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Systems chemistry

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Building on our ability to design and synthesise molecules and our understanding of the noncovalent interactions between these molecules, the chemical sciences are currently entering the new territory of Systems Chemistry. This young field aims to develop complex molecular systems showing emergent properties; *i.e.* properties that go beyond the sum of the characteristics of the individual constituents of the system. This review gives an impression of the state of the art of the field by showing a diverse number of recent highlights, including out-of-equilibrium self-assembly, chemically fuelled molecular motion, compartmentalised chemical networks and designed oscillators. Subsequently a number of current challenges related to the design of complex chemical systems are discussed, including those of creating concurrent formation–destruction systems, continuously maintaining chemical systems away from equilibrium, incorporating feedback loops and pushing replication chemistry away from equilibrium. Finally, the prospects for Systems Chemistry are discussed including the tantalizing vision of the *de novo* synthesis of life and the idea of self-synthesising and self-repairing chemical factories.

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Introduction

Chemistry has made huge strides in the past centuries and transformed our lives. The field has for a long time almost exclusively focussed on the challenges of forming covalent bonds (*i.e.* on synthesis) and of determining the structure of specific molecules. Emphasis has been firmly on making and

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studying pure compounds and much of the education we provide to our current chemistry students today still echoes this. The advent of supramolecular chemistry, now 50 years old,¹ marked the next step up in complexity in chemistry by focusing on interactions between molecules. Learning about the nature of these interactions and how they may be exploited to construct specific assemblies has paved the way to the now emerging field of Systems Chemistry.²

In Systems Chemistry, the focus is on emergent properties that derive from complex systems of interacting and/or inter-converting molecules. Nature already provides stunning examples of functions that can result from chemical complexity. Autonomous cell function and ultimately Life provide prime examples. Yet, there may be many more emergent properties and functions that can be called into being by exploiting the imagination and creativity natural to chemists.

At this moment Systems Chemistry is still in its infancy. We are yet to learn most of the design rules, master the underlying basic chemistry, and develop analytical tools in order to successfully design new emergent behaviour. In this review we will illustrate the state of the art and the breadth of the field by a number of rather diverse examples. We will then focus on a select set of challenges that the field currently faces and conclude with a view towards the still under defined but promising future of the field.

Illustrations of the state of the art

It is difficult to do justice to the breadth of the Systems Chemistry field in a relatively short review. Rather than aiming for a comprehensive discussion of the state of the art, we have chosen to provide an illustration of some of the current frontiers in the field, biased somewhat towards supramolecular systems.

A. Transient dissipative non-equilibrium self-assembly

In biology, many important self-assembly processes are powered by chemical fuels that keep living systems from death. Microtubules,

for example, use guanosine triphosphate (GTP) as fuel, whereas actin filaments use adenosine triphosphate (ATP) to control where, when, and for how long assembly takes place. Without a fresh influx of chemical fuels (provided, for example, by the mitochondria), the cell relaxes to the thermodynamic equilibrium.

At first glance it may appear wasteful to continuously dissipate energy to sustain a self-assembled structure, but such dissipation has some important consequences. Firstly, it enables decoupling the mechanical properties of the assembly from its dynamics. For microtubules, the dissociation rate of tubulin-GTP is almost 3000 times lower compared to its hydrolysed diphosphate analogue, tubulin-GDP.³ This means that a microtubule is robust when fuelled by GTP (with persistence lengths of up to 8 μm for a long microtubule),⁴ whereas it can quickly disassemble when GTP is converted to GDP. The result is a strong yet dynamic structure, a combination which is not easily achieved through other means!

Secondly, dissipative self-assembly using chemical fuels allows forming structures that are not determined by their thermodynamic stability, but rather by the rates of assembly and disassembly.⁵ Note that the relevant rates are not necessarily the inherent self-assembly rates (*i.e.*, the on/off rates in absence of the chemical fuel) but rather the rates of reaction (or non-covalent binding) of the chemical fuel with the assembling building block. Furthermore, optical driving to achieve dissipative non-equilibrium assembly is notably different from chemically fuelled systems (see discussion by Astumian).⁵

In recent years, several synthetic chemically fuelled systems have been reported. Van Esch and co-workers presented a general strategy to obtain transient hydrogels with tuneable lifetimes. The first step is fuelled methylation of carboxylic acid(s) **1a**, **2a**, or **3a** into methyl ester **1b**, **2b**, or **3b** (Fig. 1), which have reduced electrostatic repulsion, and can self-assemble into bundled fibres.⁶ Over time, however, the esters spontaneously hydrolyse back to the corresponding acids, leading to fibre disassembly (and release of waste product CH_3OH). Using confocal microscopy, fast collapsing fibres were observed, similar to catastrophic collapse

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the design and optimisation of autocatalytic reaction networks in non-equilibrium materials, devices or sensors.

