Composing a molecular symphony

ter Harmsel, Matthijs

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Chapter 1

Chemical Oscillators

In this chapter the field of chemical oscillators is reviewed. A definition of chemical oscillators is given and possible applications of chemical oscillators are summarized. Existing chemical oscillators are divided into categories based on the underlying chemistries and selected examples for each category are discussed. Lastly, the work performed in this thesis is placed in the context of the state of the art and the content of each chapter is briefly discussed.

Chemical oscillators are chemical reaction networks (CRN) that show periodic variations in the concentration of one or more components of the reaction mixture (Fig. 1a). Oscillations can arise when positive feedback, where a product accelerates its own formation, is coupled to negative feedback, where a product is consumed.

Chemical oscillators are a common feature of living systems. Oscillators regulate key cellular functions such as metabolism and cell division as well as larger scale processes such as circadian rhythm and heartbeat.[1] In the latter half of the 20th century the first artificial chemical oscillators were discovered serendipitously. Since then, synthetic chemical oscillators have been developed for potential applications in analytical chemistry[2], personalized drug delivery[3,4], and for systems that emulate biological properties such as quorum sensing[5], homeostasis[6] or periodic movement[7,8].

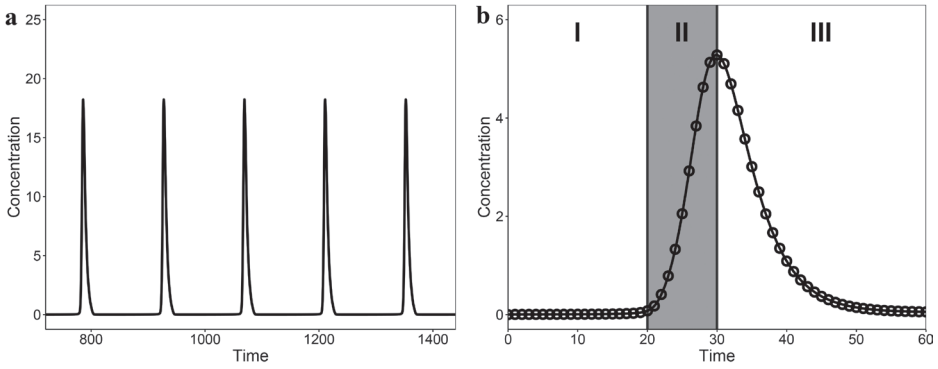

Figure 1 | Chemical oscillators a, *Concentration versus time profile of a chemical oscillator,* ***b,*** *A single pulse showing the three phases:* ***I*** *Lag phase,* ***II*** *Exponential growth,* ***III*** *Decay.*

An oscillation consists of a sequence of pulses of growth and decay. If we look at a single pulse, we can divide it into three parts (Fig. 1b). First, there is a lag phase (**I**) during which not much seems to be happening. Looks can be deceiving however, because during the lag phase the system is slowly building up until it reaches a tipping point. At the tipping point we reach the second phase, exponential growth (**II**), during which the concentration rapidly shoots up because of positive feedback. As the system runs out of substrate, the exponential growth slows down and a maximum is reached. Then negative feedback takes over and the concentration decays back to the baseline (**III**).

Chemical oscillators can be found both in closed systems, where there is no exchange of matter with the environment, or open systems, where there is a

constant flow of matter in and out of the system. In the following sections the most relevant examples of oscillators in closed and open systems will be discussed.

1.1. Oscillators in closed systems

The Belousov-Zhabotinsky Reaction

The first synthetic chemical oscillator was discovered accidentally by Boris Belousov in 1951 when he was trying to find an inorganic version of the Krebs cycle. He struggled to get his research published, because he could not support his findings to the satisfaction of the reviewers, something a lot of us can relate to. Eventually, after some encouragement from Simon El'evich Shnol, Belousov published his findings in a little read, non-reviewed journal. Then Schnoll's graduate student Anatol Zhabotinsky investigated the reaction in detail and published his findings in 1961, finally allowing the wider Soviet world to get to know this fascinating reaction. It would take longer yet, until 1968 to be exact, for the Belousov-Zhabotinsky (BZ) reaction to be known in the west.[9]

The BZ reaction is deceptively simple. The recipe requires only potassium bromate, potassium bromide, malonic acid, sulfuric acid and a metal salt – n.b. in most recipes you will find another component, this is an indicator that is added so that the oscillations can be followed visually. The underlying chemistry, however, is anything but simple.

