Patterns and Waves in Chemistry and Biology

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1 Introduction

Among the most fascinating properties of living systems is their ability to organize themselves spontaneously into various types of forms and "structures" [1]. This phenomenon of self-organization may either occur in the temporal domain yielding periodic or aperiodic sequences of repeating events, or it may take place in space, thus generating stationary or propagating patterns. In a variety of physical systems spatiotemporal patterns arise spontaneously from a state that was originally uniform and homogeneous [2, 3], and this is particularly true for a system involving chemical or biochemical reactions [4, 5, 6]. In order to form and to sustain spatial and temporal structures, a reaction system must exchange matter and energy with its environment, it must be far from thermodynamic equilibrium, and it must follow kinetic laws which contain nonlinear steps.

The requirements described above are fulfilled by all living beings and by certain biochemical and chemical reaction systems. Comparatively "simple" chemical reactions can thus be employed as laboratory models for the investigations of the generation and manipulation of structures, their dynamics, and their fate. The exact type of patterns formed varies according to the reaction conditions and the properties of the transport of chemicals. Either propagating patterns or stationary structures can be formed (fig. 1) in both, the biological systems and in the chemical mimic reactions.

This Chapter is organized as follows: In the next Section we sketch the biomimetic aspects of our approach in investigating some of the fundamental properties of living systems. Section 3 deals with the generation, dynamics, and fate of propagating circular and spiral-shaped waves in excitable media. We discuss the properties of such waves as studied in a biomimetic chemical reaction and compare them with the observations of similar phenomena in a biological context. In Section 4 we describe a process and a
Figure 1: Examples of structures formed in reaction-diffusion systems. Left: rotating spiral-shaped waves in the Belousov-Zhabotinsky reaction are a prominent example of propagating structures, as described in Section 3. Right: stationary honey-comb structure formed in the methylene blue–oxygen–sulfide reaction in a polyacrylamide gel (PA–MBO system), as described in Section 4.

A mechanism that gives rise to stationary patterns. It follows in Section 5 a brief presentation of a biomimetic cytochrome P450 model system which shows self-organization due to an interaction between its chemical reactions and a non-diffusive type of transport. The Chapter ends with a few concluding remarks.

2 The biomimetic approach in studying basic properties of living systems

Self-organization phenomena such as pattern formation are fundamental characteristics of living systems. These phenomena are ubiquitous and, frequently, living systems of very different biological nature may develop similar types of structures. This indicates that some basic mechanisms must exist that govern the processes of self-organization and create generic features that remain the same in quite different contexts. Therefore, there is a substantial scientific interest in elucidating and understanding these mechanisms, their dynamic properties, as well as in learning which kinds of patterns they can give rise to and which implications they have for pattern formation in the realm of biological systems.

The approach presented in this chapter consists in studying "simple" chemical reaction systems that possess the properties and features so far recognized as being essential to induce spontaneous self-organization. In living systems, self-organization requires that
a system (i) be open, i.e. that it constantly exchanges matter and energy with its environment, that (ii) it be reactive (like in all metabolic events), and that (iii) it be excitable, oscillatory, or bistable. These conditions can also be met in chemical reactions that follow nonlinear kinetic laws, and that contain a chemical species which promotes its own production (the so-called activator) and another component that limits the production of the activator (the inhibitor). It is important to note that the necessary conditions for the occurrence of spontaneous self-organization in reactive systems are exclusively concerned with the properties of the reaction system as a whole. At the molecular level, there is no need for any structural correspondences or similarities between the components involved in the biological and the mimetic reactions.

In the following Sections, we present results obtained in investigations of chemical reaction systems that show excitable behaviour. They were used to study various questions concerning the basic mechanisms of self-organization and pattern formation, the generic dynamics shown by the autonomous reaction systems, and their to manipulations and control algorithms. The investigated chemical reactions were found to mimic the behaviour and the dynamics of a vast variety of seemingly unrelated, biological entities. In other words, the purely chemical reactions are biomimetic models in the sense that they allow a clearer, more direct and less complicated study of the phenomena that are common for excitable living systems. Thus, research using such biomimetic model reactions generates important knowledge applicable to the basic properties of excitable biological systems. This can be seen in figs. 1–5: whereas figs. 1a and 3 depict propagating circular and
spiral-shaped waves observed in the biomimetic reactions, figs. 2 and 5 show examples of similar propagating waves that occur in various biological context.