Fig. 1 (A) Chemical structures of gelators. (B) Self-assembly to form transient hydrogels (red fibres) using dimethyl sulfate (DMS) as chemical fuel. Waste products are CH₃OH and CH₃SO₄⁻. Reproduced from ref. 6 with permission from the American association for the advancement of science, copyright 2015.

observed for microtubules. A clear link between the mechanical gel stiffness, and the amount/addition of chemical fuel was found, which further indicates that fuelled self-assembly is also of interest in synthetic systems, and not just in living beings.

Prins and co-workers have taken a different approach, where the chemical fuel ATP binds non-covalently to three building blocks simultaneously, and in this way, induces self-assembly.⁷ For surfactant molecule **C**₁₆TACN·Zn²⁺ binding of ATP leads to vesicle formation (Fig. 2).^{7a} An enzyme (potato apyrase) slowly breaks down ATP to ADP and AMP, both of which have a lower binding affinity to the building blocks. The result is that the assembled vesicles ("self-assembled state", Fig. 2) are only formed transiently. Next, the authors used the vesicle bilayer to carry out a chemical (substitution) reaction, which, just as the vesicles themselves, is transient in nature. In this way, timed chemical reactivity was achieved.

So far, however, all the supramolecular systems above are in transient dissipative states, through batch-wise supply of fuel, whereas natural systems are in sustained non-equilibrium steady-states through a continuous supply of fuel. Some important factors to achieve conditions allowing non-equilibrium steady-states are outlined below, in the section on current challenges.

B. Chemically fuelled molecular motion

The control of motion is another important aspect of biology⁸ and chemistry, highlighted also by the award of the 2016 Nobel Prize in chemistry for the development of molecular machines.⁹ While in biology motion is in many cases chemically fuelled (harnessing energy deriving from ATP hydrolysis or chemical gradients), the development of synthetic systems operating by the same principle has been achieved only recently.

Leigh reported the unidirectional motion of a ring (shown in blue) on a cyclic track (black with green stations; diameter of

Fig. 2 Transient vesicle formation using ATP as chemical fuel. Multivalent interactions between the building block **C**₁₆TACN·Zn²⁺ and ATP enable vesicles to form. Enzymatic breakdown of ATP leads to vesicle disassembly during a decay phase. Waste products are ADP, AMP, and phosphate ions (P_i). Reproduced from ref. 7a with permission from the Nature publishing group, copyright 2016.

about 2 nm) fuelled by Fmoc chloride (Fig. 3).¹⁰ The ring occupies one of the two stations on the track. Hopping of the ring between the stations, driven by Brownian motion, requires the cleavage of (one of) the bulky Fmoc groups (red) on the track. This cleavage (mediated by Et₃N) occurs with a rate that is independent of the position of the ring. At the same time, the Fmoc groups are re-attached through the continuous addition of Fmoc chloride to the reaction mixture. Unlike cleavage, re-attachment is dependent on the position of the blue ring and occurs faster when the blue ring is further away from the site of reaction. Thus, starting from the top in Fig. 3, the ring can initially move clockwise or anti-clockwise with equal probability, but re-attachment of the Fmoc group is faster on the anticlockwise site. The design of the track is such that the site anticlockwise to the station that the ring occupies is always more remote than the clockwise site, resulting in an overall sustained clockwise motion of the ring. Thus, the system relies on an information ratchet mechanism, in which the position of the blue ring dictates the rate of re-attachment of blocking groups.

The ring keeps moving in an overall clockwise direction as long as the Fmoc chloride fuel is provided. Importantly, the

Fig. 3 Unidirectional movement of a ring on a track fuelled by Fmoc chloride. Overall movement is clockwise as the re-attachment of the Fmoc blocking groups is faster (less hindered) on the site anticlockwise (most remote) to the station that the ring occupies. Modified from ref. 10 with permission from the Nature publishing group, copyright 2016.

removal and attachment of the Fmoc groups is not an equilibrium process, both steps are distinct and irreversible reactions.