The BZ reaction oscillates through a combination of three main pathways (Fig. 2a). There is a positive feedback loop which oxidizes the metal center while producing $HOBr_2$, an energy dissipation pathway which reduces the metal center and a negative feedback loop which is destroying $HOBr_2$. Even this extremely simplified overview of the BZ reaction is already much more complicated than the reactions organic chemists deal with on a day-to-day basis. The real BZ reaction is an order of magnitude more complex still. There are over eighty reactions occurring simultaneously. Of these eighty at least ten are essential to obtain oscillatory behavior (Fig. 2b).[10]

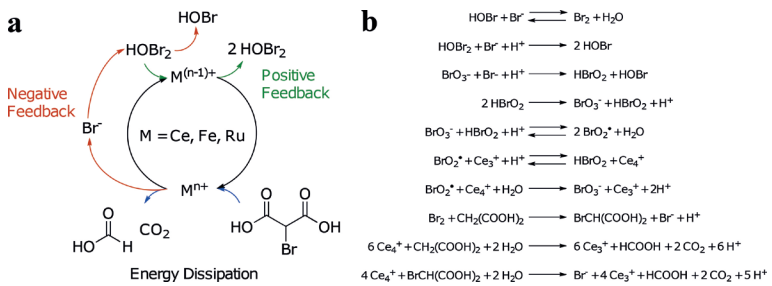

Figure 2 | The Belousov-Zhabotinsky reaction. a, *The three reaction pathways that produce oscillations in the BZ reaction: positive feedback (green), energy dissipation (blue), and negative feedback (orange).* ***b,*** *The ten essential reactions needed to obtain oscillatory behavior.*

The unique oscillatory behavior of the BZ reaction has inspired a plethora of systems that use the BZ reaction to perform other functions in an oscillatory fashion. Not all of these systems can be described in detail here, but an overview is given of a few examples that highlight the diversity of applications that chemical oscillators can have. There are applications of the BZ reaction in chemical computing[11–14], and in emulating biological properties such as quorum sensing[5], and active materials.[15–18]

The BZ reaction can be used to construct simple logic gates[11], complex logic gates[12], and even a chemical computer with memory and pattern recognition.[13] To demonstrate how far chemical computing with oscillators has come Jerzy Gorecki and Ashmita Bose made a network of BZ oscillators that was able to recognize the Japanese flag with 95% accuracy.[14]

Kenneth Showalter's group showed that by immobilizing the transition metal catalyst of the BZ reaction on beads in a catalyst free solution these beads can synchronize their oscillations rapidly through the exchange of signaling molecules. This kind of behavior is similar to that observed for micro-organisms such as yeast, both are examples of quorum sensing.[5]

The core of the BZ reaction is an oscillation between two oxidation states of a metal ion. Yoshida realized that if he used ruthenium tris-bipyridine complexes immobilized in a polymer gel then the oxidation of these complexes would change the volume phase transition temperature of the gel as well as the swelling ratio because the hydrophilicity of the polymer chains increases in the oxidized Ru^{3+} state and decreases in the reduced Ru^{2+} state. If these polymer gels are placed in a solution with BZ reagents they can therefore exhibit periodical swelling/deswelling behavior.[15,16] This autonomously swelling and deswelling gel has been used for a

wide range of functional materials.[17] Here I'd like to highlight an example where this gel was used to transform chemical energy into physical movement (Fig. 3). Through the BZ reaction a gel actuator undergoes sequential stages of bending and stretching which on a ratchet floor allows the gel to perform unidirectional movement.[18]

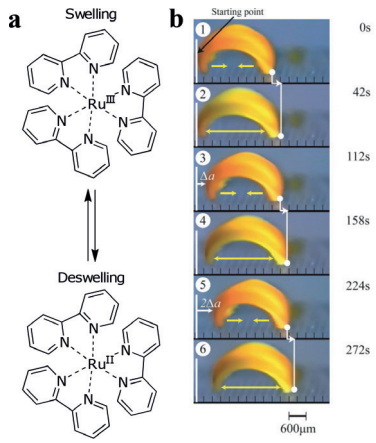

Figure 3 | Self walking gel. a, *Ruthenium(II)/(III) oscillation that induces swelling/deswelling in polymer gels.* ***b,*** *Directional movement powered by the BZ reaction. Reproduced with permission, Copyright © 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.[18]*

Biomolecule batch oscillators

Nature is no stranger to oscillations.[1] In an effort to emulate nature's oscillators with minimal systems, batch oscillators based on nature's molecules have been developed.[19,20] Fujii and Rondelez, made a chemical oscillator using DNA and DNA polymerization/depolymerization enzymes that can be described with the same mathematics that describe oscillations in the populations of animals.[19]

Fujii and Rondelez constructed a chemical oscillator based around complementary DNA strands and DNA polymerization-depolymerization enzymes (Fig. 4). Their design is based on a pair of autocatalytic reactions, a predator and a prey. The system contains three DNA strands, a template, a prey that multiplies using the template, and a predator that multiplies by consuming the prey. The template strand is the only stable strand in the system. The other two strands, predator and prey, are dynamically produced and destroyed.[19]