3 Propagating circular and spiral-shaped waves

Travelling circular and spiral-shaped waves are widespread structural elements in a large variety of shapes created by nature. They are characteristic phenomena observed in many spatially extended excitable systems, which are capable of transmitting a local pulse of excitation into a resting, not yet excited neighborhood. Rotating spirals and propagating circular waves are found in thin layers and at surfaces of an increasing number of biological processes (fig. 2). They are involved in the aggregation of amoebae in the slime mould Dictyostelium discoideum [7, 8, 9], they occur in heart tissue [10, 11], and during the fertilization of oocytes of Xenopus laevis [12, 13], as well as in the glycolysis in yeast cell extracts [14, 15], and during the spreading depression in chicken retina [16, 17]. In addition to these biological examples, propagating spirals and circular waves are also known to occur in excitable chemical reactions. One of them, the Belousov-Zhabotinsky reaction, has developed into an excellent laboratory model for the study of the dynamics of oscillating reaction systems, as well as for spatiotemporal pattern formation in excitable media.

3.1 The Belousov-Zhabotinsky reaction

In the Belousov-Zhabotinsky (BZ) reaction, the reduction of bromate by malonic acid (or another CH-acidic organic compound) is catalyzed by a suitable metal catalyst (generally ferroin, cerium(III), or ruthenium tris(bipyridine)) in strongly acidic solution. The BZ system shows a rich variety of dynamical behaviours, which include periodic and deterministic chaotic oscillations, excitability, and the ability of forming spatiotemporal patterns.

Although the reaction mechanism of the BZ reaction is complicated [18] and not yet known in all details, the basic mechanistic and kinetic features of this reaction are well established [19], and discussed in detail elsewhere [20, 21]. The basic reaction mechanism has been simplified to yield the so-called Oregonator model which contains only three dynamic variables [22], namely the autocatalytic species HBrO₂, the oxidized form of the metal catalyst (which is the observable in experiments), and the inhibitor bromide. When studying phenomena connected to excitability, one of these three variables can be eliminated, so that the dynamics of the BZ reaction is described by the differential equations

\[
\frac{du}{d\tau} = \frac{1}{\epsilon} \left( u - u^2 + f_v \frac{u - q}{u + q} \right),
\]

(1)
\[ \frac{dv}{dr} = u - v, \]  
(2)

according to Tyson and Fife [23]. The two variables \( u \) and \( v \) play the roles of the scaled, dimensionless concentrations of the activator and of the inhibitor, respectively; \( r \) stands for the dimensionless time, while \( q, \epsilon, \) and \( f \) denote parameters derived from the reaction kinetics, initial concentrations, and the stoichiometry of the reaction.

### 3.2 Excitability

For the purpose of pattern formation, it is very important that the BZ reaction can be prepared such that the resulting state is excitable. In a homogeneous system, the reaction dwells at a quiescent, stationary state, called the resting or excitable state. Upon a perturbation, the BZ system can react in two ways: (i) it rapidly relaxes back to the resting state, if the amplitude of the perturbation is low, or (ii) it forms a single large-amplitude oscillation if the amplitude of the perturbation exceeds a certain threshold. In this case, the BZ system is excited, and it makes a large oscillatory cycle, thus amplifying the perturbation. Once the maximum of the cycle is reached, the system enters the so-called refractory phase which is characterized by a "slow" approach to the original resting state. During this refractory phase it cannot respond to any new stimuli, even if the stimuli are large enough to exceed the threshold. These properties of the BZ reaction are very similar to the behaviour known from nerve cells, which also can be found to dwell in a resting state, to be excited, or to be in a refractory state.

In an excitable solution of the BZ system placed in a spatially extended container, say, a Petri dish, a local pulse of excitation may either be induced by locally decreasing the concentration of bromide upon a brief immersion of a silver wire into the BZ solution or it may arise spontaneously. If we only monitor the dynamics at the point of the excitation, we will follow the sequence resting (excitable) state \( \rightarrow \) excited state \( \rightarrow \) refractory state \( \rightarrow \) resting (excitable) state. However, in spatially extended systems, the chemicals located at the excited spot will not only react according to eqs. (1) and (2), but they will also diffuse into the adjacent area. Thus, the description of spatially extended reacting systems must also account for diffusion. This leads to reaction-diffusion systems including Fick's diffusion terms, so that the equations for the BZ system in an extended reactor now read

\[ \frac{\partial u}{\partial t} = D_u \nabla^2 u + \frac{1}{\epsilon} \left( u - u^2 + f v \frac{u - q}{u + q} \right), \]  
(3)

\[ \frac{\partial v}{\partial t} = D_v \nabla^2 v + u - v, \]  
(4)

where \( D_u \) and \( D_v \) are the diffusion coefficients of the activator \( u \) and the inhibitor \( v \), respectively. In spatially extended excitable systems \( u \) plays the role of a fast propagator,
while \( v \) acts as a slower control variable. In order to generate propagating circular or spiral-shaped waves the diffusion coefficient of the activator must be larger or roughly equal to that of the inhibitor, i.e.

\[
D_{activator} \geq D_{inhibitor}
\]

(5)

or, in the present notation \( D_u \geq D_v \).

