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Review

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# Exploitation of feedback in enzyme-catalysed reactions

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**Abstract:** Some cellular systems, such as yeast, bacteria and slime mould, display dynamic behavior including switches and rhythms driven by feedback in enzyme-catalysed reactions. The mechanisms of these processes have been well investigated and recent attention has turned to generating similar responses in synthetic biocatalytic systems, with a view to creating bioinspired analogues for applications. Here we discuss how feedback arises in the reaction mechanisms of some enzyme-catalyzed reactions *in vitro*, the behaviour obtained and the emerging applications. These autocatalytic reactions may provide insights into behaviour in cellular systems as well as new methods for drug delivery, sensing and repair that can be exploited in living systems.

**Keywords:** autocatalysis • enzymes • reaction networks • systems chemistry • bioinspired

## 1. Introduction

Enzymes are biological macromolecules that are used as catalysts in nature. They have been harnessed in applications varying from sensors and chemical or material synthesis to food production and fermentation. They typically work under mild conditions and are sustainable catalysts readily sourced from plants, micro-organisms and animals. Drawbacks such as a lack of stability and narrow range of operating conditions can be overcome with advances in protein engineering as well as through immobilisation techniques[1]. Thus it is likely that their application will only continue to grow.

Although not typically exploited in applications, enzymes-catalysed reactions often contain feedback in their mechanism[2]. Positive feedback results in an increase in rate as the reaction progresses and typically manifests as a sigmoidal or S-shaped rate curve instead of the usual hyperbolic curve obtained with Michaelis-Menten kinetics. This arises through effects such as allosteric regulation, where the binding of activators or inhibitors influences the enzyme activity.

Feedback plays an important role in biological functionality[3]. The presence of feedback can result in chemical oscillations, a switch-like response above a threshold signal, and it also provides a mechanism by which behaviour can be rapidly synchronised across a population of cells. Processes such as frog cell maturation[4], glycolytic oscillations in starving yeast cells, circadian rhythms, synchronisation of activity in bacteria (quorum sensing) and cellular aggregation in starving slime mould[5] all exploit feedback. The role of certain enzymes in these processes, such as phosphofructokinase (PFK) in glycolysis, is now well understood.

There has been much interest in the design of reaction networks that produce feedback-driven behaviour, both theoretically[6] and experimentally through genetic manipulation of cells; for example, genetic circuits were designed to create a toggle switch[7], chemical oscillations[8] and synchronisation of oscillations in E. coli[9]. Such studies have provided further insight into the role of feedback in certain biological processes. There is now a move towards engineering functional reaction networks in synthetic biology with technological applications in bio-sensors, bio-fuel cells or bio-refineries for complex chemical transformations[10].

In chemical systems, acid/base feedback was used to develop pH oscillators that drive periodic changes in pH

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responsive polymers[11], precipitation[12], or even DNA[13], creating materials that directly transform chemical energy into mechanical motion. However, bromate redox oscillators were mainly used that are harshly oxidative. Recently autocatalysis has been designed in systems based on biological molecules such as DNA[14], peptides[15] and enzymes[16] presenting new opportunities in terms of potential applications, for example, in living systems. However, there are still relatively few illustrations of the exploitation of feedback in bio-catalytic reactions.

In this review, we explore the mechanisms of feedback in some enzyme-catalysed reactions and associated dynamics that have been obtained *in vitro*. We also discuss some of the drawbacks to working with enzyme-catalyzed reactions. Finally, we highlight some of the current and potential applications of enzyme-driven feedback in the area of smart or functional materials.

## 2. Mechanisms of feedback

The most widely studied enzyme oscillators are phosphofructokinase (PFK) in glycolysis and the peroxidase-oxidase reaction and detailed models have been developed for both of these. Other mechanisms of feedback were discovered many years ago, such as the auto-activation of certain proenzymes, and have been revisited in recent studies. More generic mechanisms were developed from simple models, including the pH-driven autocatalysis in enzyme-catalyzed ester or amine hydrolysis discussed in detail below.