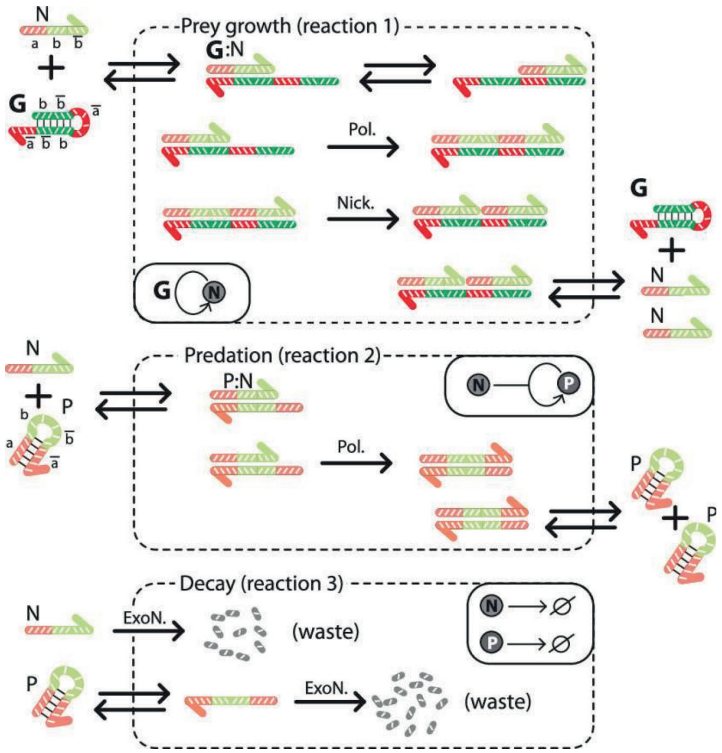

Figure 4 | The DNA-enzyme oscillator constructed using a predator-prey reaction network. *The network is built up using DNA polymerization-depolymerization reactions. Complementary sequence domains are indicated using color-coding. The template G (in strong colors) is the only stable sequence in the system. Other oligonucleotides (predator P and prey N, in light colors) are dynamically produced and degraded. Reprinted with permission ACS Nano* ***2013****, 7 (1), 27–34, Copyright © 2012 American Chemical Society.*[19]

The system oscillates as follows. The prey strand binds to a template strand, is polymerized to twice its length, and subsequently cut in two. Thereby, from one prey strand two prey strands are created, causing an exponential rise in the concentration of prey. Simultaneously, the predator binds to the prey and through polymerization transforms the prey into another predator – a very scary predator indeed – creating two predator strands from one predator strand and one prey strand, causing an exponential rise in the concentration of predators while causing the concentration of prey to diminish. When there are many prey, the concentration of predators rises rapidly, depleting the concentration of prey. Without prey to feed upon, the predators die out and, because there are now no predators consuming them, the prey are once more able to reproduce and their concentration rises exponentially. In turn, this provides plentiful food for the predators, which reproduce, consuming the prey once more. These cycles continue until the system runs out of energy, which

in this case is the activated nucleotide building blocks which are the materials used for the DNA polymerization reactions.

These dynamic oscillations of predator and prey DNA strands are almost identical to the oscillations found in the populations of predator and prey animals. This system did not only show oscillations for a single predator-prey pair. Upon combining different sets of predators and prey the resulting oscillations could be strengthened, showing symbiosis – another important feature of biology – or the oscillations could enter a chaotic regime instead. This work exemplifies that the mathematics describing oscillations are conserved across length scales and that we can take inspiration from other, often only distantly related, fields of science when designing chemical oscillators.

1.2. Oscillators in open systems

The Belousov-Zhabotinsky reaction has been established for almost a century, and yet there exist very few examples of oscillators in closed systems. The reason for this is simple. Oscillators in closed systems function through complex, intricate reaction networks that result in alternating pathways of production and destruction. To design such a system from scratch is a massive challenge, if not utterly impossible. Using nature's building blocks (DNA & enzymes) we can build complex systems, such as batch oscillators, but these are not synthetic oscillators, rather they are clever re-arrangements of cellular machinery.

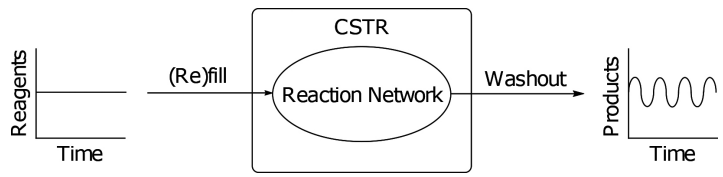

Figure 5 | Oscillations in flow*. An oscillator in flow is kept out of equilibrium by a constant supply of energy in the form of fresh reagents. The reagents are fed into the reactor at a constant rate but due to the chemical reaction network inside the reactor the products are produced in an oscillatory fashion.*

To design a synthetic oscillator we can use a system where the out-of-equilibrium character of the reaction network is not an emergent property of the chemical reaction network but is applied to the system from outside. We can do this by using an open system where energy, in the form of fresh reagents, is continuously

supplied (Fig. 5). This supply of energy is usually coupled with a constant removal of waste products. This setup of a reactor with a continuous inflow of reagents and a continuous removal of products is called a Continuous Stirred Tank Reactor (CSTR). In such a setup the reactor has a constant volume of liquid inside it, in contrast to the flow reactors typically used for flow photo-chemistry for example, where the reactor itself is a long tube and does not have a standing volume of liquid.

