Review

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Dissipative Self-Assembly of Peptides

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Abstract: In molecular self-assembly, molecules interact with each other by non-covalent interactions to form larger structures. The process occurs in-equilibrium, which means that molecules can leave the assembly and reassemble elsewhere, but that occurs on average with equal rates. For self-assembly, peptides have proven to be a particularly useful building block, in part because of their versatility. Biology also uses self-assembly to create functional materials. For example, components of the cytoskeleton, like actin filament and microtubules, are self-assembled proteins. In other words, biology uses the same building blocks and design rules as supramolecular chemists to create functional structures. However, biological assemblies are vastly more complex than their synthetic counterparts. The discrepancy is in part because proteins are more complex than the peptides we use as building blocks. Another contributing factor to the difference in complexity is that most assemblies in living systems exist out of equilibrium. To use peptides for complex functions as biology does, we should understand and be able to create peptide assemblies out of equilibrium. In this work, we review recent advances towards the creation of peptide assemblies that exist out of equilibrium driven by an external energy source.

Keywords: Peptide · Fuel Driven · Dissipative Self-assembly · Dynamic Self-assembly · Self-assembly

1. Introduction

Molecular self-assembly refers to the spontaneous organization of molecules into larger structures driven by non-covalent interactions under equilibrium conditions.[1] Over the past decades, the field has gained significant attention, in part because of the use of self-assembly to create functional materials.[2] Particularly successful examples include the ubiquitous liquid crystals in LCDs or micelles in detergent. More recently, the use of peptides as building blocks for supramolecular materials has proven powerful in part because of their versatility. The library of amino acids can be combined in practically infinite ways to form peptides.

Biology also makes use of molecular self-assembly to create functional structures. For example, the components that comprise the cytoskeleton, including the actin filaments and the microtubules, are assemblies of proteins.[3] In other words, when it comes to the creation of functional structures, biology and supramolecular chemists use a similar approach and are governed by similar rules. However, biological assemblies are vastly more complex than synthetic ones. Biological assemblies, like the cell, can self-replicate, move autonomously and are regulated over space and time. The discrepancy in the level of complexity between synthetic and biological structures is in part because proteins are intrinsically more complex than the peptides we use as building blocks. Moreover, the cell comprises numerous different types of building blocks assembled in their respective structures. However, the higher complexity and the combination of multiple building blocks does not fully account for the complex behavior of the cell. In other words, even if we were to combine thousands of well-designed complex building blocks in equilibrium, we will not end up with a self-replicating, autonomously regulated sophisticated structure like the cell. Another major contributing difference between biological and synthetic assemblies is their thermodynamic state. While most synthetic assemblies exist in-equilibrium or close to equilibrium, living systems exist exclusively out of equilibrium.[4] Residing out of equilibrium is a requirement for life. Once equilibrium is reached, life is over. For supramolecular chemists to use peptides for complex functions as biology does, we should understand and be able to create peptide assemblies out of equilibrium.

In this review, we thus discuss advances towards the creation of peptide assemblies that exist out of equilibrium driven by an external energy source. We start with recent examples of peptide assemblies in equilibrium. Then we introduce the notion of dissipative non-equilibrium self-assembly after which we focus on the dissipative (dynamic) self-assembly of peptides.

2. Self-Assembly of Peptides

Peptides possess several properties that make them especially attractive as building blocks for supramolecular materials. For

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example, they offer an immense structural diversity due to the practically infinite possible combinations of amino acids that can be used to form a peptide sequence. Amino acids show different physicochemical properties and self-assembling propensity depending on the charge, hydrophobicity or size of their side chain, allowing the engineering of the desired assembly.[5] As a result, the morphologies that peptides can form upon assembly are diverse and include fibers,[6] vesicles,[7] nanobelts,[8] nanotubes[9] or colloids[10] among others.[11,12] Moreover, in recent years, design rules for the self-assembly of peptides have become available.[5] The wide range of assemblies from relatively simple building block demonstrates the power of peptides to design complex nanostructures from the bottom up. Peptides are synthetically accessible because of the well-established, robust and efficient solid phase peptide synthesis.[13] Moreover, peptides can be chemically modified by conjugation with aromatic groups or aliphatic tails which can further enhance their propensity for self-assembly. In this regard, fluorenyl protected peptides,[14–18] lipopetides[19,20] and peptide amphiphiles[21–24] have been extensively studied.