### 2.1 Phosphofructokinase

The principal metabolic pathways involved in energy production in cells are glycolysis and the citric acid (Krebs) cycle. In 1951, Belousov discovered oscillations in the oxidation state of a metal ion catalyst, while trying to create an inorganic analogue of the Krebs cycle, in what became probably the most well-known chemical oscillator, the Belousov-Zhabotinsky reaction[17]. A decade later, damped oscillations were reported in fluorescence of NADH following injection of glucose into a suspension of yeast cells[18]. Oscillations were also found in cell-free extracts, demonstrating that membrane processes are not involved.

Glycolysis is a sequence of ten enzyme-catalyzed reactions that convert sugars into pyruvate:

$$\text{Glucose} + 2\text{ADP} + 2\text{P}_\text{i} + 2\text{NAD}^+ \rightarrow 2\text{Pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+ \quad (1)$$

The oxidation of glucose provides energy to convert ADP to ATP – the primary source of energy storage in cells. The overall conversion involves an energy investment phase in which ATP molecules are consumed, followed by an energy generation phase in which ATP is produced. The key regulatory step is conversion of fructose-6-phosphate (F6P) to fructose-1,6-biphosphate (FBP) catalysed by the enzyme phosphofructokinase (PFK):

$$\text{F6P} + \text{ATP} \xrightarrow{PFK} \text{FBP} + \text{ADP} \quad (2)$$

Glycolytic oscillations can be explained by considering the allosteric nature of PFK[19]. PFK is inhibited by ATP and activated by AMP, which is formed primarily from ADP by enzyme adenylate kinase (AK):

$$2\text{ADP} \xrightarrow{AK} \text{AMP} + \text{ATP} \quad (3)$$

If the cell is ATP low/ ADP high, PFK is activated and glycolysis takes place. If the cell is energy rich (ATP high/ ADP low), PFK is inhibited and the glycolytic pathway shut down.

Despite the fact that reduced models with the PFK enzyme alone explain the basic dynamics[2], oscillations have not been reported in experiments with this enzyme only demonstrating that the other enzymes in glycolysis do play an important role, perhaps in broadening the range of conditions for which oscillations might be observed.

### 2.2 Peroxidase

Peroxidases are a group of non-specific enzymes that catalyse oxidation of substrates such as NADH by $H_2O_2$ or $O_2$; the term oxidase being reserved for reactions involving the latter. The peroxidase oxidase (PO) reaction is important in all living systems for control of oxidative intermediates and for the production of lignin, a polymer of aromatic alcohols used to strengthen plant cell walls. Oscillations in the PO reaction were first reported in 1965 when oxygen was bubbled through a solution of NADH, peroxidase and acetate[20].

Most of the dynamical behaviour has been studied using horseradish peroxidase (HRP), a heme-containing enzyme, which catalyses oxidation of NADH:

$$2\text{NADH} + 2\text{H}^+ + \text{O}_2 \xrightarrow{HRP} 2\text{NAD}^+ + 2\text{H}_2\text{O} \quad (4)$$

This reaction involves only one enzyme, however the full mechanism is still not known and involves free radicals in maybe 40 elementary steps with 20 – 30 species[21]. The complexity arises from the fact that horseradish peroxidase has five enzymatic states which differ according to the oxidation state of the heme group at the active site[22]:

$$\text{coIII - coI - coII - Per}^{3+}\text{- Per}^{2+} \quad (5)$$

Models[23] that capture the main dynamic features of the reaction involve successive reductions which convert coI to Per3+ and result in autocatalytic production of NAD radicals by "chain-branching",

$$\text{NAD}\cdot \rightarrow \text{nNAD}\cdot \quad n > 1 \quad (6)$$

which is ultimately checked by the dimerization, or "chain-terminating," reaction:

$$2\text{NAD}\cdot \rightarrow (\text{NAD})_2 \quad (7)$$

Hence the key feedback species is NAD· in this case.