At the same time, Feringa reported a chemically fuelled molecular motor.¹¹ The system shown in Fig. 4 makes clever use of the fact that Pd can selectively react with C–H or C–Br bonds depending on the oxidation state of the metal. The position of the Pd centre is dictated by the chiral sulfur centre. The bond to Pd enables the otherwise hindered rotation of the upper benzene ring. Unidirectional rotation is achieved by a series of independent stepwise reactions (requiring a change in reaction conditions for the various steps): (i) introduction of Pd by C–H activation; (ii) reintroduction of the C–H bond; (iii) introduction of Pd by oxidative addition and (iv) reintroduction of the C–Br bond.

Also in this system, it is essential that the introduction and removal of Pd is not an equilibrium process but occurs through irreversible chemically fuelled steps.[†] And also here it is essential

[†] In principle also reversible reactions can be used for constructing multistep molecular machines, but then conditions must be changed to make the individual steps happen in a specific direction.

Fig. 4 Unidirectional rotation enabled sequential introduction and removal of Pd(II) and Pd(0).¹¹

that an information ratchet mechanism⁵ is implemented (this time by the experimenter choosing which chemical step to perform at which point in the cycle).

Where these systems show motion of specific molecular subcomponents with respect to each other, chemically fuelled macroscopic motion has also been achieved by repetitive swelling and deswelling of a gel, fuelled by the Belousov-Zhabotinsky oscillating reaction.¹²

C. Compartmentalised chemical networks

One of the grand challenges of Systems Chemistry, namely producing synthetic life, might be fulfilled through the design of a collection of molecules, a ‘network’, that is simple enough to self-organize, yet sufficiently complex to accommodate the essential properties of a living organism: compartmentalisation, replication and metabolism, all maintained out-of-equilibrium. Attempts at ‘stripping-down’ bacterial cells to their minimum components suggest that hundreds of genes and many more different proteins and other molecules are needed to maintain proper function. Complementary to this top-down approach, minimal life may also be approached bottom-up, starting from isolated components contained within compartments.¹³ A fascinating idea was put forward, suggesting that a simple synthetic cell model, affording several life-like functions, should include replicator molecules, and potentially other catalysts, operating inside a replicating membrane vesicle (Fig. 5).¹⁴ The replicators and membrane vesicles can be spontaneously formed by self-assembly of simple molecules, and encapsulation of the replicators within the compartment is easily achieved. Co-localisation of the chemical network components is expected to speed up reactions, while partial separation from the environment outside the barrier affords selective availability of reactants for different network pathways. Furthermore, energy and material transport allow for out-of-equilibrium growth of the replicating molecule and vesicles, as well as providing mutation and/or competition landscapes for further Darwinian evolution.

After studying separate synthetic systems presenting minimal self-replication or vesicle growth and division, scientists have recently turned their focus to systems that can combine both

Fig. 5 Proposed pathway for the synthesis of a cell.¹⁴ Independently developed (RNA) replicators and self-replicating vesicles, are combined into a single network, enabling rapid evolutionary optimization of the system.

activities, aiming to develop new features resulting from the encapsulation, in terms of reactivity and selectivity towards the best fit molecule.

A recent illustration is provided by Adamala and Szostak,¹⁵ who studied a system that consists of fatty-acid vesicles containing a simple dipeptide catalyst, which catalyses the formation of a second dipeptide. The product dipeptide binds to the vesicle membranes, which imparts enhanced affinity for fatty acids and thus promotes vesicle growth. The catalysed dipeptide synthesis proceeded with higher efficiency in vesicles than in free solution, which further enhanced fitness. Their observations suggest that in a replicating protocell with an appropriate (RNA) replicator, ribozyme-catalysed peptide synthesis might have been sufficient to facilitate Darwinian-like evolution. A related replicating vesicle system, also studied by Szostak *et al.*, enabled a primitive homeostatic behaviour, demonstrating the maintenance of constant (ribozyme) catalyst activity during protocell volume change due to growth and division.¹⁶ At high concentrations of ribozyme and specific or random short oligonucleotide binders, catalysis was inhibited. However, dilution activated the ribozyme *via* inhibitor dissociation, thus maintaining near-constant ribozyme specific activity throughout protocell growth. These results suggest that a simple mechanism of short oligonucleotides reversibly inhibiting functional RNAs, could be applied, instead of sophisticated modern regulatory mechanisms.

