1

Panta Rei: Everything Flows (*Heraclitus, 535–475 BC*)

1.1 HISTORICAL PERSPECTIVE

Change and motion define and constantly reshape the world around us, on scales from molecular to global. Molecules move, collide and react to generate new molecules; components of cells traffic to places where they are needed to participate in and maintain life processes; organisms congregate to perform collective tasks, produce offspring or compete against one another. The subtle interplay between change and motion gives rise to an astounding richness of natural phenomena, and often manifests itself in the emergence of intricate spatial or temporal patterns.

Formal study of such pattern-forming systems began with chemists. Chemistry as a discipline has always been concerned with both molecular change and motion, and by the end of the nineteenth century the basic laws describing the kinetics of chemical reactions as well as the ways in which molecules migrate through different media had been firmly established. At that point, it was probably inevitable that sooner or later some curious chemist would 'mix' (as the profession prescribes) these two ingredients to 'synthesize' a system, in which chemical reactions were coupled in some nontrivial way to the motions of the participating compounds.

This was actually done in 1896 by a German chemist, Raphael Liesegang.[1] In his seminal experiment, Liesegang observed that when certain pairs of inorganic salts move and react in a gel matrix, they produce periodic bands of a precipitate (Figure 1.1).

Chemistry in Motion: Reaction–Diffusion Systems for Micro- and Nanotechnology Bartosz A. Grzybowski

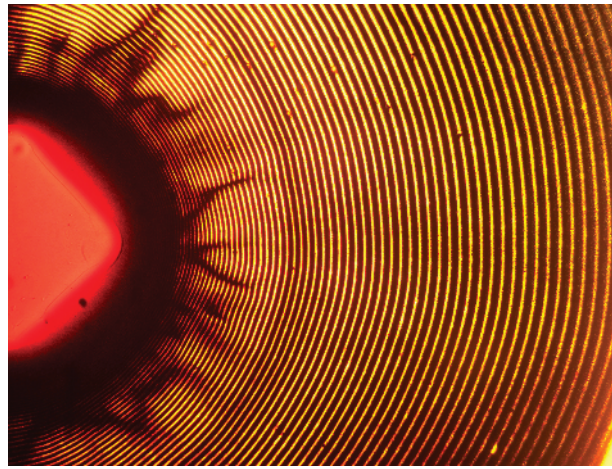

Figure 1.1 Classical Liesegang rings. A small droplet of silver nitrate (red region on the left) is placed on a thin film of gelatin containing potassium dichromate. As $AgNO_3$ diffuses into the gel and outwards from the drop, it reacts with $K_2Cr_2O_7$ to give regular, periodic bands of insoluble $Ag_2Cr_2O_7$. The bands in this picture are all thinner than a human hair

The surprising aspect of this discovery was that there was nothing in the mechanism of a chemical reaction itself that would explain or even hint at the origin of the observed spatial periodicity. Although Liesegang recognized that the patterns had something to do with how the molecules move with respect to one another, he was unable to explain the origin of banding, and the finding remained – at least for the time being – a scientific curiosity. By the early 1900s, however, examples of intriguing spatial patterns resulting from reactions of migrating chemicals in various arrangements had become quite abundant. In 1910, a nearly forgotten French chemist, Stéphane Le Duc, catalogued them in a book titled *Théorie Physico-Chimique de la Vie et Générations Spontanées* (Physical–Chemical Theory of Life and Spontaneous Creations),[2] in which he also alluded to the potential biological significance of such structures. Although his analogies between patterns in salt water and mitotic spindle or polygonal salt precipitates and confluent cells were certainly naive, Le Duc's work was in some sense prophetic. Several decades later, when his static patterns were supplemented by structures varying both in space and time, changing colors and propagating chemical waves, the analogy to living systems became clear. The ability to recreate life-like behavior in a test tube fuelled interest in migration/reaction systems. Chemists teamed up with biologists, physicists, mathematicians and engineers to explore the new universe of reactions in motion. Theory caught up, and several new branches of science – notably, nonlinear chemical kinetics and dynamic system theory – flourished. Mathematical tools and computational resources became available with which to model and explain a

wide range of previously puzzling phenomena, including the formation of skin patterns in certain animals, or the functioning of cellular skeleton. By the end of the last century, migration/reaction systems were certainly no longer considered a scientific oddity, but rather a key element of the evolving world.