Chemical oscillators in open systems all use the same basic design principle. At their core there is a reaction that has some sort of positive feedback – the reaction rate increases as the reaction progresses. Next to this there is a source of delayed negative feedback, which is removing the products of the reaction. This creates a single pulse. This combination of reactions is then placed in a CSTR, which prevents the system from reaching equilibrium and allows it oscillate.[21] Delayed in this context means that the negative feedback should become dominant after positive feedback has taken place. Imagine a system where such a delay does not exist, any formed molecule will quickly be destroyed by the negative feedback and no oscillation will take place. The negative feedback thus needs to be delayed with respect to the positive feedback. In kinetic terms it needs to be slower so that the positive feedback reaction(s) can outcompete the negative feedback until the substrate of the positive feedback reaction(s) has been nearly consumed.

These straight-forward design principles have been used to create chemical oscillators based on inorganic salts[21–23], biomolecules such as enzymes[24], small organic molecules[25–27], as well as systems where one of the reactions in the chemical reaction network is replaced by physical motion[28] or supramolecular assembly[29,30].

In the remainder of this chapter selected examples from these classes of oscillators, their applications, and the advantages and disadvantages of using a certain class of oscillators will be discussed.

Inorganic (pH) oscillators

The first oscillators in open systems were based, perhaps unsurprisingly, on the same oxyhalogen chemistry as the BZ reaction.[23] Theses early inorganic oscillators will not be discussed in detail but what came after, inorganic pH oscillators[22], will be highlighted instead.

Inorganic pH oscillators are a subset of the wider family of inorganic chemical oscillators where the core oscillating component of the chemical reaction network

is H^+. It is relatively easy to use pH to control other processes and thus this type of chemical oscillator holds particular promise as a basis for further applications.[21]

Inorganic pH oscillators are typically based around the following set of reactions (Fig. 6a). There is an oxidation reaction of a certain substrate (S_1) that produces H^+ and proceeds faster at low pH. The reaction itself lowers the pH by producing H^+ and thus this oxidation will accelerate as more substrate is consumed, providing positive feedback to the system. There is a second oxidation reaction which consumes H^+, thereby increasing the pH again, providing negative feedback.[21] An illustrative example of such a pH oscillator is the iodate-sulfite-ferrocyanide oscillator (Fig. 6b).[31]

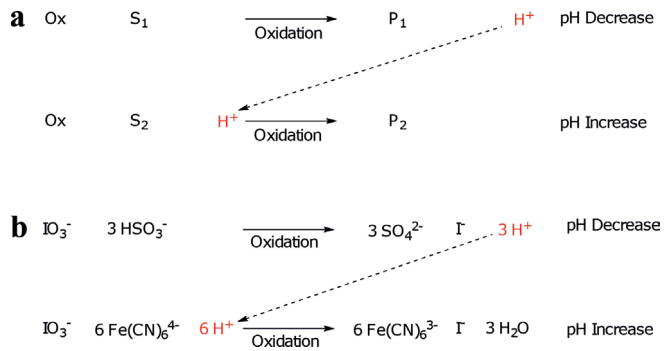

Figure 6 | pH oscillators a, *The design scheme for an inorganic pH oscillator. The first oxidation consumes H^+, and thus increases the pH. The second oxidation produces H^+, and thus lowers the pH.* ***b,*** *The iodate-sulfice-ferrocyanide oscillator which follows the design scheme for an inorganic pH oscillator. Adapted from J. Am. Chem. Soc.* ***1986****, 108 (11), 2826–2830.*

Inorganic pH oscillators generate and consume H^+ and can thus be made to interface directly with biological and synthetic systems that use or are responsive to pH. Using this principle model systems have been made that can control gel growth[16], and periodic drug release[32]. Typically, an oxidant has to be used limiting potential applicability. Additionally, in living systems any pH change will be quickly counteracted by the living system itself. Indeed, even minor changes in pH can have fatal consequences. The likelihood of pH oscillators being used for drug delivery are therefore rather slim.

Recently, pH oscillations were also observed in a palladium catalyzed carbonylation reaction.[33] In a follow up study it was shown that this reaction could be used for periodic release of fluorescein from a chitosan gel.[4,34] Although the system uses palladium and carbon monoxide, and can thus never be used in a clinical setting due to toxicity concerns, this discovery is nonetheless promising. It opens the possibility

of more of such oscillators being present in catalytic systems that have heretofore been overlooked.

Bio-molecule oscillators

Oscillators play an important role in biological systems.[1] We saw before that using life's machinery it is possible to construct a chemical oscillator that will function in a batch system. Here, a much simpler system that uses only enzymes in a CSTR to generate chemical oscillations will be discussed.[24]