The wide range of morphologies combined with design rules has enabled supramolecular chemists to develop functional materials based on peptide assemblies. A particularly successful material is a fibrillar scaffold that helps the body to regenerate tissue after trauma or disease, *i.e.*, peptide-based materials for regenerative medicine.[25,26] Another successful peptide-based material is an organocatalyst that shows a vastly higher activity compared to its non-assembled building counterpart.[27–29] The ordered structure of the building block into the self-assembly is also of great interest for energy applications.[30]

3. Dissipative Self-Assembly

To create molecular assemblies from peptides or proteins, biology uses the same non-covalent interactions as supramolecular chemists. Somehow, biological structures are vastly more intricate and can perform drastically more sophisticated functions. The complexity of biological assemblies can, in part, be explained, by the higher complexity of the building blocks compared to synthetic ones. Moreover, biological structures like the cell, contain numerous different building blocks that can sort into their respective assemblies. However, the complex mixture of assemblies does not fully account for the complexity of the cell. Another crucial feature that explains the difference between natural and synthetic assemblies is that biological assemblies almost always exist out of equilibrium, while synthetic ones typically reside in equilibrium. In this section, we explain the difference between biological and synthetic assemblies from a thermodynamic point of view.

Biological examples, like the cytoskeletal actin fibers or microtubules, do not exist in equilibrium. Their self-assembled state represents a non-equilibrium state that is thermodynamically unfavored. Without externally applied energy these self-assembled filaments rapidly collapse into their non-assembled thermodynamically more favored state. Thus, to sustain the self-assembled state, the cell has to supply the assemblies with energy continuously. In the case of these cytoskeletal networks, the energy comes from a chemical reaction cycle that hydrolyzes ATP or GTP (adenosine triphosphate or guanosine triphosphate) and is coupled to the self-assembly process.[31,32]

In the reaction cycle, two chemical reactions regulate the assembly process (Figure 1).[33] First, an activation reaction activates a precursor molecule by reacting it with a molecule of chemical fuel. The reaction is irreversible and activates the precursor for self-assembly. The irreversibility implies that the fuel is "burnt" and cannot be reused. A second reaction spontaneously deactivates the activated building block and reverts it to the precursor state while releasing waste molecules. The net reaction is thus the irreversible conversion of fuel into waste. In the process of consuming fuel, the reaction cycle forms a transient building block for assembly. In the case of the microtubule network, the building block is the heterodimeric protein tubulin that reacts with GTP to form activated tubulin that can self-assemble. In the deactivation reaction, the GTP is hydrolyzed releasing guanosine diphosphate (GDP) and inorganic phosphate.



Job Boekhoven is a Rudolf Mößbauer assistant professor at the Institute for Advanced Study and Department of Chemistry of the Technical University of Munich. He received his M.Sc. degree in Chemistry in 2008 at the University of Groningen. In 2012, he received his Ph.D. for his work in the group of Prof. Jan van Esch and Prof. Rienk Eelkema at Delft University of Technology. He pursued his academic career as a Rubicon postdoctoral fellow in the group of Prof. Sam Stupp at Northwestern University from 2012 to 2015. Boekhoven's current work focusses on the development of supramolecular materials via non-equilibrium self-assembly.



Marta Tena-Solsona graduated in Chemistry in 2010 and obtained her master's degree in Pharmaceutical Chemistry in 2011 at Universitat Jaume I (Spain). In 2015, she got her Ph.D. at the same university supervised by Prof. Beatriu Escuder and Juan Felipe Miravet. In 2016, she joined Prof. Boekhoven's Lab at the Technical University of Munich where she is currently working as a Marie Skłodowska-Curie fellow. Her main interests focus on the development of synthetic dissipative materials driven far from equilibrium by chemical reaction networks.

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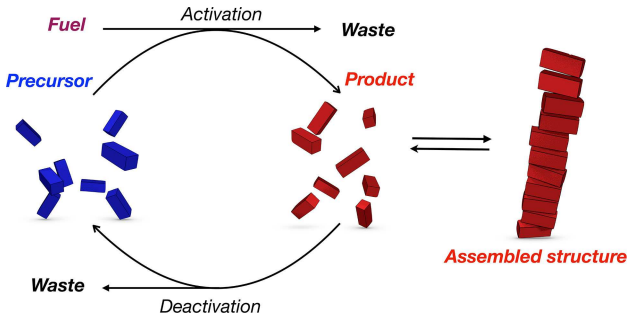

Figure 1. A general scheme for a chemical reaction cycle that drives dissipative self-assembly. The precursor (blue) is activated into the product (red) by the irreversible conversion of fuel into waste. Simultaneously, a deactivation reaction spontaneously deactivates the product into the initial precursor. Both activation and deactivation reactions follow different pathways. In its limited lifetime, the product can assemble in a dissipative assembly.