The partial differential equations (3) and (4) can be solved numerically for a discretized space, so that the observed spatiotemporal dynamic behaviour of the BZ reaction can also be predicted and confirmed by numerical simulations.

### 3.3 Propagating circular waves

The typical scenario for the propagation of waves in the BZ reaction can be sketched as follows: A local perturbation causes a localized fast increase of \( u \) giving rise to a diffusive flux of \( u \) into the neighbourhood. If the concentration of \( u \) increases above a certain threshold, it triggers its own production in an autocatalytic fashion, and the system leaves the resting (excitable) state and becomes excited. At the same time, a small increase in the concentration of the control variable \( v \) takes place, and the system switches to the refractory phase in which \( u \) is small and the value of \( v \) decreases slowly. The diffusive flux constantly transports a fraction of the locally formed \( u \) and \( v \) to adjacent regions, so that the wave propagates in space, provided \( u \) exceeds the threshold in these adjacent areas. Since the quasi-two-dimensional reaction media are isotropic, the excitation wave propagates away from its origin with constant speed and constant amplitude. Thus, we observe the generation of a circular propagating wave (fig. 3). Its spatial concentration profile typically shows a steep front and a much smoother wake.

If a travelling circular wave is not generated by a single event, but by a pacemaker (e.g. some small nucleation site in the experiment, or a dust particle), the origin will emit a series of concentric circular waves which form a target-like pattern. The velocity \( c \) of the wave propagation in reaction-diffusion systems depends on the frequency \( \nu \) by which new wave trains are generated [24]. Generally, one finds monotonically decreasing dispersion relations \( c(\nu) \), where \( c \) saturates at low frequencies. In addition, there is an upper limit for the frequency by which new waves can be emitted, since each wave train is closely followed by a refractory phase, where the reaction medium is not capable to respond to new stimuli. The exact value of the maximal frequency reflects the specific dynamics of the excitable reaction medium.

In two (and three) dimensionally extended reaction media, the local propagation velocity also depends on the local curvature of the reaction front. The normal velocity \( N \) obeys the eikonal equation
Figure 3: Formation of different types of propagating waves of excitation in a spatially extended Belousov-Zhabotinsky reaction medium. At the right side, a pacemaker emits propagating concentric circular waves. At the left, a pair of counterrotating spiral-shaped waves is formed in the same experiment.

\[ N = c - D_u \kappa \]  \hspace{1cm} (6)

where \( c \), \( \kappa \), and \( D_u \) are the velocity of the planar wave front, the local curvature, and diffusion coefficient of the autocatalytic propagator \( u \), respectively [24]. Equation (6) quantifies the effect of the increased diffusion of \( u \) to a spot located in front of a concave front segment (\( \kappa < 0 \)) and the decreased fluxes into the "larger" areas in front of convex wave segments (\( \kappa > 0 \)). Furthermore, there is a critical curvature \( \kappa_{\text{crit}} = \frac{c}{D_u} \), below which wave propagation can no longer occur.

Another interesting feature of propagating waves in excitable reaction-diffusion systems is the mutual annihilation of two propagating waves upon collision. This is again due to the fact that each wave of excitation is followed by its own refractory phase. Upon collision the wave front travels into the refractory region of the other wave, where it cannot respond to the stimulus. Hence, the propagating waves can no longer be sustained, and thus vanish upon collision.

3.4 Spiral-shaped waves

Spiral-shaped waves (fig. 3) are formed by perturbations of propagating circular excitation waves. Usually, this occurs when a segment of the circular BZ wave hits an obstacle or a small domain which is not excitable. Such an obstacle causes the annihilation of the wave in the confined, non-excitatory segment (or at the obstacle), while the remainder of the wave continues propagating. Once the wave moves past the obstacle, it has two open
Figure 4: Spiral-shaped waves in the Belousov-Zhabotinsky reaction. The figure shows a digital overlay of a sequence of six successive images which cover one revolution of the spiral. The black spots represent the spiral cores, which are not invaded by the waves of excitation. In the figure we also see several territories that are governed by different spirals. Each of these territories consists of an area that is covered by a sequence of concentric windings emitted by a spiral centre. The borders between such domains are marked by cusps that are formed upon collision of two windings emitted from two different spirals.

ends, the local curvature $\kappa$ of which is much larger than that of any segment within the reaction front. Thus, following the eikonal equation (6) the normal velocity $N$ at the open ends is much smaller than that of the travelling wave. Consequently, the open ends lag behind the body of the propagating front, so that the wave starts curling up into a spiral. The open ends turn into so-called spiral tips. Since a defect generates a pair of open ends, the emergence of a pair of counterrotating spirals is usually observed (fig. 3).