#### 2.3 pH autocatalytic enzyme networks

A more generic form of feedback can be obtained through the bell-shaped rate-pH curve of enzyme-catalysed reactions (Figure 1a). In the simplest scenario, this rate behavior can be explained as a result of two weak acid equilibria (with constants $K_2$ and $K_1$) associated with the active form of the enzyme, EH[24]. At low pH, ($H^+ > K_1$) the $EH_2^+$ form

dominates while at high pH ($H^+ < K_2$) the predominant form is $E^-$. The substrate can bind to all enzyme forms, however the product is only generated from binding with EH. Therefore the maximum rate and optimum pH will arise when the concentration of EH is at a maximum i.e. when the pH lies between $pK_1$ and $pK_2$.

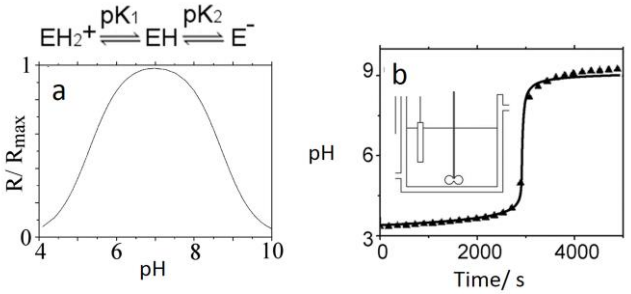


Figure 1. (a) Bell-shaped rate-pH curve characteristic of enzyme catalyzed reactions and corresponding protonation equilibria of the enzyme (b) Change in pH in a closed reactor for the urea-urease reaction.

In 1973, a mechanism was proposed for autocatalysis in the papain-catalysed hydrolysis of benzoyl-L-arginine ethyl ester (BAEE) that exploited the bell-shaped rate-pH curve [25]. The reaction has an optimum pH of 7 and produces an acid. If the pH is initially higher than 7, the decrease in pH as the reaction proceeds results in rate acceleration. In a 1d reaction-diffusion model, oscillations in pH were obtained in an enzyme-bound membrane in contact with a bath of substrate at pH 10.

A general scheme for feedback via this mechanism can be constructed for enzyme-catalysed ester or amide hydrolysis:

$$EH + S \leftrightarrow EHS \rightarrow EH + P_1 + P_2 \quad (8)$$

where S is an ester or an amide and $P_1$ is a carboxylic acid or an amine, generating acid and base autocatalysis respectively. The enzyme rate $V$ follows a modified Michaelis Menten expression of the form:

$$V = \frac{V_{max} S}{(K_M + S)\left(1 + \frac{K_2}{H^+} + \frac{H^+}{K_1}\right)} \quad (9)$$

where $K_M$ = Michaelis constant, $V_{max} = k_1 E_T$ where $k_1$ is the turnover number and $E_T$ is the total amount or concentration of enzyme. The term containing $K_2$ and $K_1$ accounts for the modulation of the rate with pH.

To date, this mechanism of feedback has been implemented experimentally in only a few systems[2, 25]. Some tentative results were obtained with papain in a membrane that were difficult to reproduce[26]. Feedback was demonstrated later with another enzyme, urease[16]. Urease is a large (typically >500 kDa for jack-bean) nickel-based enzyme that catalyses the decomposition of urea:

$$CO(NH_2)_2 + H_2O \xrightarrow{urease} 2NH_3 + CO_2 \quad (10)$$

The reaction has a maximum rate at pH 7 and the base ammonia raises the solution pH:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \quad (11)$$

With an initial pH of 4, rate acceleration is observed accompanying the increase in solution pH (Figure 1(b)). This is possibly the simplest example of feedback in an enzyme-catalysed reaction with only three components necessary: enzyme, urea and acid, and a reaction rate that follows equation (9).