The consequence of using a reaction cycle to drive the assembly and disassembly of nanostructures is that chemical reactions regulate the morphology of the assemblies. That starkly contrasts the thermodynamic control of assemblies in equilibrium. For example, when a finite amount of fuel is added to the precursor, the reaction cycle will produce self-assembling building blocks for a finite amount of time. The consequence is that assemblies emerge, but also will decay when the cycle has run out of fuel. In other words, the lifetime of the assemblies and its associated material properties can be regulated by the kinetics of the reaction cycle.[34] Another unique material property of these dissipative molecular assemblies is that their building blocks are formed dynamically. The building blocks are constantly deactivated while new ones are formed. Consequently, the emerging materials can be self-healing. Externally applied damage can be repaired by the dynamically formed building blocks. Moreover, due to the continuous formation of the building blocks, the assemblies can undergo dynamic behavior if they exert minimal feedback on their reaction cycle. For example, if the assemblies enhance their formation and slow down their deactivation, exciting behavior like oscillations can spontaneously emerge.[35]

4. Dissipative Self-Assembly of Peptides

The field of synthetic molecular dissipative self-assembly is still young, yet different types of molecular structures have been explored including vesicles,[36] liquid crystals[37] and assemblies of nanoparticles.[38–41] Given the diversity of the assemblies that peptides can form, peptides and peptide-derivatives have been a successful class of molecules to serve as building blocks for dissipative self-assembly. Multiple examples have been reported over the past decade, which is the topic of this section. Some of the examples we mention are based on amino acid-derivatives but are strictly not peptides. We have decided to include those since they are generally governed by the same rules as those for the dissipative self-assembly of peptides. The peptides we discuss range from pure peptides, to peptides functionalized with hydrophobic groups that facilitate self-assembly like benzyl-, naphthyl-, fluorenyl- and perylene-based groups or aliphatic tails. We divided this section based on the peptide's functionalization. We first describe the dissipative assembly of peptides functionalized with aromatic groups, after which we describe peptides functionalized with aliphatic groups. We close the section with the dissipative self-assembly of unmodified peptide sequences.

4.1. Dissipative Self-Assembly of Peptide Sequences with Aromatic Groups

The first example of molecular dissipative self-assembly driven by a chemical reaction cycle was reported by Eelkema, van Esch, and coworkers.[42,43] The precursor in the reaction cycle comprises N,N'-dibenzoylcystine (DBC, Figure 2a). The carboxylic acids in the DBC precursor are crucial in the chemical reaction cycle that drives its dissipative self-assembly. The DBC was dissolved in pH-basic media such that the carboxylic acids were deprotonated. A methylating

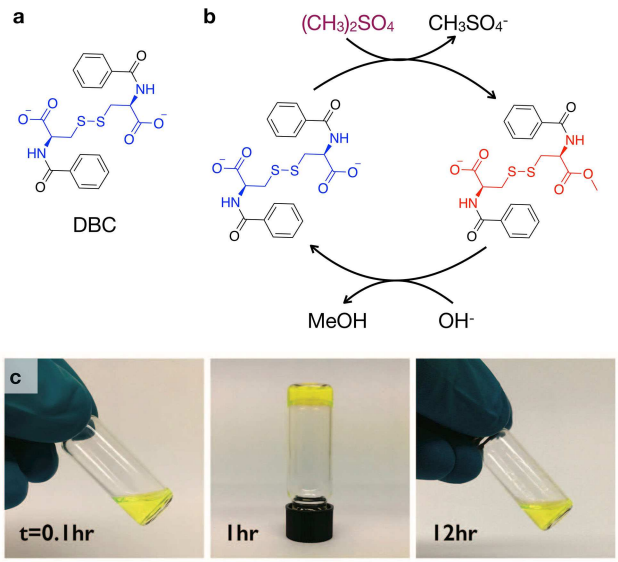

Figure 2. Dissipative self-assembly driven by the hydrolysis of methylating agents. **a)** The molecular structure of N,N'-dibenzoylcysteine (DBC). **b)** The chemical reaction cycle in which DBC is converted into its transient methyl ester driven by the hydrolysis of methylating agent dimethylsulfate. Simultaneously, the hydrolysis of the methyl ester in the aqueous media restores the initial dicarboxylate precursor. **c)** Photographs of the transient hydrogels formed by the methyl-ester of DBC, in the first minutes, 1 hour and 12 hours after addition of methylating agent as fuel. Adapted from ref. [43]. Copyright 2015, American Association for the Advancement of Science.