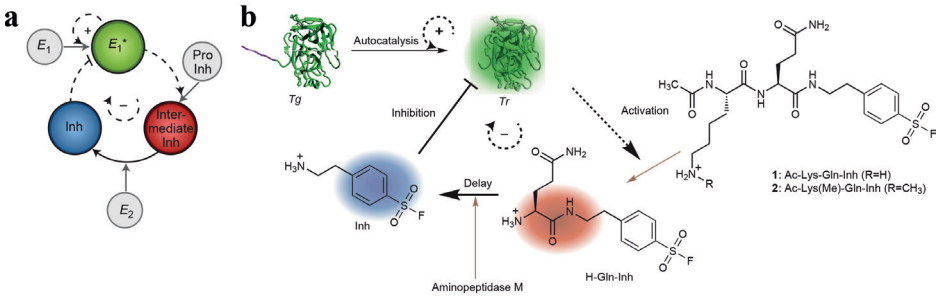

Figure 7 | An enzymatic oscillator. a, *Schematic network layout of the enzymatic oscillator based on autocatalytic production and delayed inhibition of an enzyme* ***b,*** *Detailed reaction network showing the cleavage reactions which activate the inhibitor. Reproduced with permission from Springer Nature. Copyright © 2015, Nature Publishing Group.[24]*

In 2015 the Huck group designed an enzymatic oscillator centered around the autocatalytic activation of trypsin (Fig. 7).[24] Trypsin is a protease which can be inactivated by attaching an additional small peptide chain to it – this deactivated form of trypsin is called trypsinogen. Trypsin itself can cleave this chain and thus the activation of trypsin from trypsinogen is autocatalytic. The negative feedback is a covalent inhibitor for trypsin. Crucially, the inhibitor is not introduced directly but rather with several amino acids attached. Trypsin itself can remove some of these amino acids but leaves some on. This last part is cleaved of by a secondary enzyme, aminopeptidase. This two-step activation builds the necessary delay into the system allowing sustained oscillations to be achieved. This second cleavage also allows additional control over the dynamics of the system. Using the building blocks of this enzymatic oscillator it was possible to obtain temporal control of gel formation[35] and make an adaptive enzymatic reaction network.[36]

Bio-molecular oscillators are, by definition, compatible with all biological systems and thus hold great promise in the area of therapeutics. It is more difficult to couple other non-enzymatic reactions to such an oscillator because of the requirement

to work in buffered water. Most organic reactions, and almost all transition metal catalyzed reactions, do not function in water. Additionally, tuning a bio-molecular system is difficult, and often prohibitively expensive in terms of both time and resources. It is especially this lack of tunability which is prohibiting development of enzymatic oscillators for further applications.

Organic oscillators

Oscillators based on small organic molecules offer several advantages over enzymatic and inorganic oscillator systems. Small organic molecules can be made from readily available building blocks, they can function in non-aggressive conditions, and can be easily tailored with the power of organic chemistry. Despite these apparent advantages very few examples of oscillating system based solely on organic molecules exist.

The first oscillator to use small organic molecules as one of the core components was Annette Taylor's hybrid organic/inorganic pH oscillator.[25] The core of this oscillator is the reaction between methylene glycol and sulfite (Fig. 8). This reaction produces hydroxide and is base catalyzed. During the course of the reaction the pH increases and this in turn accelerates the reaction. This provides the positive feedback that is needed to obtain oscillations. The base catalyzed hydrolysis of gluconolactone to gluconic acid is used to bring the pH back down again. Using this system sustained oscillations between pH 7 and 10 were obtained in a CSTR over a period of 20 minutes. The authors propose applications of this pH oscillator in (bio)sensing and responsive polymers, however except for a system showing micelle to vesicle transitions[37], no applications of this system have been reported unfortunately.

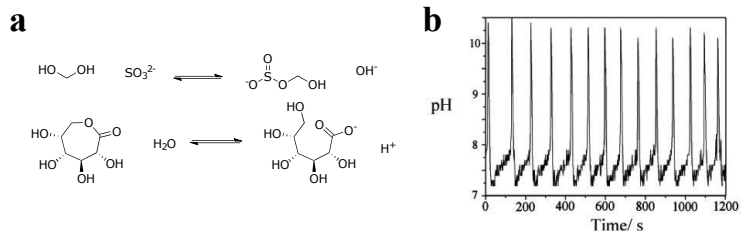

Figure 8 | An organic based pH oscillator. a, *The design of the hybrid organic/inorganic pH oscillator showing the autocatalytic reaction between methylene glycol and sulfite which increases pH and the base catalyzed hydrolysis of gluconolactone which decreases pH.* ***b,*** *Large amplitude pH oscillations obtained in a CSTR using the hybrid organic/inorganic pH oscillator. Adapted with permission from J. Phys. Chem. A* ***2007****, 111 (4), 549–551, Copyright © 2007 American Chemical Society*

The first oscillator that uses solely small organic molecules was reported by Semenov *et al.* in 2016.[26] Instead of a single autocatalytic reaction the oscillator is based around a reaction network which amplifies the concentration of free thiol groups (Fig. 9). The species that is oscillating is therefore also not a single compound but rather a single functional group. Negative feedback is provided by conjugate addition reactions. Crucially, there are two conjugate additions, one fast and one slow. The fast reaction creates a lag phase before exponential growth can take place and the slow reaction brings the concentration of free thiol back down to the baseline after exponential growth has taken place. The oscillation is initiated by the slow hydrolysis of alanine thio-ester. Since the initial report Semenov *et al.* have used the power of organic chemistry to modify the initial oscillator to produce gaunidines[38] and peptides[39]. These works suggest that chemical oscillators could be used in combination with dynamic combinatorial chemistry to access unexplored chemical space.