A fully developed spiral assumes the geometry of an Archimedean spiral [25]. Spiral-shaped waves rotate around a small, quiescent but excitable area, called the spiral core, the circumference of which is located tangentially to the spiral tip (fig. 4). However, the spiral core cannot be invaded by the excited wave because the local curvature $\kappa$ of the spiral tip reaches the critical value $\kappa_{\text{crit}}$. Thus, the spiral core is a small quiescent region, where the reaction conditions remain virtually unchanged throughout the experiment [25, 26] (fig. 4).

A spiral core acts as an organizing centre. It pins the origin of a spiral-shaped BZ
wave to a small confined interval of the reaction medium. In addition, it is a pacemaker: a spiral wave rotating at a constant velocity winds up once per revolution around the core. Two consecutive wave trains (which are connected by the spiral geometry) are therefore separated by a constant wavelength $\lambda$ and a given frequency $\nu$. The dispersion relation, i.e. the dependence of the velocity of the wave propagation $c$ from the rotation frequency $\nu$ (see Section 3.3), is also valid for BZ spiral waves.

An important feature of rotating spiral waves is their ability to "conquer" a certain territory within the excitable medium (fig. 4). On the one hand, this is due to the fact that a spiral core acts as a pacemaker which emits one additional winding of the spiral per revolution. On the other hand, a BZ wave of excitation can be destroyed by (i) either hitting an obstacle or a patch which is not excitable, or by (ii) a collision with another wave of excitation. If a spiral wave collides with a single circular wave of excitation, the circular wave and the outermost winding of the spiral are annihilated. The subsequent windings of the spiral are then capable of successfully propagating into the domain originally occupied by the circular wave. However, if two spirals of the same frequency collide (and this is the most frequently observed situation in experiments), the outermost windings will be annihilated always at the same location of the reaction medium. This process creates stable boundaries between the domains of the excitable medium governed by the respective BZ spirals. The capability of determining a territory with stable boundaries is illustrated in fig. 4 which shows an overlay of a sequence of successive images taken during one full revolution around the spiral core. Here, the territories are visualized by sequences of concentric windings emitted by the spiral centres, while the cusps formed at the junctions of two circular segments determine the borders separating domains governed by the individual spirals.

The dynamics of spiral tips has been studied intensively. In the BZ reaction, spiral waves performing rigid rotations around the core as well as meandering spirals have been observed [27, 28]. The tip behaviour of cerium(IV)-catalyzed BZ reaction media has been studied in detail by Nagy-Ungvarai et al. [29]. Their data show a complex dependence of the tip trajectories on the initial concentration of sulfuric acid and on the elapsed reaction time. With time and for a fixed value of initial concentrations, a continuous variation of the dynamics is recorded, which typically leads to increasingly larger spiral tip trajectories. A noteworthy change in the shape of the tip trajectories is encountered for $[\text{H}_2\text{SO}_4]_0 = 0.2$ M and ~70 min of reaction time, where the trajectory changes from a hypocycloidal to an epicyclic [29].

A major topic of current research interest consists in the manipulation and control of the dynamics of spiral-shaped waves in the BZ reaction, as recently reviewed in [30]. In the BZ reaction control and perturbation experiments are generally performed either
by applying an external electric field or by illuminating a BZ medium that contains the photosensitive catalyst ruthenium(tryis(bipyridine)). While an external electric field affects the motion and mobility of all ionic compounds in the solution, light causes the enhancement of the production of the inhibitor bromide. Thus, the dynamics induced by these two types of manipulations can be very different.

When an electric field is applied to a propagating BZ wave, the velocity of the waves is altered: waves moving towards the anode are accelerated and those moving towards the cathode are slowed down [31]. At high electric field strengths, waves may even reverse their sense of propagation or split into two waves [32]. Electric fields cause a deformation of the spiral geometry and they induce a drift which can be used for controlling the dynamics in the reaction medium, e.g. by inducing the collision of two spirals, which are mutually annihilated [33].