Another example of pH-driven feedback in an enzyme catalysed reaction involves glucose oxidase (GO). The GO enzyme is a flavin-containing glycoprotein which catalyses oxidation of glucose in a ping-pong type mechanism:

$$GO_{ox} + \text{glucose} \rightarrow \text{D-glucon-}\delta\text{-lactone} + GO_r \quad (12)$$
$$GO_r + O_2 \rightarrow GO_{ox} + H_2O_2 \quad (13)$$

Gluconolactone spontaneously hydrolyses to produce gluconic acid and the reaction has a pH optimum from 5 - 6, thus feedback may be possible through the bell-shaped rate pH curve. Vanag et al.[27] replaced oxygen with ferricyanide which resulted in an alternative source of acid feedback, since the reduction of ferricyanide produces acid and is catalysed by acid.

### 2.4 Proteases

The proteases trypsin and pepsin are part of a family of protein-cleaving enzymes that aid in the digestion of food in humans and other mammals. They are produced from an inactive form, a zymogen or proenzyme, in order to prevent degradation of proteins within the cell, then released into the stomach or intestine.

As early as 1939 it was reported that the addition of pepsin to the proenzyme pepsinogen accelerated the rate of loss of pepsinogen[28]. A mixed reaction mechanism with both first order and autocatalytic removal of pepsinogen was later proposed, with the pathway depending on the pH[29]. At pH < 3, the first order, intramolecular pathway dominates, whereas at pH = 4, the intermolecular reaction is more favorable.

The activity of trypsin was also observed to increase exponentially in time explained by production of trypsin (Ti) from the inactive form, trypsinogen (Tg), in a simple autocatalytic process[30]:

$$Tg + Ti \rightarrow 2Ti \quad (14)$$

More complicated scenarios that consider how activity is experimentally measured have also been considered[31].

All proteolytic enzymes are synthesized in an inactive form, and in some cases activation may not involve the enzyme product but a type of self-activation also referred to as autocatalysis[32]. In this case, dimerization of the proenzyme results in removal of inhibitory subunits. This mechanism of activation does not constitute feedback.

### 2.5 Hydrogenase

Hydrogenases are a group of enzymes mainly found in bacteria and archaea that catalyse the oxidation of dihydrogen as well as the reverse process[33]:

$$H_2 \leftrightarrow 2H^+ + 2e^- \quad (15)$$

These nickel and iron-based enzymes are used for energy cycling in microorgansims and may have played a role in the evolution of metabolism in early life[34]. They are also of significant interest as a result of renewable and clean energy applications centered around hydrogen[35]. Hydrogenases may provide a replacement for platinum based catalysts in $H_2/O_2$ biofuel cells or be exploited in hydrogen sensors.

There are three main classes of hydrogenase according to the transition metal content at the active site: [NiFe], [FeFe] and [Fe] alone. The [NiFe] hydrogenase has been isolated from organisms including *Thiocapsa roseopersicina* and the enzyme activity is quantified *in vitro* in the presence of a redox partner such as benzyl viologen. This enzyme displays multiple redox and coordination states and the details of the transitions between states are still the subject of debate[36]. The reaction involves two parts, an activation process from resting states Ni-A (unready) and Ni-B (ready) followed by heterolytic $H_2$ cleavage involving short-lived active states $Ni_a$-S, $Ni_a$-C, $Ni_a$-R and possibly $Ni_a$-L:

$$\text{Ni-B} \rightarrow \text{Ni}_a\text{-S} \leftrightarrow \text{Ni}_a\text{-R} \leftrightarrow \text{Ni}_a\text{-C} \quad (16)$$

Activation involves a lag phase, the length of which depends on the enzyme concentration and can last several hours[37]. These are hallmark features of an autocatalytic process requiring initiation by a few active hydrogenase molecules. A second autocatalytic process has been proposed within the fast catalytic cycle[38]. This appeared to involve activation by hydrogen to the Ni(I) state followed by further $H_2$ addition. An alternative mechanism has been invoked in order to explain the experimental results in which one of the enzyme forms catalyses its conversion[39].