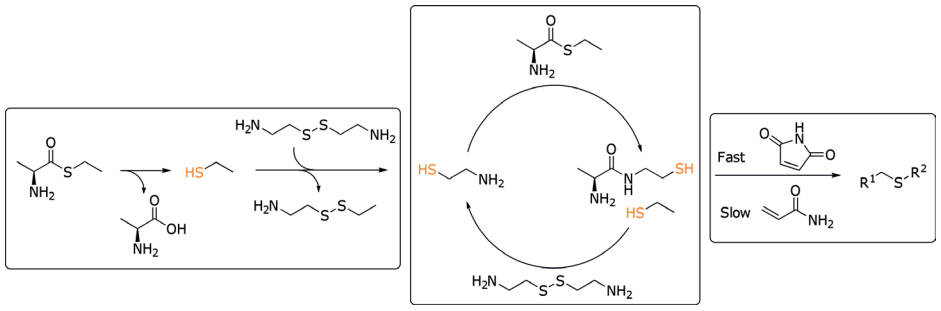

Figure 9 | The thiol oscillator. *The oscillation is initiated by the hydrolysis of alanine thio-ester which forms ethanethiol. This then enters the thiol-amplifying reaction network, leading to exponential growth of the concentration of thiols. These thiols then react in a conjugate addition reaction, thereby removing them from the reaction network. There is a fast conjugate addition, which creates a lag phase before exponential growth occurs, and a slow conjugate addition, which returns the concentration of thiols back to the baseline after exponential growth has finished. Adapted from Nature* ***2016****, 537 (7622), 656–660.*

Supramolecular oscillators

In recent years, oscillators that use supramolecular assembly as a key component of the oscillating reaction network have been developed.[29,30] When working with supramolecular assemblies it is much more difficult – if not impossible – to use a CSTR. It is quite hard to make a fiber flow. The two systems described here are performed in semi-batch conditions. Semi-batch means that there is a constant inflow, but no removal of products. Over time the reactor thus accumulates waste materials, preventing oscillations in these systems from being fully sustained.

Nevertheless, in both cases oscillations sustained over a significant period are obtained.

Supramolecular assembly often follows a nucleation-and-growth mechanism. After an initial seed is formed there is a rapid assembly towards the final structure. This mechanism has the first two phases of an oscillation built into it, a lag phase followed by exponential growth. The group of Hermans used this nucleation-and-growth mechanism to construct a chemical oscillator that uses supramolecular assembly at its core.[29] The oscillator uses perylene diimide (PDI) which in aqueous buffer self-assembles into supramolecular polymers (Fig. 10). Upon reduction to PDI^{2-} the stacks fall apart into mostly monomers and some small assemblies. The reduced PDI^{2-} is then oxidized back to neutral PDI through atmospheric oxygen, allowing the assembly to start again, thus completing a single oscillation.

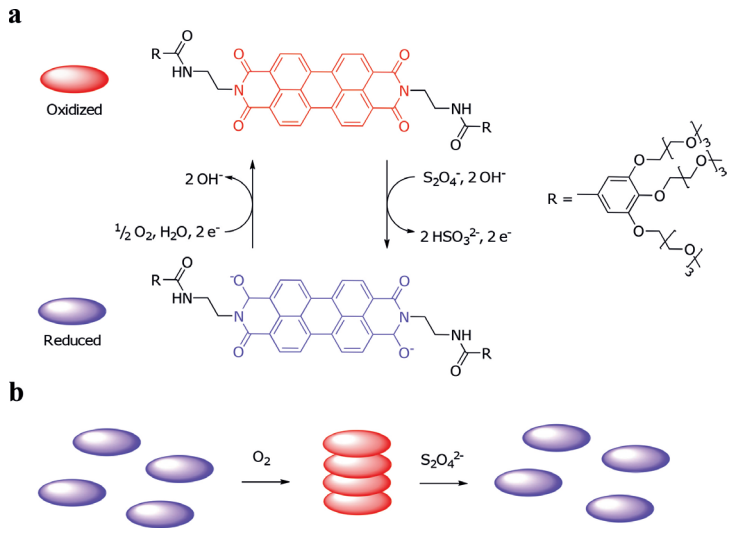

Figure 10 | The supramolecular oscillator. *PDI assembles into supramolecular polymers in aqueous buffer. Upon reduction by thiosulfate PDI^{2-} forms which cannot assemble, thus destroying the stacks.* ***a,*** *the PDI monomers and the oxidation and reduction reactions.* ***b,*** *a schematic overview of the assembly and disassembly process. Adapted from Nat. Nanotechnol.* ***2018****, 13 (11), 1021–1027.*

Next to oscillation, the Hermans' group was also able to show traveling fronts and pattern formation, which are normally only features of batch oscillators. This work shows that using supramolecular assembly at the core of chemical oscillators allows for the construction of new and exciting systems with properties that are otherwise very hard to achieve.[29]

Another way to use supramolecular assembly in an oscillator is to use physical autocatalysis, instead of chemical autocatalysis. In physical autocatalysis a reaction is accelerated not through catalysis but because the product causes a physical change that increases the rate of the reaction.