Szathmáry, Griffiths and co-workers have very recently highlighted a different aspect associated with compartmentalisation of replicating networks. Through theory, simulation and experiments they have shown that repeated cycles of mixing and transient compartmentalisation (inside droplets, Fig. 6), accompanied by selecting the compartments based on the activity of the contained ribozymes, is sufficient to maintain

Fig. 6 Selection of RNA molecules compartmentalised in aqueous droplets in an inert carrier oil. Functional RNA molecules replicate in the droplets, and catalyse a fluorogenic reaction. Droplets containing active ribozymes were sorted, and the RNA from the sorted droplets is pooled before the next round of selection. The protocol enables chemical evolution and prevents takeover by parasitic mutants. Reproduced from ref. 17 with permission from the American association for the advancement of science, copyright 2016.

functional replicators.¹⁷ This scheme provides a simple conceptual solution to allow the evolution of molecular complexity even before the appearance/construction of the first ‘formal’ protocells.

D. Designed oscillators

Rhythms are ubiquitous in biology and a common emergent feature of a network of coupled chemical reactions. The discovery of oscillations in an inorganic analogue of the Krebs cycle, the Belousov–Zhabotinsky reaction, and the subsequent elucidation of its mechanism, was soon followed by an extraordinary series of papers from Epstein and collaborators on “the systematic design of chemical oscillators”.¹⁸ The design strategy required selection of a reaction that produces its own catalyst, an autocatalyst or activator (positive feedback), and an inhibitor species that removed the activator (negative feedback). The reactions were performed in a flow reactor to maintain the system far from equilibrium and the dynamical behaviour mapped out in a cross-shaped phase diagram (Fig. 7a). These activator–inhibitor networks (Fig. 7b) typically displayed four different types of behaviour in flow: high or low activator steady states; co-existence of a low and high activator state *i.e.* a bistable switch, where the concentration of activator depended on whether the flow rate was increased or decreased (memory) and oscillations. Experimentally the feedback was almost entirely based around inorganic redox processes with halogen- or sulfur-based oxo-acids.

A new generation of chemical oscillators is now appearing involving large biological molecules such as enzymes and DNA.¹⁹ Feedback is prevalent in enzyme catalysed reactions in biology but commonly involves allosteric regulation rather than direct autocatalysis. In the system introduced recently by

Fig. 7 (a) Sketch of a continuous stirred flow reactor (CSTR) used to maintain activator–inhibitor reactions far-from-equilibrium and typical cross-shaped phase diagram indicating values of flow rate and inhibitor concentration for which oscillations, bistability and steady states in activator are observed. (b) General representation of an activator–inhibitor network in which the activator catalyses its own production and the production of inhibitor subsequently suppresses activation. (c) Simplified activator–inhibitor network showing the key species in the trypsin enzyme oscillator in ref. 19a. (d) Simplified network representation of the thiol–maleimide oscillator in ref. 20. Figure (c) and (d) were adapted from ref. 19a and 20 respectively, with permission from Macmillan Publishers, copyright 2015 and 2016, respectively.

Semenov *et al.* the enzyme trypsin was itself produced autocatalytically from trypsinogen.^{19a} The negative feedback was implemented through a series of reactions activated by trypsin that led to production of an inhibitor (Fig. 7c). The activator–inhibitor network topology is not sufficient for oscillations: the negative feedback must be delayed with respect to the positive feedback. In contrast to earlier work, the authors chose small molecular inhibitors that were amenable to chemical modification, thus allowing them to control the necessary delay between activation and inhibition through synthesis of suitable precursors. In addition, the authors employed a microfluidic reactor to follow the reaction reliably over the course of many hours with a high degree of control at low flow rates; a feat not possible with the large continuous stirred tank reactors of the 1980s.

A designed oscillator based on autocatalysis of simple organic molecules had not been reported until very recently.²⁰ In this system, cysteamine was produced autocatalytically from a thioester and dialkyl disulfide substrate (Fig. 7d). The small organic thiol was removed through two separate processes: one that delays onset of autocatalysis (reaction of thiol with maleimide) and one that becomes important at high concentrations of thiol (with acrylamide). This network shares in essence the activator–inhibitor topology of its inorganic predecessors and it also displays a cross-shaped phase diagram in a flow reactor; as the inhibitor inflow concentration was increased, so oscillations in thiol were obtained. But the chemistry involved is more relevant to living

systems. Metabolic oscillations that occur in starving yeast cells and slime mould are driven by enzymatic processes. This new system introduces the possibility of complex information processing with biologically relevant molecules, without the need for enzymes.