In the photosensitive BZ reaction, illumination causes an increase of the concentration of the inhibitor. By illuminating a spot of the reaction medium with a laser, small non-excitable patches can be induced. Thus, a wave front travelling past such an "obstacle" creates a pair of counterrotating spirals [34]. Laser-induced non-excitable spots can also be used to anchor a spiral within the reaction medium and to control its wavelength. Slowly moving laser spots are a tool for either moving a spiral in the reaction medium or for removing spirals by induced collisions [34]. In addition, a vast variety of light-induced perturbations that are modulated in time are applied to BZ media. This kind of manipulations lead to various interesting types of dynamics which are described in detail elsewhere [30].

3.5 Propagating circular and spiral-shaped waves in biological systems

In the following, we briefly present four of the many biological systems where propagating circular and spiral waves are involved. We outline the physiological relevance of such propagating waves in the selected systems and show the parallels to the BZ reaction, thus underlining its biomimetic character.

The glycolysis is a universal pathway in biological systems which generates metabolic energy by converting glucose into pyruvate in an enzymatic reaction cascade. Under appropriate metabolic conditions the glycolysis behaves as an excitable system. Experiments performed in spatially extended extracts of yeast cells show that glycolysis may sustain the formation and propagation of concentration waves of NADH and protons [14]. This is remarkable, since the concentration of NADH (and NADPH) is routinely used as a direct measure for the metabolic activity of a tissue, thus indicating that there are waves of metabolic activity in the cell extracts. Recently, similar circular NAD(P)H and proton waves were also found to propagate along the direction of the cell orientation within elon-
gated, living neutrophil cells [35]. In cells of this type, glycolysis plays a predominant role in the energy supply [35]. The propagation velocities of the NAD(P)H and pli waves in the cells [35] were found to be similar to that measured in yeast cell extracts [14]. As in the BZ reaction, a collision of waves leads to their annihilation [14, 35].

Propagating intra- and intercellular concentration waves of calcium ions are a general physiological phenomenon occurring in most cells and tissues. Ca$^{2+}$ waves are observed in a variety of cell types [36], including hepatocytes (liver cells) [37, 38], cardiac myocytes [39], and oocytes of Xenopus laevis [12]. The calcium waves have an eminent physiological importance, since Ca$^{2+}$ ions are "second messenger" molecules, i.e. structurally unspecific molecules which are universal carriers of a diversity of physiological information. Travelling waves of Ca$^{2+}$ concentration thus provide a means of communication within a cell or a tissue. The information content of Ca$^{2+}$ signals is encoded in the frequency of the signals, as seen in hepatocytes where different stimulating hormones induce Ca$^{2+}$ signals of different periodicity [38, 40]. If a rotating spiral is formed on a hepatocyte [37], the spiral periodically emits Ca$^{2+}$ wave trains at a certain frequency. The nature of the hormone that induces Ca$^{2+}$ release has an influence on the rotation frequency of the spiral and hence on the periodicity of the calcium signal [38]. This behaviour resembles that of BZ spirals which are created around cores of different diameter (Section 3.4).

An example for the physiological importance of Ca$^{2+}$ signalling via spiral waves is found in Xenopus laevis oocytes. When one of these frog eggs is fertilized, it emits a spiral-shaped Ca$^{2+}$ wave that eventually travels over the entire cell [12]. If Ca$^{2+}$ release is suppressed by addition of suitable Ca$^{2+}$ inhibitors, the cell is no longer capable of undergoing the embryogenetic cell division cycles, although it has been fertilized [41].

Excitation waves of eminent importance can also be observed on the heart. The normal heart beat is induced by the sinus node at a frequency of $\sim 1$ Hz. However, perturbations of the normal beat can give rise to cardiac arrhythmias. Some type of arrhythmias are induced by spiral-shaped waves of excitation rotating on the heart muscle [10]. Since arrhythmias are potentially fatal, the elimination of undesired spirals has become an important issue of research. At present the emergency treatment to suppress rotating spirals on the heart muscle consists of administrating a single, severe electrical shock on the thorax of the patient in order to reset the heart to its normal beat. Given that the defibrillation procedure is extremely painful and that it might cause tissue damage, there is a hope that efforts undertaken to study the control of spiral waves in biomimetic systems may lead to the development of new methods of controlling and annihilating cardiac spiral waves which are less invasive and more effective than defibrillation [11]. Many of the suggested control procedures have been tested previously in excitable media, like the BZ reaction.