The Fletcher group devised a system where a surfactant is formed from a two-phase system where one reactant is soluble in the aqueous phase and one non water soluble reactant creates a separate phase. Once the concentration of surfactant reaches the critical micelle concentration (CMC) the surfactant self-assembles into micelles. These micelles can take up the apolar reagent into and disperse it in the aqueous layer, effectively increasing the surface area between the two layers and thereby increasing the rate of surfactant formation.[40]

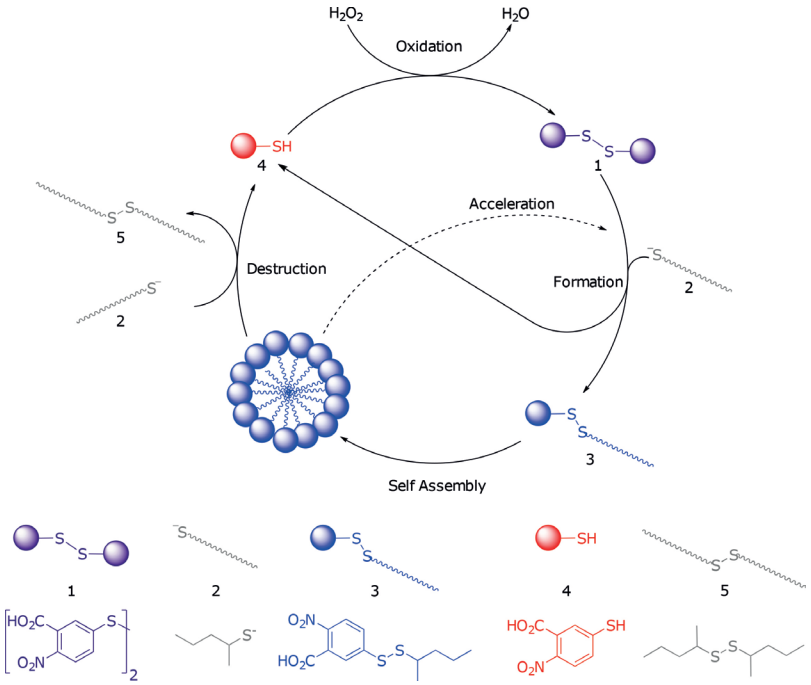

Figure 11 | The micelle oscillator. *Through thiol exchange reactions a surfactant **3** is formed which accelerates its own formation via physical autocatalysis. Once the feedstock **1** is depleted a second thiol exchange reaction takes over which forms the apolar **5** and the thiol **4**, consuming **3** and destroying the micelles. Via oxidation **4** is transformed back into feedstock **1** and the oscillation resets. Adapted from Nat. Chem, 14, 805-810, **2022**.*

Recently, Fletcher's group was able to adapt this system in such a way that chemical oscillations could be obtained (Fig. 11).[30] The oscillator uses thiol-exchange chemistry. Polar **1** performs a thiol exchange reaction with apolar **2**, thereby forming

surfactant **3**. Once the CMC is reached this surfactant forms micelles, accelerating the formation of **3**. Once **1** runs out, **3** reacts instead with apolar **2** to form the apolar dithiol **5** and polar thiol **4**, destroying the micelle in the process. Then, **4** is oxidized back to **1** by hydrogen peroxide, thus arriving back at the starting point of the oscillation. Although no applications of such transient micelle systems exist at the moment the authors do show that it is possible to transiently dissolve apolar dye in aqueous solution. This suggests that perhaps a type of transient phase-transfer-catalysis could be possible.

Mechano-chemical oscillators

The final class of chemical oscillators pushes the definition of a chemical oscillator to its limits. The group of Aizenberg developed a system where catalysts are immobilized at the tips of thermo-responsive hydrogel pillars made of polymers with a low LCST (Lowest Critical Solution Temperature) which are immersed in a tow phase environment (Fig. 12). The substrates of the catalytic reaction are only present in the top layer, and so catalysis only occurs when the hydrogel pillars are fully extended. Now, when the reaction occurs heat is generated at the tip of the pillar and the shape of the pillar changes to the bent state. By bending the catalyst is moved to the bottom layer and the reaction stops. As the local temperature drops, and the pillar extends again. With the catalyst is in the top layer again, the cycle repeats. They named this system SMARTS (self-regulated mechanochemical adaptively reconfigurable tunable system).[28]