Current challenges in systems chemistry

As the examples above illustrate, most functional potential and most novelty is found in chemical systems that are out of equilibrium. One can distinguish between different types of non-equilibrium systems, including kinetically trapped systems that reside in a metastable state, or systems that are in transition from a higher to a lower chemical potential. The most interesting systems, however, are those in continuously dissipative non-equilibrium steady-states. Such states are able to do work and show behaviour that, in isolation, would defy the second law of thermodynamics. But, as these systems are not isolated but linked to an energy input, behaviour such as unidirectional movement, population of thermodynamically disfavoured assemblies and oscillations, discussed in the previous section, are made possible. The first requirement for such continuously dissipative systems is the presence of concurrent processes of formation/addition and destruction/removal of molecules and/or molecular assemblies. Such chemistry is underdeveloped. Once feedback is coupled to a formation/destruction system exciting functional behaviour may emerge. Examples of functions could be centrosome centering²¹—the process by which growing microtubules push onto the cell walls to find the geometric centre of the cell—using fuelled artificial supramolecular polymers, or self-replicating systems showing bistability. Below we will first discuss the challenges with implementing formation/destruction systems and on how to maintain non-equilibrium steady-states. We will then focus on engineering feedback systems and replication networks.

A. Formation–destruction chemistry

The simplest way of building dissipative systems is by employing flow reactors (see below). In such systems, a high-energy fuel may be continuously added while the “destruction” occurs in the form of physical removal of some of the reaction mixture (as employed in the oscillating system shown in Fig. 7c). However, systems in which bond formation and breakage occur concurrently are often more elegant and versatile. In the above section on examples illustrating the state of the art, we already encountered several systems where function relied on the continuous formation and breaking of the same chemical bonds (attachment and cleavage of Fmoc blocking groups in Fig. 3 and formation and cleavage of bonds to Pd in Fig. 4). Note that it is essential that the formation and destruction paths are not each other’s microscopic reverse, as they are in a simple equilibrium process, because the formation and/or destruction reaction needs to be coupled to another chemical process which converts a high-energy reactant (the fuel) to a

same time. Systems in which synthesis and cleavage occur at different conditions (such as for the system in Fig. 4) may appear somewhat less attractive as they need constantly changing conditions that often require continuous external interference. However, they may still be attractive, for example when changing conditions are generated by coupling to an oscillator internal to the system, or when using a systems design in which the non-compatible conditions for formation and destruction are spatially separated.

B. Maintaining dissipative non-equilibrium steady states

Above we reviewed a number of chemically fuelled systems with the exciting prospect of making microtubule-like synthetic materials, or continuously operating molecular motors. In biological processes, often the cleavage of the “high-energy (phosphate–phosphate) bond” of ATP (or GTP) is said to be utilized to fuel dissipative processes. However, such statement is an oversimplification.²⁷ ATP hydrolysis can only drive a process or perform work when the concentrations of its waste products—that is, ADP (adenosine diphosphate) and Pi (inorganic phosphate)—are away from their equilibrium ATP/ADP ratio (10^{-7}). In the cytosol, the cell manages to keep the latter ratio 10^{10} times in favour of ATP, leading to a significant driving force (-57 kJ mol^{-1}) of fuelled processes. To maintain this force, however, ADP and Pi need to be removed continuously. In other words, the addition of fuel and removal of waste are both of importance for obtaining non-equilibrium steady states. In the field of non-linear dynamics such steady states are often sustained in a semi-batch or continuous stirred tank reactor (CSTR, *cf.* Fig. 7a).²⁸

In recent examples of self-assembling systems driven by chemical fuels, the semi-batch approach has been used, leading to transient optical, materials, or catalytic properties.^{6,7,26,29} The result of working in semi-batch conditions is that the system experiences “fatigue”, leading to a damped response upon addition of more chemical fuel.