Last, but not least, we present another biological system where spiral waves play an
Figure 5: Spiral-waves of cAMP concentration observed during the aggregation of social amoebae in the slime mould *Dictyostelium discoideum*. The picture was taken using dark-field optics. In the bright areas, which are characterized by high cAMP concentrations, cells move chemotactically towards the reaction centre (see fig. 6), while in the dark bands no directed cell motion takes place.

important role in intercellular communication (fig. 5). *Dictyostelium discoideum* cells live as solitary amoebae in the soil where they feed on bacteria. Upon starvation the cells start a developmental programme that leads to the aggregation of amoebae into multicellular colonies. Some of the cells behave as aggregation centres and begin to periodically produce and release cyclic adenosine monophosphate (cAMP) into the extracellular medium. Neighbouring cells detect the diffusing cAMP and are stimulated to also produce and secrete cAMP. This autocatalytic process results in a wave-like propagation of cAMP from cell to cell. Since cAMP is degraded by extracellular enzymes, the *Dictyostelium* system is excitable. The refractory phase ensures that the signal propagates outward [7, 8].

For *Dictyostelium* amoebae, cAMP is a chemoattracting signal transmitter. Amoebae move towards the region of high cAMP concentration by chemotaxis [7, 8, 9] (fig. 5). Since the aggregating centres emit concentric or spiral-shaped cAMP waves (fig. 5), they induce an periodic inward chemotactic motion of the cells. This process eventually causes the amoebae to gather at the wave emitting centre. Here the cells aggregate to form a multicellular slug (fig. 6), thus entering in the next stage of the life cycle of the slime mould *Dictyostelium discoideum*. When more than one aggregation centre exists, each of them emits a cAMP spiral wave. Upon collision these waves annihilate (fig. 5), and since they usually have similar frequencies, the annihilation occurs always within a narrow area.
Figure 6: Spatial distribution of *Dictyostelium discoideum* amoebae during different stages of aggregation. The pictures were obtained using brightfield optics and depict the area surrounding the spiral core, i.e. the aggregating centre. Left: at an early stage of aggregation, a vortex-shaped structure is formed by the cells which chemotactically follow the cAMP spirals (fig. 5). The vortex surrounds the spiral core. Right: At a later stage of cell aggregation, a cell colony (called pseudoplasmodium) begins to form a three-dimensional slug.

This mechanism, which we also have seen to operate for BZ spirals (fig. 4), causes the formation of aggregation territories and clearly defines the location of their borders [15].

4 Turing structures

The self-organized structures described so far consist of propagating waves which constantly move within the reaction medium. This Section is devoted to the spontaneous evolution of patterns that are stationary in time and in space. These stationary structures are formed in media where the reactions are coupled with diffusive transport.

4.1 Idea and concept

The generation of stationary patterns in reaction-diffusion systems has been predicted by A. M. Turing in his pioneering publication on "The chemical basis of morphogenesis" as early as in 1952 [42]. Based on theoretical considerations, Turing postulated that regular stationary patterns can evolve spontaneously if the diffusion coefficient of the autocatalytically active species (the activator) is (much) slower than that of the component that reacts with it (the inhibitor), so that

\[
D_{\text{activator}} \ll D_{\text{inhibitor}}.
\] (7)
Note that the generation of stationary Turing patterns calls for the opposite relation between the diffusion coefficients (eq. 7) than that required for the formation of propagating waves (eq. 5).

The evolution of a Turing pattern from a homogeneous medium usually starts at a point which is affected by a small local perturbation. Let us assume that the perturbation locally enhances the rate of the reaction producing the autocatalyst and the inhibitor. The two products will start to diffuse into the neighbourhood of the activated spot. However, since $D_{\text{activator}} \ll D_{\text{inhibitor}}$ (eq. 7), the concentration of the activator increases locally, while the inhibitor "rapidly" spreads out into the vicinity of the perturbed spot. This long-range inhibition prevents the activator of effectively invading its neighbourhood. The predominance of the inhibitor in the vicinity, however, has a limited range, so that further spots can be formed at a certain distance of the original spot. The resulting pattern arranges itself in well defined geometrical shapes, and it is characterized by a fixed average distance (wavelength) between the patterns formed.

### 4.2 Model reactions

It took almost four decades since Turing's predictions until the first Turing structures were obtained in experiments [43]. One of the problems that had to be overcome was to find a reaction system that has an activator which has a sufficiently low diffusivity, so that the condition imposed by eq. (7) can be met. At present, three reaction systems are used for studying Turing patterns: the chlorite–iodide–malonic acid (CIMA) reaction [43], a derivative of it, the chlorine dioxide–iodide–malonic acid (CDIMA) reaction, and the methylene blue–oxygen–sulfide reaction in a polyacrylamide gel (PA-MBO) [44]. In these reaction systems, the low values of $D_{\text{activator}}$ are achieved either by forming a bulky complex between the activator and a bulky ligand (as in the CIMA and CDIMA systems, where iodide is complexed in a triiodide–amylose complex) or by trapping the activator in a very tight gel matrix (as in the PA-MBO system).