### 2.6 Kai proteins

Living organisms tend to exhibit daily cycles in metabolic activity, hormone production and other physiological processes. In general, these circadian rhythms are thought to arise from transcription-translation feedback in which clock proteins negatively regulate gene expression[40]. Cyanobacterial circadian ryhtmns are regulated by three genes that encode for KaiA, KaiB and KaiC proteins[41]. Remarkably a post-translational oscillator was constructed *in vitro* from KaiA KaiB and KaiC proteins in the presence of ATP alone suggesting that, at least for cyanobacteria, a genetic mechanism is not required for time-keeping[42].

In experiments, oscillations were observed in the amount of phosphorylated KaiC. Various models[43] have been proposed that revolve around the phosphorylation and dephosphorylation properties of KaiC as well as monomer exchange - this protein exists as a hexamer and exhibits autokinase and autophosphatase activities. Binding with KaiA accelerates the rate of phosphorylation of KaiC whereas KaiB inhibits action of KaiA and promotes return to the dephosphorylated state. An autocatalytic regulation mechanism is typical for protein kinases showing autophosphorylation.

## 3. Feedback-driven behavior

The presence of feedback is typically characterized by a time-lag before rapid conversion of substrate to product in a closed (batch) reactor or damped oscillations. If the system is maintained far-from-equilibrium by constant supply of substrate (or slowly decaying substrate) then feedback can manifest as a sigmoidal signal-response curve where, for example, the signal corresponds to substrate concentration and the response to the product concentration.

The switch-like responses from simulations of a two-variable model of the urea-urease reaction in an open reactor are shown in Figure 2[44]. The reactor exchanges substrate and acid with the surroundings at a rate dictated by rate constants $k_H$ and $k_S$ respectively. At high enzyme concentrations, a sigmoidal switch in pH is obtained as substrate is increased (Figure 2b). A sigmoidal signal-response is sometimes referred to as an ultrasensitive switch[3, 45]. A small change in substrate concentration can lead to a large, amplified change in product concentration. This switch is reversible – increasing the signal or decreasing the signal results in the same response.

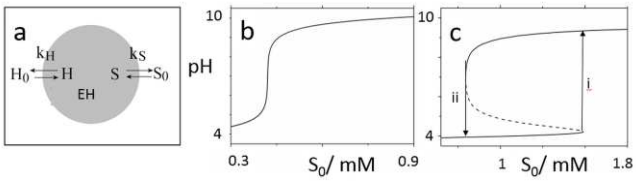


Figure 2. (a) Model scheme for pH driven feedback in an open reactor containing total enzyme concentration, EH, and constant supply of substrate and acid from the surroundings. (b) Sigmoidal switch in pH with increasing $S_0$ at high EH in simulations. (c) Bistable switch in pH with increasing $S_0$ at low EH in simulations. Adapted from ref [37].

At low enzyme concentrations, another type of switch occurs when the system is capable of displaying bistability. In this case a sharp discontinuous response is obtained as the signal is increased above a threshold level (Figure 2ci). Such a switch is robust in the sense that following the increase to high pH, small changes in the substrate concentration do not result in dramatic changes in the response. Instead the signal must be decreased to much lower levels (Figure 2cii) in order for the pH to drop back down. Between the two transition points, two different values of the pH can be achieved for the same substrate concentration depending on the system history, in a phenomenon known as hysteresis.

At intermediate enzyme concentrations, a subcritical Hopf bifurcation is obtained and oscillations in pH arise. The reaction displays a cross-shaped phase diagram in enzyme-substrate space (Figure 3a); the form of which is observed in many inorganic chemical oscillatory systems[46]. The region of oscillations in substrate-enzyme space separates a low conversion state (low pH) from a high conversion state (high pH) and represents a generic response that arises at low substrate. Biologically, such a response is indicative of when cells, e.g. yeast, are starving[47].