For example, Nitschke and co-workers developed a copper based metallosupramolecular system fuelled by triphenylphosphine (Fig. 8A).^{29b} Their system could be refuelled up to six times. Prins and co-workers⁷ used ATP as a trivalent chelator to drive self-assembly of nanoparticles or vesicles (Fig. 2). The waste product in their case is divalent ADP and monovalent AMP (adenosine monophosphate), which can act as competitive binders, and thus can disrupt the self-assembly when performing multiple transient self-assembly cycles (7 times refuelled, Fig. 8B). George and co-workers²⁶ used direct addition of ATP (or addition of phosphocreatine as a chemical fuel to produce ATP) that acts as a multivalent binder to induce transient helicity in assembling naphthalene diimides, which could be refuelled with a damped response (see last entry in Table 1 and Fig. 8C).

There is still quite a lot to be learned from traditional (non-assembling) oscillators studied in CSTRs, or from the cell where transport of ATP, ADP, and Pi across the mitochondrial membrane keeps the system in a non-equilibrium steady state. The recent advent of microscale CSTRs (*cf.* Fig. 7) could help achieving better control over dissipative non-equilibrium

	Ref.
<p>Photochemical reactions</p> <p> </p> <p>23</p> <p> </p> <p>24</p>	
<p>Thermal reactions</p> <p> </p> <p>6 and 25</p> <p> </p> <p>10</p>	
<p>Enzymatic reactions</p> <p> </p> <p>26</p>

Fig. 8 Examples of fatigue in (transient) dissipative self-assembled systems. (A) Aliquots of triphenylphosphine are added. (B) Shots of ATP to refuel twice. (C) Additions of ATP lead to a damped amplitude in the system's response. Partially adapted from ref. 29b, 7a and 26, respectively. Asterisks indicate when an aliquot of chemical fuel is added. Fig. 8C was reproduced from ref. 26 with permission from Wiley-VCH, copyright 2017.

self-assembly (especially considering the cost of enzymes in normal size CSTRs). The behaviour of supramolecular self-assembly might be quite different under conditions of continuous fuelling, as compared to the current (semi-)batch-fuelled experiments. That is, in the current systems fuel is added in separate shots to a stirred reactor, but more interesting behaviour is expected when the chemical fuel is added using a constant inflow.

C. Feedback systems

Feedback plays a vital role in the spatial and temporal control of chemical signals in living systems. There has been much speculation as to how feedback might be exploited in applications, particularly when the output of the chemical process is coupled to material formation or mechanical motion; then reaction networks could be used to precisely control a physical process in time and space.³⁰ Expansion of the repertoire of chemical feedback processes is required in order to fully exploit these opportunities.

Autocatalysis has been obtained serendipitously with a wide range of small inorganic molecules such as hypobromous acid or iodide ion.²⁸ The substrate is completely converted to autocatalyst, albeit in multiple steps (and in some cases followed by degradation of autocatalyst). In a closed batch reactor the positive feedback process is characterised by exponential growth with an abrupt end point as a result of substrate depletion. Under open conditions, these systems exhibit sharp switches in state in response to substrate concentration. However, these well-known

clock reactions tend to involve harsh inorganic oxidising agents that limit their application.

In some reactions, the positive feedback is much weaker as the autocatalytic species is produced in a reversible step and alternative pathways become viable as the reaction proceeds (such as glycoaldehyde from tetrose in the formose reaction³¹). The sharp switch is not obtained in this case. The efficiency of autocatalysis *via* dimer formation can also be limited by a high binding constant. This appears to be a feature of some systems involving larger, organic autocatalysts.³² One particular area of interest is the development of asymmetric autocatalysis, both as a means to explain the origin of chirality in biology and also as a strategy for the production of enantiopure compounds.³³ Amplification of the pyrimidyl alcohol in the Soai reaction is the prototypical example and mechanistic analysis suggests that this reaction proceeds *via* dimers which are themselves are catalysts. It is difficult to achieve such effects by design.

Strategies producing H^+ or OH^- autocatalytically have been more successful as reactions involving acid (base) catalysed acid (base) production are common: for example, ester or amide hydrolysis and dehydration of carbohydrates.³⁴ Another approach uses the bell-shaped rate-pH curve of enzymes coupled with acid/base production.^{34b} The urease catalysed decomposition of urea has a maximum rate at pH 7; if the initial pH is low, rate acceleration is obtained as base is produced raising the pH. This reaction can be tuned to display a spatially propagating or temporal switch from acid to base. Control of the pH using in-built reaction networks creates some exciting possibilities for applications when coupled with acid/base catalysed self-assembly or polymerisation processes.³⁵

Fewer synthetic systems have been developed involving purely negative feedback. Recently one such example was demonstrated with a coordination-coupled deprotonation of a hydrazone switch.³⁶ Feedback was obtained in response to zinc(II): above a threshold, a cascade of reactions was initiated leading to production of an imine that sequestered zinc(II) and switched the cascade of reactions off. Hence the imine inhibited its own production. This approach may result in a new design strategy for negative feedback loops.