Recent investigations of the PA-MBO reaction indicate that the pattern forming mechanism active in this reaction system is much more complex than that postulated by Turing [45, 46]. In this reaction system, the Turing mechanism is accompanied by viscoelastic deformations of the gel matrix which are induced by the chemical reaction [46]. Although the elucidation of these new effects is of great interest from the point of view of physics, the complications associated with it require some care when using the PA-MBO system as an experimental model to generate Turing-like patterns.
4.3 The patterns

When examined in quasi two-dimensional layers of gels containing the reactants, the three reaction systems described above spontaneously develop a variety of Turing patterns of different geometrical shape, depending on the individual reaction conditions used. The reactions are all known to form stationary patterns that consists of stipes with a certain wavelength or circular spots where each spot has six neighbours which are a hexagonal geometry. Indeed, two different hexagonal states are known to evolve: dark spots formed on a bright background (the so called $\pi$ hexagons) and bright spots on a dark background (the $\pi^*$ hexagons). Under slightly different reaction conditions other structures can evolve, like spots with neighbours arranged in a rhombic grid, stipes, zig-zag patterns, honeycomb structures (fig. 2), chevron-like patterns, and so called "eyes" (either dark or bright circles with a bright or dark centre).

The most prominent feature of Turing structures is that they remain stationary in time and space, as long as the reaction conditions are kept constant. The effect of perturbations on the stationary patterns are also a topic of investigations [47]. A physiologically meaningful type of perturbation consists in applying an electric field to a reaction medium in which Turing patterns are formed. Figure 7 shows hexagonal patterns that are perturbed by such an external field, giving rise to domains of stripes within the original hexagonal patterns. Thus, changes in the geometry and shape of the stationary patterns can be induced by an external control parameter, the electrical field strength.

4.4 Biomimetic aspects of Turing patterns

The biomimetic quality of Turing’s suggestions on "the chemical basis of morphogenesis" [42] seems to be obvious at a first glance. The postulate which is based on purely theoretical arguments has been proven to hold in experiments if the appropriate chemical reaction systems are used. The requirement of a slowly diffusing activator and a faster diffusing inhibitor are a feature that is well conceivable to occur in biological systems, where the activator could be a bulky protein and the inhibitor a much smaller, and therefore more mobile molecule. Another aspect which seems to underline the good biomimetic character of the Turing mechanism lies in the structure of the stationary patterns that it is capable of generating. The stationary patterns closely resemble those seen in the pigmentation of some animals and plants. All the shapes of patterns produced by Turing’s mechanism are also found in living systems [48].

However, the big draw-back from the biomimetic point of view is the lack of conclusive evidence showing that such patterns in living systems are indeed formed due to the concepts put forward by Turing. Whether a Turing mechanisms is responsible for the generation of gradients of genetic material in Drosophila embryos remains a controver-
Figure 7: Left: hexagonal patterns formed in the CDIMA reaction. Three domains with π hexagonal patterns are separated by defects. Right: patterns formed by applying an electric field to the π hexagons: note the intercalation of domains of stripes in the original hexagonal patterns.

sly discussed, still open question. On the other hand, no conclusive evidence has been presented yet that definitively eliminates the Turing mechanism as the origin of pattern formation.

Nevertheless, Turing's ideas for the spontaneous evolution of stationary structures [42] represent a conceivable and appealing concept for pattern formation which is based on very basic assumptions. As such it represents a very valuable physical and biomimetic concept.

5 Self-organization arising from reactions coupled to non-diffusive transport

So far we have investigated chemical model systems which form spatiotemporal patterns due to interactions of nonlinear chemical reactions with diffusion. In this Section we briefly introduce a supramolecular chemical reaction system that shows self-organization in time in a spatially homogeneous environment. In the present case, self-organization arises from an interplay of chemical reactions with a non-diffusive kind of transport.

The system under investigation is a synthetic supramolecular reaction which is designed as to reproduce the reactivity, the kinetics, and the most prominent structural features encountered in the natural cytochrome P450 system. The components of this biomimetic model were specially designed and synthesized in order to reproduce these properties from
Figure 8: Schematic representation of the cytochrome P450 mimic system. The sequence of redox cycles involved, and the structures of the key components (the manganese porphyrin 1 and the Rh complex 2) are depicted. Picture taken from [49].

its natural counterpart [49, 50, 51].