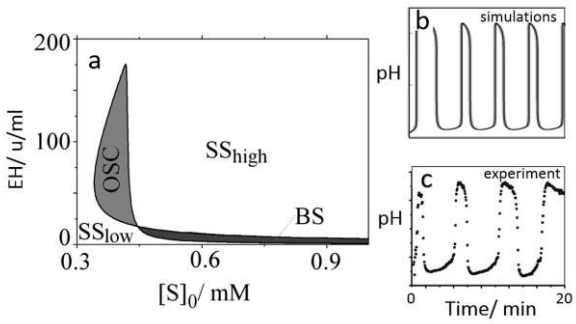


Figure 3. (a) Cross-shaped phase diagram with pH driven feedback in an open reactor where $SS_{high}$ = high pH steady state, $SS_{low}$ = low pH steady state, OSC = oscillations and BS = bistability. (b) pH oscillations in simulations (c) pH oscillations in the experiment.

In experiments in which urease, urea and acid were added to a continuous-flow stirred tank reactor (CSTR), the bistable switch was obtained and oscillations in pH were observed (Figure 3c) but were difficult to stabilize[16]. Further investigations suggested that differential transport rates are required for oscillations in the model, and these cannot be achieved in a flow reactor[48]. Therefore, it appears that another mechanism was involved in the experimental oscillations, possibly transfer of the product ammonia to the gaseous phase. In addition, when working with small amounts of urease dissolved in water for the stock solutions, the urease activity also noticeably decays over the course of hours[49]. Since the oscillations and bistability occur at low levels of enzyme, these behaviours are expected to be more difficult to reproduce without precise knowledge of the enzyme activity in a given experimental run. Bistability was also obtained in a flow reactor with glucose oxidase that was later difficult to reproduce[50]. Precise characterization of enzyme activity in stock solutions is essential if the range of parameters for which dynamical behaviour is obtained is narrow. Alternatively, methods must be sought for increasing the oscillatory domain in phase space for robust oscillations with the pH-driven feedback mechanism. Sustained oscillations have been found in yeast cells and cell-free extracts when the reaction was performed under flow conditions[51]. More complex behavior has been studied extensively with the peroxidase oxidase reaction under semi-batch conditions, such as chaotic oscillations and birythmicity[52].

An interesting feature of systems with feedback is that they can display pH or temperature compensation such that oscillatory periods are relatively unaffected by external changes in these factors. This feature has been observed in some inorganic chemical oscillators where it was demonstrated to arise as a result of competing chemical processes that control the length of the oscillatory cycle[53]. The two governing processes responded to a change in temperature in the opposite way, hence overall the cycle time barely changed when the temperature was raised from 25 to 33 °C. The KaiC *in vitro* oscillator also shows such behavior[42]. This is an extremely important defining characteristic of circadian rhythms[54].

When systems that display feedback are spatially distributed, for example in a petri-dish, then autocatalysis can propagate through the medium with constant velocity of the order of mm/min. These reaction-diffusion fronts (the spatial equivalent of a switch) or waves (if the system can display oscillations) result in a faster mechanism for transfer of autocatalytic species in space than by diffusion alone. Glycolytic waves have been obtained in yeast extracts in an open spatial reactor[55] and rotating spirals of NADH and protons were also imaged[56]. Fronts have been observed in the urea-urease reaction in a thin layer in a petri-dish that converted the medium from acid to base[57]. Constant velocity fronts have also been observed in the hydrogenase catalyzed oxidation of hydrogen under buffered conditions[39]. Fronts and waves represent an important signaling mechanism in cellular biological systems such as bacteria and slime mould.

**4. Coupling nonlinear enzyme reactions with materials**

Recently there has been an increasing interest in the coupling of biocatalytic or bio-based feedback systems with materials, with a view to exploiting the mechanisms for emergent behavior employed by natural systems in applications[58].

Urease has been widely used to generate dynamic behavior[59] – the reaction is simple and the enzyme is ubiquitous being found in numerous plants and organisms such as bacteria. In one example, the base produced by the urea-urease reaction was used to catalyse the addition of a tri-thiol to a diacrylate[60]. Performed in a batch reactor, this resulted in a time-lapse gelation process, the timing of which depended on the initial composition of the reaction. The process is one-pot and aqueous phase, involving simple benign components that are biofriendly. In addition, the reaction could be initiated locally producing gel fronts that propagated with constant velocity, curing the entire medium. The features of this system are attractive to applications such as adhesives, in which a liquid formulation is required upon mixing, followed by rapid curing.