There are limited reports of oscillators with organic or biological molecular feedback. Ironically, the design methodology often involves breaking the system into sub-components, testing each of the modules separately, then combining them in the hope of finding emergent behaviour. However, these systems are far from plug-and-play. Once all the components are present it is not possible to independently vary the rates of individual reaction steps nor eliminate unwanted side reactions. A combinatorial strategy could be employed to a greater extent, with many conditions and potential inhibitors screened simultaneously. This will improve the success rates in finding optimal conditions for certain dynamical behaviour.

Oscillations with biocompatible molecules could be used to perform useful functions such as periodic drug delivery in living systems. The reaction networks may be tuned for desired behaviours such as temperature compensation – when rhythms are constant in a changing environment. Designed “clocks”

have the potential to out-perform their biological counterparts, mimicking, for example, pancreatic cells in the pulsed delivery of insulin but with a more robust mechanism, or generating a circadian rhythm when the host cells have failed. However, the challenge here is to produce systems that oscillate robustly, over a wide range of parameters and in the presence of many interfering chemical agents, if these oscillators are to find applications.

D. Replication networks

Self-replicating systems are among the most investigated systems showing positive feedback (autocatalysis). Self-replicating systems also address grand challenges in Systems Chemistry, including the origin of life and the *de novo* synthesis of life.

Several highlights have been realized using synthetic replication networks of different characteristics – originally by exploiting networks under kinetic control,³⁷ and more recently with continuously dynamic mixtures³⁸ incorporating the (auto)catalytic processes, where the emergence of replicators was guided by a sometimes complex interplay between replication efficiency and the thermodynamic stability of the replicators.³⁹ Small replication networks can be readily developed from bio-organic molecules,⁴⁰ as well as from purely synthetic organic compounds.⁴¹ The general scheme through which self-replication takes place is shown in Fig. 9a.

Replication networks can display various motifs, such as cross-catalytic replication, hubs, and sub-network modularity. Furthermore, they are adaptive and respond to chemical and physical triggers⁴² and allow for the emergence of functional, kinetically-trapped and energy-dissipating architectures. Since the design and construction parameters are increasingly well-understood for several replicating systems, current challenges involve developing more complex functions, with potentially transformative implications for adjacent fields such as material science, evolutionary biology and biomedicine. We mention below several different directions that may benefit from replication chemistry and construction of small networks.

Design of self-synthesizing materials. The preparation of molecular assemblies that can promote their own formation by accelerating the synthesis of building blocks and/or assembly of their super-structures was recently realized^{38c,43} and the replicating assemblies are called 'self-synthesizing materials'. At present these materials do not feature any functionality beyond the ability to replicate. The design of functional self-synthesising material might be achieved through the incorporation of relevant groups into the replicating molecules affording activity that is orthogonal in nature to the replication chemistry. Most appealing functions would be those relevant to the development of artificial cells, such as supramolecular catalysis and electron transfer.

Design and analysis of replication networks far from chemical equilibrium. The gradual increase in network complexity from one studied model to the next may facilitate the emergence of function beyond replication and may pave the way to early chemical evolution and *de novo* life. However, in contrast to recent progress in developing supramolecular assemblies out of equilibrium, replication systems have mostly been studied at equilibrium or en-route to an equilibrium state.

(a) Minimal self-replication cycle

(b) Bistability in peptide replication

(c) Spontaneous replicator diversification

Fig. 9 (a) Typical autocatalytic cycle applicable to most self-replication reactions and emergent properties resulting from peptide self-replication: (b) bistable behavior observed for thiodipeptides far from equilibrium. (c) Spontaneous diversification in self-assembly driven self-replication. Oxidation of a mixture of two dithiol building blocks functionalized with two different peptides results in a mixture of disulfide macrocycles from which a first set of self-replicating hexameric rings emerges. This first set of mutants cross-catalyses the formation of a second set of hexamer rings with different building blocks composition, suggesting an ancestral relationship between the two sets of mutants.