The biomimetic system consists of a manganese porphyrin (1) which is incorporated deeply into the lipophilic region of a phospholipid bilayer as shown in fig. 8. The latter is formed of a zwitterionic phospholipid to which an amphiphilic rhodium complex (2) is intercalated in the concentration ratio of [phospholipid]:[2] = 500:1. Due to electric repulsion the charged substrates (formate and NADPH) are not capable of penetrating into the hydrophobic domain of the phospholipid bilayer. The aqueous phase contains the substrates sodium formate (or NADPH) and oxygen. In the synthetic system the porphyrin 1, the rhodium complex 2, and the sodium formate mimic the enzyme cytochrome P450, the NADPH-cytochrome P450-reductase, and NADPH, respectively.

The supramolecular reaction system has been shown to catalyze the epoxidation of alkenes, a typical reaction for cytochrome P450. In addition, the turn-over numbers obtained for the synthetic supramolecular mimic were found to resemble those observed in
Figure 9: Temporal oscillations of the oxidation state of the artificial enzyme 1. The absorbance at 435 nm monitors the concentration of the manganese(III)porphyrin. Picture taken from [49].

reactions of the natural counterpart [50]. Thus, the supramolecular reaction system can be considered as a good biomimetic model for the natural cytochrome P450 system.

Preliminary results from studies of the dynamics of the artificial cytochrome P450 model system reveal that under certain reaction conditions, it shows oscillatory kinetics [49]. The oxidation state of the membrane-bound model enzyme 1 oscillates periodically between its manganese(II)porphyrin and its manganese(III)porphyrin states [49] (fig. 9). Such dynamics has not yet been observed in reactions with cytochrome P450. However, when the natural cytochrome P450 system is perturbed by a single light pulse, the reaction products were found to be formed periodically and to accumulate in a step-wise fashion [52, 53]. These results are consistent with oscillatory dynamics, and may indicate that oscillations also occur in reactions of natural cytochrome P450. However, a confirmation of oscillations in the natural cytochrome P450 system still needs experimental verification.

The involvement of the Rh complex 2 is crucial for the artificial cytochrome P450 system. In the absence of 2 no reduction of the manganese(III)porphyrin 1 (the model enzyme) was observed [51]. The complex 2 emulates the functions of the NADPH-cytochrome P450-reductase: first, it is reduced by the substrate (i. e. it accepts electrons from the substrate), then it transfers these electrons to the synthetic enzyme 1, which is intercalated in the hydrophobic region of the lipid bilayer. While the Rh moiety of the oxidized form of 2 carries a net positive charge, and therefore is located in the aqueous phase, its reduced form is hydrophobic. It is postulated that the Rh moiety shuttles between the aqueous phase and the hydrophobic membrane, thus mediating electrons from
the substrate to the artificial enzyme [51]. It seems unlikely that this transport process which mediates electrons from the aqueous phase to the hydrophobic porphyrin 1 via the Rh complex 2 can be described by normal, isotropic diffusion. So far, however, the nature of the mechanism of this electron transport across the membrane has not been investigated in detail, despite of its crucial role for the generation of oscillations. Experiments along these lines are currently in progress.

6 Concluding remarks

Spontaneous self-organization in space and in time is one of the characteristic properties of living systems. Using "simple" biomimetic chemical reaction systems, we have presented basic mechanisms which, in reactive systems, lead to the formation of propagating or stationary patterns, as well as to self-organization in time. In all cases, pattern formation is due to an interplay between the nonlinear reaction kinetics and the transport of molecules. The characteristics of the transport process play a prominent role in determining the type of patterns: diffusive transport has been shown to either induce propagating waves of excitation (when \( D_{\text{activator}} \geq D_{\text{inhibitor}} \), eq. 5) or stationary patterns (when \( D_{\text{activator}} \ll D_{\text{inhibitor}} \), eq. 7). Furthermore, a non-diffusive kind of transport in combination with nonlinear chemical reactions is also capable of promoting self-organization, as shown for the biomimetic cytochrome P450 model system.

The biomimetic systems presented in this Chapter were designed with the goal of reproducing the key properties that allow spontaneous self-organization. These properties impose conditions on the reaction kinetics and on the transport mechanism, however, they do not require any structural similarities on the molecular level between the chemicals of the biomimetic system and that of its natural counterpart.

Biomimetic reactions have been shown to be convenient laboratory models for the study of the rules governing the formation, properties, dynamics and fate of patterns that arise in biological systems. Biomimetic systems are expected to play an important role in further investigations aimed at effectively and conveniently controlling the dynamics of propagating spiral-shaped waves. In addition, the studies of biomimetic model reactions may open up new areas of research which are not (or very difficulty) accessible when using natural systems. As an example, we should like to point out emerging efforts to investigate the interactions found to occur between polymeric networks or lipid bilayers, at the one hand, and biochemical reactions, at the other. This research relies on the use of biomimetic systems with the challenge to elucidate further self-organizing principles operating in biological systems.
References