An important feature of enzyme-catalysed reactions is that the catalyst can be encapsulated in gel beads or particles, a feat which has otherwise only been achieved with one particular inorganic chemical oscillator (the BZ reaction)[61]. As depicted in Figure 4, a pH autocatalytic network can be maintained far from equilibrium by trapping the enzyme in the particle and placing the particle in a bath of reagents. Theoretically, all of the behaviours described above are possible; a bistable switch from a low conversion to high conversion state in response to signal (substrate) concentration, oscillations in a group of particles, propagating waves. Experimentally, bistability and waves have been reported, but no oscillations to date[62]. The main reason for this may be insufficient differential diffusion of acid and substrate in the gel matrix but also the activity was found to decay in time thus alternative supports are required. However, the fact that the response involves a change in pH has been exploited by employing a pH sensitive support material that results in the particle changing size in response to substrate[63]. This represents a move towards smart materials that may, for example, amplify a response to a signal by internal reaction resulting in a transfer of chemical energy into mechanical motion.

Autocatalytic enzyme reactions may also be tuned to spontaneously develop states such as a stationary pH gradient (a Turing bifurcation, proposed theoretically for morphogenesis, illustrated in Figure 4c)[46]. This might be exploited in self-motion of pH sensitive microparticles by diffusiophoresis which currently requires particles with an asymmetric distribution of catalyst[64].

Oscillations have been obtained recently with trypsin by combining the autocatalytic generation of this species with negative feedback through designed small molecule trypsin inhibitors[65]. The output of this reaction was used to drive a number of other processes – including periodic production of chymotrypsin and the disassembly of coacverates constructed from a polycation and polyanion.

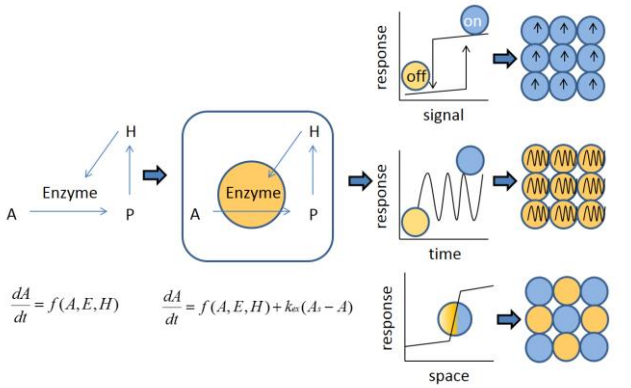


**Figure 4.** Design of enzyme reaction networks that are autocatalytic in acid/base; (b) compartmentalisation of the reaction by preparation of enzyme-loaded microparticles; (c) generation of a switch to low/high pH states, a pH gradient or periodic pH pulses in response to a chemical signal (d) characterisation of collective behaviour such as a synchronised switch

## 3. Outlook

Although enzymes have been used in many applications[66], the wealth of behaviour associated with autocatalytic reactions and feedback has yet to be truly exploited. Methodologies exist for the design of autocatalysis in enzyme reaction networks, but feedback has been explored more *in silico*[2, 25] than *in vitro*.

Applications of enzyme-catalysed reactions include sensors, drug delivery devices and bio-reactors[67] and logic gates in devices such as microfluidic reactors or bio-fuel cells[68]. Enzymes offer specificity, efficiency and biocompatibility; feedback gives potential advantages such as amplification of a chemical signal above a threshold (transistor); fast response to a signal; robust[69] response over a wide range of conditions and in the presence of noise; irreversibility[4] so the switch remains on when the signal is removed; adaptation[70] so the switch turns off in the continued presence of the signal; periodic release[71] of a chemical or synchronised activity overcoming diversity in a group of particles and temperature or pH compensation. All of these features are well investigated in biological cell models but would be novel in applications utilizing enzymes *in vitro*. However, in order to exploit feedback in enzyme-catalyzed reactions, methods for more robust performance and enhanced stability must be sought.

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