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agent was added as a chemical fuel which converted part of the carboxylates in their corresponding methyl-esters (Figure 2b). Due to the pH-basic media, the methyl ester hydrolyzed, which constituted the deactivation in the reaction cycle. In other words, the hydrolysis of methylating agents transiently formed the methyl-ester of DBC.

In its precursor state, DBC was well-soluble. In contrast, the transient methyl-ester of DBC was able to self-assemble into hydrogel-forming fibers (Figure 2c). The self-assembly was a result of hydrogen bonding and π-π interactions.[44] Taken together, when fuel was added to DBC, it formed the transient product that self-assembled into gels. Due to the transient nature of the product, the gels were also transient. When the system was running low on fuel, the hydrogels decayed and reverted to liquids. Moreover, their lifetime could be regulated by the amount of fuel added to the system and by the pH of the media. The authors also showed that the dissipative gels were self-healing early in the cycle. When the system was supplied with a batch of fuel, and the activation of the building blocks was outcompeting the deactivation, the hydrogels could be destroyed by a rheometer, and they would spontaneously reform and continue their cycle. In contrast, when the fuel was running low, and deactivation was faster than activation, the gels were not self-healing.

Naphtyl[45] and fluorenyl[34,46–48] protecting groups are commonly used in combination with short peptide sequences. The aromatic groups increase the hydrophobicity and ability to undergo aromatic interactions of the sequence thereby driving self-assembly. As an example, our group recently reported a family of short Fmoc-protected peptides and amino acids which were successfully applied as precursors in a chemical reaction cycle (Figure 3a).[34,46] The reaction cycle is driven by the hydrolysis of EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide). The precursors were designed such that the C-terminus of the peptides contained an unprotected aspartic or glutamic acid. Consequently, the precursor was endowed with two carboxylates in proximity, *i.e.*, the C-terminus and the carboxylate side group. The dicarboxylate precursor was well soluble in aqueous buffer at pH 6. Addition of the condensing agent EDC as fuel converted the dicarboxylate into its corresponding anhydride in the activation reaction (Figure 3b). Simultaneously, the anhydride product was hydrolyzed to the precursor, which constituted the deactivation. The half-life of the anhydride was in the range of half a minute. The short half-life did not hinder it from self-assembling. Due to the loss of the anionic carboxylates upon activation, the product assembled into fibers, spherulites or colloids depending on the molecular design. However, due to the short half-life of the anhydride, the assemblies were dynamic and only temporarily present. The kinetic control of the system allowed us to tune and predict the lifetime of the assemblies by changing the concentration of initial fuel added to the precursor. In these experiments, we found that the precursors could undergo subsequent fueling cycles without loss of function, and the material could thus be recycled. These experiments also demonstrated that the accumulation of waste, as a result of fuel hydrolysis, did not affect the self- assembly behavior drastically.

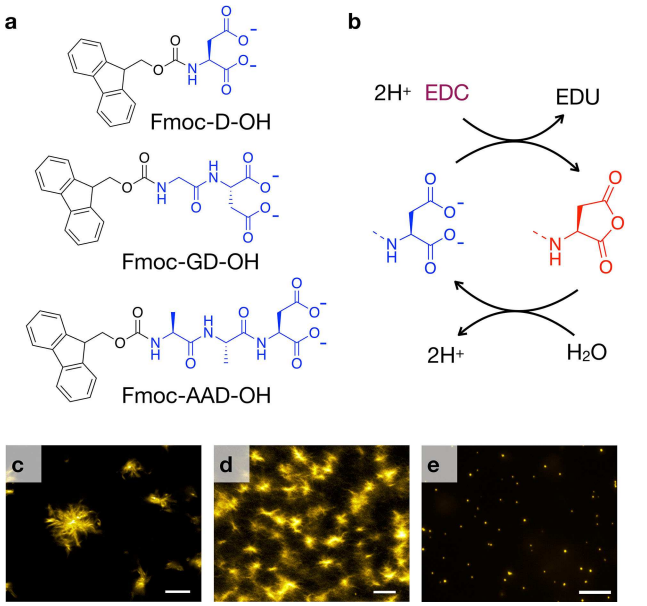

Figure 3. Dissipative self-assembly driven by the hydrolysis of carbodiimides. **a)** The molecular structures of the precursors Fmoc-D-OH, Fmoc-GD-OH and Fmoc-AAD-OH. **b)** The chemical reaction cycle that converts the precursors into an anhydride-based product driven by the consumption of condensing agent EDC. **c)** Fmoc-D assembles into spherulites driven by the hydrolysis of EDC as observed by confocal microscopy. **d)** Fmoc-AAD assembles into fibers driven by the hydrolysis of EDC resulting into temporary hydrogels. **e)** Fmoc-GD self-assembles into colloids driven by the hydrolysis of EDC. All scale bars correspond to 10 μm. Adapted from ref. 46. Copyright 2017, Nature Publishing Group and adapted from ref. [34]. Copyright 2018, The Royal Society of Chemistry.

