangoni-type microfluidic flow. Brownian motion, on the contrary, moved the particles away from aggregation. Tens to thousands of unfunctionalized nanoparticles exhibited rich and complex behavior, including adaptation and self-healing responsiveness to the changing environment, depending on the perturbation strength, competition, and self-replication to make copies of the adjacent domain, and displayed self-regulation to persist their overall structure far from equilibrium.

All living matter requires a boundary to contain and protect the self from the environment. Therefore, compartmentalization can be considered as an indispensable element of life, and it has become an important tool for out-of-equilibrium systems. Polymersomes, which are bilayer vesicles made from amphiphilic block copolymers, are versatile nanocapsules with tunable properties such as permeability, flexibility, and size. These features make polymersomes ideal compartments for mimicking life-like architectures. Recently, we demonstrated the construction of self-adaptive nanoreactors, based on pH-responsive polymersomes consisting of a pH-responsive poly(2-diethyl amino ethyl methacrylate) (PDEAEMA) block. This system was based on an earlier developed “breathing” microgel system. During the formation of polymersomes, urease, which can tune the pH change, and HRP, which served as a model enzyme, were encapsulated. At high pH, the polymersomes shrank because of deprotonation of PDEAEMA, giving rise to impermeable membranes of the nanoreactors, and substrates were strongly hindered to penetrate the polymersomes, turning the nanoreactors in an “off” state. The introduction of chemical fuel (HCl and urea) enabled a rapid pH decrease, thereby resulting in a size increase of the polymersomes. Thus, the polymersomes became permeable and the substrate was able to pass through the membranes, allowing the “ON” state of the nanoreactors. Over time, a gradual increase in pH was observed due to the formation of ammonia via the action of urease on urea, and the nanoreactors were switched off again. Several nanoreactor “OFF→ON→OFF” cycles could be obtained upon repeated introduction of fuel, endowing the nanoreactors with cell-like “breathing” features.

More recently, Che et al. developed an ATP-mediated transient system using bowl-shaped polymer vesicles named stomatocytes (Figure 7). We demonstrated that, upon loading the stomatocytes with catalytic species in the cavity,
they were able to convert chemical energy into kinetic energy, thereby endowing the nanoparticles with motility. In our previous work, we showed that the motion of the stomatocytes can be steered by temperature or by fuel in low concentrations via one- or two-enzyme systems; however, motion did not show transient behavior in contrast to analogs found in nature. In this specific case, Pt-loaded PEG-b-PS stomatocytes were decorated with polyeisine chains (PLL), which can dynamically interact with ATP, noncovalently forming hydrophobic complexes on the stomatocyte surface; this allowed a decrease of the nanometer-sized opening of the stomatocyte cavity. As a result, the ATP-PLL interaction blocked substrate access. Potato apyrase, an enzyme that can hydrolyze ATP to AMP was added to the stomatocytes to remove ATP from the system, which led to the recovery of the original open state of the system. In this work, the stomatocyte nanomotors showed a regulated transient velocity profile, which demonstrates that adaptive self-assembled systems can be created with not only regulated structure, but also function.

4. CONCLUSION AND FUTURE PERSPECTIVES
Adaptivity is a natural phenomenon scientists are trying to incorporate in man-made molecular systems. This field of research has been traditionally the domain of systems chemists and synthetic cell scientists. The recent developments in this area are highly intriguing; self-regulated polymeric assemblies have been created with transient properties. Dissipative, biomimetic out-of-equilibrium architectures have been designed. What is also interesting to note is that the first steps have been made toward nanomaterials with functional adaptivity, instead of only structural regulation.

Although seemingly unrelated, the area of nanomedicine can benefit much from these developments in synthetic biology. The field has come to the realization that static nanocarrier systems are often not the most optimal solution for efficient drug delivery. Particles should be able to adapt to the environment, in order to interact solely with the target cells and tissues. This requires spatiotemporal control over specific features such as size and shape. In contrast to natural alternatives, synthetic polymers can be designed with adjustable and fine-tunable chemical and mechanical properties to control self-assembly, biodegradability and biocompatibility. The first adaptive polymeric particles have successfully been tested in a biological context, showing that there is indeed much to gain when features can be changed on demand. In almost all reported cases this involves an irreversible property change. Although this is already a major step forward when compared to the static counterparts, and much more work is still to be done, spatiotemporal control would be even more desired; particles that dynamically change their size, shape, and surface charge can more effectively overcome the different biological barriers nanomedicine faces; self-regulated nanoparticles that only will become activated when requested by the environment would increase drug efficacy or allow immunomodulation; integration with living cells, thereby tuning in to the cell’s homeostasis would be of great interest for nanoparticles applied in enzyme replacement strategies. These adaptive polymeric nanoparticles can more easily be realized by adopting the concepts developed in the field of out-of-equilibrium assembly. This interaction will, on the other hand, also provide this curiosity-driven research field with clear application potential.

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Notes
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