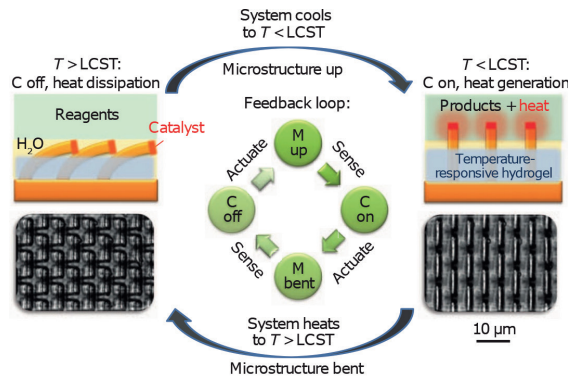

Figure 12 | The mechano-chemical oscillator. *A schematic overview of the mechano-chemical oscillator showing the feedback loop between catalysis (C) and mechanical motion (M). The mechanical motion of temperature responsive hydrogel pillars is coupled to an exothermic reaction catalyzed by a catalyst immobilized on the tip of the pillars. Schematic side views and microscopy images are shown of the active and inactive state. Reproduced with permission from Springer Nature. Copyright © 2012, Nature Publishing Group.* [28]

Although arguably the system is not a chemical oscillator – there are no oscillating concentrations – the catalytic activity is oscillating instead, in its effect – the periodic availability of a catalyst – it is the same as a chemical oscillator. The source of the oscillations is very different from the other systems discussed before. Here, there is no chemical reaction network – in fact there is only a single chemical reaction – but the feedback in the system comes from mechanical movement. The authors found that through the oscillatory generation of heat the local temperature could be controlled within a relatively tight window. Control of the local environment (homeostasis) is a feature of life and could be used to create exciting new chemical systems.

1.3. This thesis

The preceding chapter reviewed the many different classes of chemical oscillators that have been developed. Each class of oscillators has its own unique set of advantages and disadvantages. An ideal chemical oscillator should be modular, easily tailored to new requirements, compatible with other systems – i.e. operate under mild conditions – and be able to perform an application without the need for extensive re-optimization.

Some of the currently known chemical oscillators can satisfy one or two of these requirements but so far none can meet the challenge of the last requirement. The reason for this is that the chemical oscillators developed to date do not catalyze or control a second chemical reaction *in situ*. Rather, any application is performed using stoichiometric reactions. In a stoichiometric reaction the reagent is consumed and thus it is no longer available to oscillate.

The result of this is that the oscillator is greatly affected by performing an application and the oscillator has to be optimized once more. If an application is performed catalytically however, the reagent is still available to the oscillator and thus extensive re-optimization is no longer needed. The creation of a system centered around an oscillating, but free catalyst opens up a whole new category of applications for synthetic chemical reaction networks and allows the construction of time-controlled complex systems with intricate functions driven by autonomous periodic catalysis. Creating this type of system was the goal of the research described in this thesis.

An attractive strategy to achieve this would be to use catalytically active small organic molecules (i.e. organocatalysts) at the core of an oscillatory system. The

operational simplicity of organocatalytic reactions, the robustness and the non-toxicity of the catalysts, their broad functional group tolerance, and the diversity of small organic molecules available as catalysts[41,42] offer strong potential for the modular design and applications of organocatalytic oscillators. Moreover, such oscillators would benefit from readily available building blocks, the ability to function in non-aggressive conditions, and can be easily tailored with the power of organic chemistry.

In this thesis a catalytically active small organic molecule oscillator based on the principles of aminocatalysis was developed. The oscillator has a modular design that makes use of autocatalytic 9-fluorenylmethoxycarbonyl (Fmoc) group deprotection,[43] and acetylation reactions of amines. This oscillator is able to catalyze a second reaction which is independent on the oscillator itself. The product of the oscillator is an organocatalyst that can promote chemical reactions via iminium and/or base catalysis. The reactants that form the oscillator and those involved in the catalyzed reaction are mixed in the same reactor and form a system where each component serves a unique purpose with minimal interference between the oscillation and the catalysis. The potential application of periodic catalysis and the added value it can hold over conventional catalysis was demonstrated by obtaining useful selectivity between structurally similar substrates undergoing the same catalytic transformation.

Chapter 2 is an investigation of the mechanism of Fmoc deprotection, using a combination of DFT calculations and isotopic labeling studies to determine once and for all whether this mechanism goes through an E1cB mechanism.

Chapter 3 describes the design of the catalytically active small organic molecule oscillator and how that design evolved into a system that could perform a single pulse in batch conditions. The need for an elevated temperature and how we overcame the problem posed by an overly acidic leaving group are discussed.

Chapter 4 describes how we moved from a single pulse in batch conditions to sustained oscillations in flow. It also shows how a model was made using rate laws of all the reactions in the chemical reaction network and how that model was used to find conditions for sustained oscillations. Then an investigation of the effect of changes to the concentrations of core components of the oscillator on the oscillations is performed

Chapter 5 describes how we used our small molecule chemical oscillator to catalyze another independent reaction. It is explained how we chose a reaction for this purpose and what the requirements were for selecting a substrate. The effect of performing catalysis on the oscillations in both batch and flow conditions is discussed. Additionally, how varying the substrate of the coupled catalytic reaction affects the oscillating catalysis is investigated.

Chapter 6 describes how we used the catalytic oscillator developed in the preceding chapters to perform a function not possible with previously reported chemical oscillators. Additionally, an outlook on the future of catalytic oscillators is provided.

1.4. Literature

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