Particularly exciting in the dicarboxylate-anhydride-based example was the versatility of reaction the cycle. The precursor only needed to fulfill two requirements. It needs to have an aspartic or glutamic acid C-terminus, and a non-nucleophilic rest of the sequence. Using these design-principles, we demonstrated the broad range of different assemblies. For example, we used Fmoc-protected aspartic acid (Fmoc-D-OH), which self-assembled into spherulites upon EDC activation (Figure 3c). The spherulites had a finite lifetime and disappeared when the system restored equilibrium. The size of the spherulites was in the range of micrometers and thus able to scatter light. The consequence was that a clear solution turned turbid upon application of fuel and regained its transparency over time. We exploited the transient turbidity to use the platform as a self-erasing paper by trapping the precursor and buffer in a polymer gel. Where we applied ink (fuel) on the gel, the spherulites appeared and scattered light. Over time, the spherulites disappeared and the message self-erased.

We used Fmoc-AAD-OH, where A stands for alanine and is known to drive β-sheet formation.[49,50] The addition of

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alanine in the molecular design indeed induced β-sheet formation and resulted in the formation of fibers upon activation with fuel. The fibers were transient and disappeared over time (Figure 3d). Finally, we used Fmoc-GD-OH where G stands for glycine. Lacking the directionality of the β-sheet forming amino acids, the activated product self-assembled into transient colloids (Figure 3e).[34] The colloids could release hydrophobic molecules in a time-dependent manner. Moreover, when the hydrophobic molecules were released could be determined by the amount of fuel added to the solution.

Hermans and coworkers reported the dissipative self-assembly of a more complex precursor design containing a perylene-based aromatic core.[51] The precursor in their work is based on a derivative of 3,4,9,10-perylene diimide (PDI) conjugated with peptides (Figure 4a). Perylenediimide groups are known to self-assemble in water driven by π-π stacking and the hydrophobic effect. The perylenediimide-based core was functionalized with peptide sequences on either side. The peptide sequence ALRRASL was chosen such that it was recognized by the protein kinase A (PKA). This kinase was able to phosphorylate the serine amino acid (S) in the chosen sequence. Triethylene glycol groups were also included to increase the solubility of the precursor and to decrease bundling of the fibers which could hamper accessibility of the enzymes.

In the activation reaction, the precursor was phosphorylated on the two serine residues by PKA consuming ATP as fuel (Figure 4b). In the deactivation, the protein phosphatase (λPP) dephosphorylated the product to the initial precursor state. The activation reaction changed the precursor's overall charge from +4 to charge neutral zwitterionic. The change in overall charge induced a change in the morphology of corresponding supramolecular polymer. While the precursor self-assembled into relatively short P-helical fibers, the activated state assembled in larger M-helical fibers (Figure 4c). The process reverted when the reaction cycle had run out of fuel.

The generation of phosphate as waste was found to interfere with the assembly process. For this reason, the authors developed a membrane reactor which was able to selectively exchange fuel and waste but leave the precursor, product, and enzyme in place. The use of this reactor allowed to extend considerably the life of the system which could run continuously and form dynamic steady states. Moreover, the authors were also able to tune the steady-state level by modulating the influx of ATP.