Such systems are naturally easier to produce experimentally and investigate mathematically or computationally, but they miss a critical physical phenomenon that keeps cells alive. It is expected that in the near future emphasis will shift towards synthetic replication networks operating far-from-equilibrium, potentially running in open flow reactors. Such conditions enable Darwinian evolution and fuelled replication will enable complexification and concomitant emergence of new functions.

One recent example of an emergent property observed in a replicator network is bistability, where the stationary state concentration of a replicator was found to depend critically on its history (Fig. 9b; see also section iv and Fig. 7a for a general description of bistability).⁴⁴ Another interesting observation is the propagation of reaction-diffusion fronts of a replicator,⁴⁵ which raises the prospect of studying spatially resolved competition scenarios. Such behaviour may be further exploited for down-stream signalling and enabling more complex cascade processes in more advanced systems of replicators.

Open-ended evolution with synthetic replicators. Synthetic replicators show information transfer; *i.e.* replicators dictate the structure and functions of a newly-formed 'daughter' molecules. However, Darwinian evolution and selection for new, substantially more efficient replicators remains highly challenging. Recent research in this direction involved the design of self-sustained replication using an RNA enzyme⁴⁶ and cooperating sets thereof,^{40c} and the spontaneous diversification of sets of mutants using replicating peptides (Fig. 9c).⁴⁷ A promising direction for further studies includes the incorporation of chemical systems amenable to spontaneous mutation and the development of *in situ* selection processes resulting in amplification of the fittest in the Darwinian sense. Such selection can only take place far from equilibrium, in a regime where replication continuously competes with replicator destruction.

Conclusions and outlook

As the examples in this review illustrate, many of the current efforts in Systems Chemistry focus on developing the coupling between subsystems involving the formation and destruction of molecules, implementing feedback loops, and controlling supramolecular interactions. Yet, integrating these individual subsystems remains a challenge. We need to learn more about how to make subsystems compatible and how to couple them together. We also need to learn the design principles behind achieving desired complex functions. In principle, enhanced complexity allows for more advanced function (evident from the evolution of life) yet handling complexity remains challenging. Analysis of complex mixtures is now possible, but still far from trivial. It is proving difficult to guide complex systems into the right (often small) subset of structural and parameter space where the desired function is found. This part of space is often not readily identified intuitively or experimentally. Hence, computational modelling tools are indispensable, yet they need to remain tightly coupled with experimental systems.⁴⁸ Thus, models need to reflect the actual mechanisms and be parametrized

with fairly accurate estimates of rate and equilibrium constants of the chemical system under study, calling for a revival of physical organic chemistry and the associated analytical tools. Apart from more powerful techniques for the analysis of complex mixtures also more user-friendly numerical tools are still needed that can be directly used by chemists in the field. A first step is an iPad app for XPP,⁴⁹ software for solving, for example, differential equations (it is preloaded with standard models like FKN (Field, Körös and Noyes) that describes the Belousov-Zhabotinsky oscillator).

Another fundamental challenge in Systems Chemistry is to comprehend complex behaviour and develop an intuition for it, as well as the ability to communicate both to peers and non-experts. Inspiration might be taken from developments in synthetic/systems biology, such as the BioBricks⁵⁰ project that allows users to develop functional biological circuits from standardised parts. These considerations call for an investment in (new) tools for the visualisation of data and complex behaviour in chemical systems. Thus, progress in Systems Chemistry needs a combination of expertises (synthesis, supramolecular chemistry, physical organic chemistry, computational modelling, advanced analytical chemistry and visualisation tools), ideally bundled in an individual lab.

What can we expect Systems Chemistry to achieve in the foreseeable future? In terms of fundamental science the most tantalizing prospect is probably the *de novo* synthesis of life. Synthetic systems are being developed that capture many of the individual characteristics of life (compartmentalisation, replication, metabolism) and their integration under far-from-equilibrium conditions is currently being developed. In terms of concrete applications, the impact of Systems Chemistry is hard to predict and we can only speculate at this stage. A possible impact may be on the process of manufacturing of chemicals. Currently this process requires equipment that controls most aspects of the synthetic process, but much of such equipment may one day be replaced by nano- or microscale self-synthesizing energy efficient and clean chemical factories, capable of regulating and repairing themselves.

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