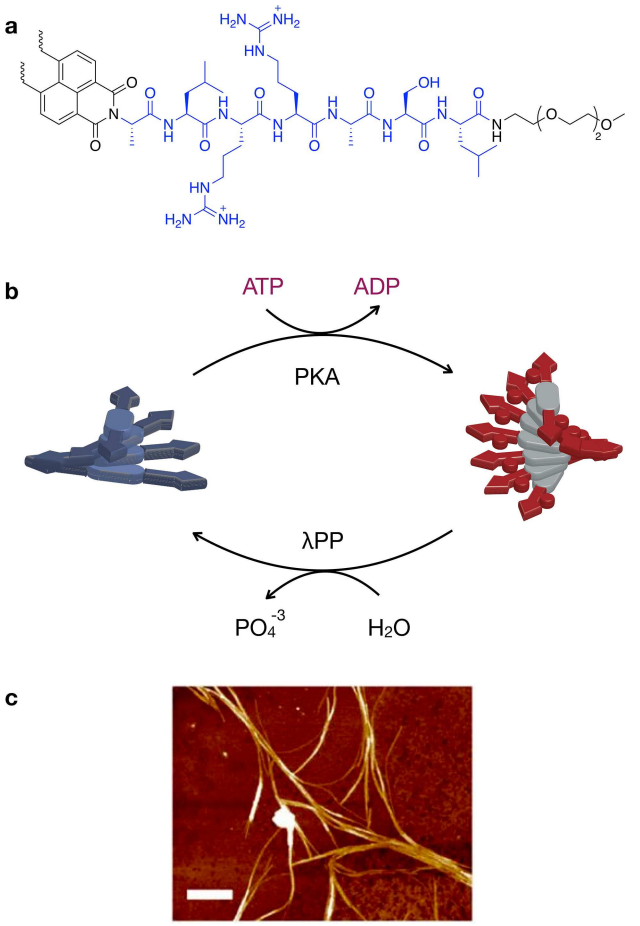

Figure 4. Dissipative self-assembly driven by the hydrolysis of ATP. **a)** The molecular structure of the precursor. The precursor contains two serine groups that can be phosphorylated. **b)** The chemical reaction cycle that drives the dissipative self-assembly. The precursor is phosphorylated by PKA at the expense of ATP. The change in overall charge induces a morphological transition from short P-helical fibers to larger M-helical fibers. Simultaneously, λPP dephosphorylates the serine residues which closes the chemical reaction cycle. **c)** Atomic force microscopy image of the phosphorylated PDI. Scale bar 500 nm. Adapted from ref. [51]. Copyright 2018, Nature Publishing Group.

4.2. Dissipative Self-Assembly of Amphiphilic Peptide Derivatives

Miravet and coworkers[52] reported an elegant example by coupling the self-assembly of peptides into hydrogels to the conversion of sugar into ethanol and CO_2 by yeast. The precursor in the reaction cycle is an N-succinylated derivative of the amino acid valine, isoleucine or phenylalanine (V, I or F, Figure 5a). On the C-terminus, the amino-acids are condensed with an aliphatic amine to yield amphiphilic peptide derivatives with a free carboxylate. The carboxylate renders the self-assembly of the peptides pH-responsive. Once the carboxylate is protonated, the building blocks self-assembled driven by hydrophobic interactions between the aliphatic tails. The precursor and sucrose were dissolved in water at pH 10. In the presence of yeast, the sucrose was converted into CO_2 and ethanol. As a consequence of the CO_2 production, the pH of the environment reduced to pH ~ 8 (Figure 5b). This pH-change was sufficient to protonate the carboxylate of the precursors and thereby induce gel formation (Figure 5c). The hydrogel was only temporary stable because of the loss of CO_2 into the atmosphere which reverted the pH

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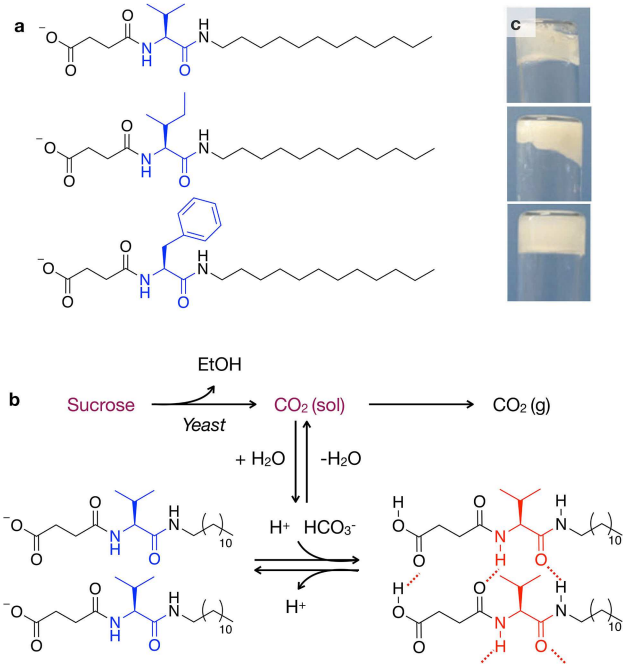

Figure 5. Dissipative self-assembly of peptide-based amphiphiles driven by the oxidation of sucrose. **a)** The molecular structure of the precursors. **b)** The chemical reactions involved in the dissipative self-assembly process converts sucrose into CO_2 and ethanol. The CO_2 temporarily decreases the pH of the solution which induces self-assembly. **c)** Self-supported hydrogels formed upon the addition of sucrose. From top to bottom, valine, isoleucine and phenylalanine derivative. Adapted from ref. [52]. Copyright 2016, The Royal Society of Chemistry.

to its original value. Further addition of sucrose allowed the gels to be reformed at least five times.

4.3. Dissipative Self-Assembly of Unmodified Peptide Derivatives

Ulijn and coworkers reported an example of transient hydrogel formation by the dissipative self-assembly of amino acids and peptides as building blocks.[53] The precursors in their work consisted of phenylalanine amide (NH_2–F–NH_2) or tyrosine amide (NH_2–Y–NH_2, Figure 6). The fuel to drive the assembly is based on the methyl ester of the dipeptide aspartic acid-phenylalanine (NH_2–DF–OMe).

In the presence of the enzyme chymotrypsin, the NH_2–DF–OMe (fuel) underwent a transacylation reaction with the amidated amino acid (precursor) to yield a tripeptide. The tripeptide rapidly assembled into hydrogels. Chymotrypsin also catalyzed the hydrolysis of the tripeptide, which constituted the deactivation. However, the deactivation of the product by hydrolysis took place at a lower rate compared to the activation, which was required to accumulate sufficient amounts of product for self-assembly. The deactivation yielded the original precursor and the free carboxylate as waste. Over

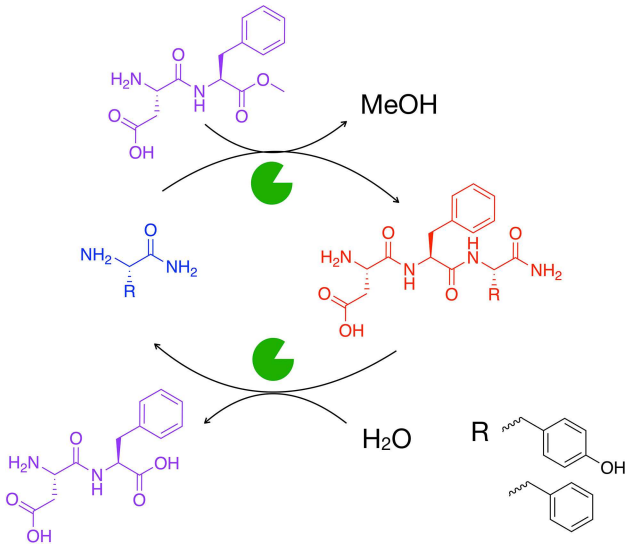

Figure 6. Dissipative self-assembly driven by the transacylation and the hydrolysis reactions in the presence of chymotrypsin. The fuel (purple) undergoes a transacylation reaction with the precursor (blue) in the presence of chymotrypsin (green). The activation forms a tripeptide which self-assembles into fibers. Simultaneously, the chymotrypsin catalyzes the hydrolysis of the tripeptide releasing the carboxylic dipeptide as waste and the amidated amino acid precursor. As a consequence of the hydrolysis, the gel collapses over time after. Adapted from ref. [53]. Copyright 2015, Wiley and Sons.

time the methyl ester fuel was consumed leading the collapse of the hydrogel.

The authors demonstrated the role of the non-covalent interactions in the formation of the temporary self-assembled structures. Depending on these interactions and their strength, the authors could control the kinetics and the lifetime of the transient nanostructures. For instance, the lifetime of the temporary hydrogels could be reduced from 24 hours to 4 hours when using tyrosine (Y) amino acid instead of phenylalanine (F). The difference was ascribed to the lower propensity of tyrosine to form assemblies in comparison with phenylalanine and the decrease in enzymatic hydrolysis activity in the presence of assemblies.

Walther and coworkers used an elegant method to create dissipative assemblies that make use of shifts in a pH value induced by a reaction cycle (Figure 7).[47,54] The authors reported a wide range of building blocks that could be coupled to the reaction cycle including peptides. Notably, the authors showed the formation of transient hydrogels by using NH_2-QQQ-F-Orn-W-Orn-F-Orn-QQ-OH as the precursor, in which Q stands for glutamine, Orn is the non-proteinogenic amino acid ornithine, F is phenylalanine and W means tryptophan.[54] The amines of the ornithine make the peptide's self-assembly pH-sensitive. The amine functional groups of the ornithine side chains are protonated at the initial acidic conditions, which makes the peptide well soluble. An increase of the pH of the media deprotonates the peptide and induces hydrogel

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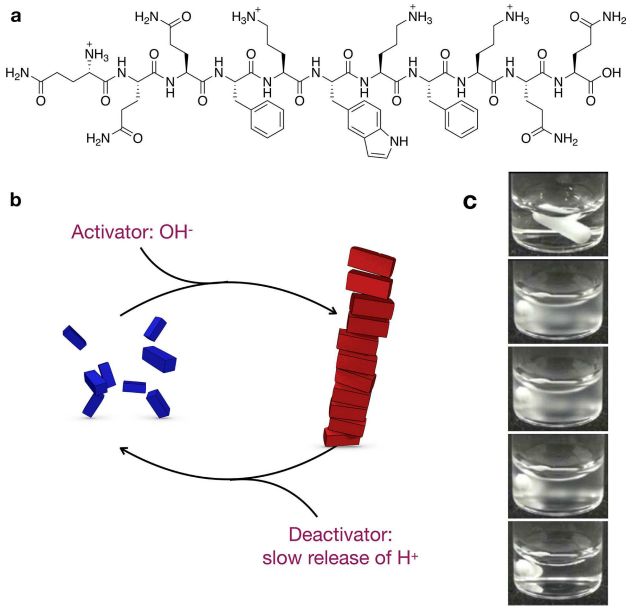

Figure 7. Dissipative self-assembly driven by the hydrolysis of esters. **a)** The molecular structure of the peptide used as the precursor. **b)** Schematic representation of the chemical reaction network. The activator, in this case, a basic buffer rapidly increases the pH deprotonating therefore the precursor. The presence of an ester in the solution which hydrolyzes into the corresponding acid bring back the pH value into acidic value protonating the amine moieties and disrupting the gel as a consequence. **c)** Time lapsed photographs of the transient hydrogel formation as a consequence of the pH change. From top to bottom: 0 seconds, 15 seconds, 5 minutes, 15 minutes, and 30 minutes. Adapted from ref. [54]. Copyright 2015, American Chemical Society.

formation. *Vice versa*, deprotonation of the peptide induces disassembly.

The authors coupled the pH-sensitive behavior of the peptide to a chemical reaction cycle that transiently shifts the pH of the media. The authors started the reaction cycle by the addition of buffer, which in this case acted as a fuel that increased the pH. However, the media contained an ester molecule that slowly hydrolyzed and brought the pH back to neutral. The lifetime of the hydrogels could be tuned from minutes to days by adjusting the reaction kinetics. As a result of their transient nature, these gels could be used to block microfluidics channels temporarily.[47]

5. Summary

In the past decades, the use of peptides to create materials has become a routine. Biology also uses peptides to create functional structures. However biological assemblies almost always reside out of equilibrium driven by energy dissipating reaction cycles. To create peptide-based assemblies with the complexity we observe in living systems, we should better understand and develop design rules for the dissipative self-assembly of peptides. Inspired by the beauty and complexity of molecular assemblies in living systems, scientists have started developing peptide-based systems that can self-assemble at the expense of energy. As we demonstrated in this review, with some exceptions, most of these peptides form fibers, and the corresponding materials are hydrogels. Researchers have studied some of the exciting properties that are a direct consequence of the dynamic nature of these assemblies, including the ability to be regulated over time, the self-healing nature of the assemblies and the dynamic formation and collapse. However, we have only just scratched the surface. We expect that fundamental studies on these materials will give valuable insights into the core principles that play a role in living systems or that have played at the origin of life. To that end, an exciting recent theoretical study predicted that when the formation of droplets was coupled to an energy dissipating chemical reaction cycle, the emerging droplets spontaneously self-divided.[55] Self-division is a hallmark of life but challenging to envision in protocells without the use of complex cell-machinery. Active droplets seem to be naturally endowed with this feat. From a translational point of view, we expect to see exciting developments towards materials for the field of healthcare. To that end, we have recently demonstrated the use of time-regulated nanoparticle assemblies to regulate the uptake of compounds by biological cells.[40] In the future, such materials can be used to regulate cell behavior with temporal control thereby the field of regenerative medicine.

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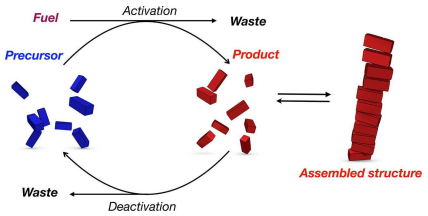

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Dissipative Self-Assembly of Peptides