And yet, despite these undeniable achievements, the nonlinear, pattern-forming chemical systems have not been widely incorporated in modern technology. Historically, the field focused on explaining the underlying physical phenomena, on model experiments in macroscopic arrangements and, more recently, on using the acquired knowledge to understand the existing biological systems. At the same time, we have not been able to apply this knowledge to mimic nature and to design new, artificial constructs that would use migration/reaction to make and control small-scale structures. Nevertheless, we argue in this book that such capability is within our reach and that migration/reaction is perfectly suited for applications in micro- and even nanotechnology. The underlying theme of this monograph is that by setting chemistry in motion in a proper way, it is not only possible to discover a variety of new phenomena, but also to build – importantly, without human intervention – micro-/nano-architectures and systems of practical importance. While we are certainly not attempting to create artificial life – a term that has become somewhat of a scientific cliché – we are motivated by and keen on learning from nature's ability to synthesize systems of chemical reactions programmed in space and time to perform desired tasks. In trying to do so, we limit ourselves to the most common and probably the simplest mode of migration – diffusion – and henceforth focus on the so-called reaction–diffusion (RD) systems.

1.2 WHAT LIES AHEAD?

Our discussion begins with illustrative examples of RD in both animate and inanimate formations chosen to emphasize the universality of RD at different length scales and the creativity with which nature uses it to build and control various types of structures and systems. Inspired by these examples, we then set a stage for the development of our own RD microsystems. In Chapters 2–4, we review the basics of relevant chemical kinetics and diffusion, set up a mathematical framework of RD equations and outline the types of methods that are used to solve them (this part is somewhat mathematically advanced and can probably be skipped on a first reading of the book). With these important preliminaries, we turn our attention to specific classes of micro- and nanoscopic systems, discuss the phenomena that underlie them and the technologically important structures they can produce. By the end of this journey, we will learn how to use RD to make microlenses and diffraction gratings, microfluidic devices and nanostructured supports for cell biology; we will see how RD can be applied in chemical sensing, amplification of molecular events and in biological screening studies. Although the examples we cover span several disciplines, we try to

keep the discussion accessible to a general reader and avoid specialized nomenclature wherever possible (after all, this book is not about some specific application, but about the generality of the RD approach to make small things). Since we envision this book to be not only instructive but also thought-provoking, we wish to leave the reader with a set of open-ended questions/problems (Chapter 11) that – in our opinion – will determine the future development of this rapidly evolving field of research. Throughout the text, we include over twenty boxed examples that are intended to highlight specific (and often more mathematical) aspects of the described phenomena. Finally, for those who would like to take a break from equations and strictly scientific arguments, we also provide some artistic respite in Appendix A, which deals with the application of RD to create microscale artwork.

1.3 HOW NATURE USES RD

Some examples of RD in nature are shown in Figure 1.2.

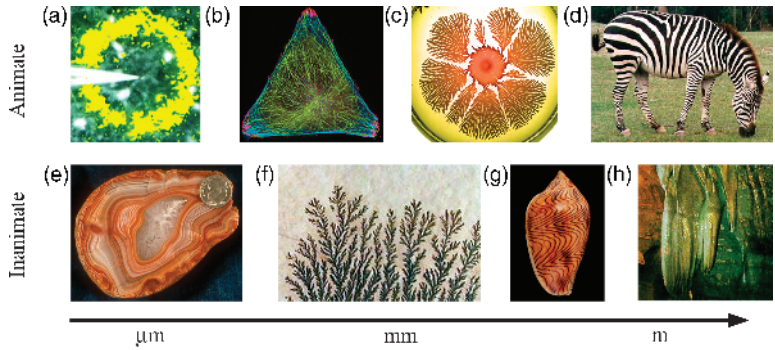

Figure 1.2 Examples of animate (a–d) and inanimate (e–h) reaction–diffusion systems on various length scales. (a) Calcium waves propagating in a retinal cell after mechanical stimulation (scale bar: 50 μm). (b) Fluorescently labeled microtubules in a cell confined to a 40 μm triangle on a SAM-patterned surface of gold (staining scheme: green = microtubules; red = focal adhesions; blue = actin filaments; scale bar: 10 μm). (c) Bacterial colony growth (scale bar: 5 mm). (d) Turing patterns on a zebra. (e) Polished cross-section of a Brazilian agate (scale bar: 200 μm) containing iris banding with a periodicity of 4 μm. (f) Dendritic formations on limestone (scale bar: 5 cm). (g) Patterns formed by reaction–diffusion on the sea shell *Amoria undulate*. (h) Cave stalactites (scale bar: 0.5 m). Image credits: (a) Ref. 6 (1997), *Science*, **275**, 844. Reprinted with permission from AAAS. (b) and (c) reprinted with permission from soft matter, micro- and nanotechnology via reaction diffusion, B. A. Grzybowski *et al*., copyright (2005), Royal Society of Chemistry (d) Ref. 30, copyright (1995), Nature Publishing Group. (e) Ref. 39, copyright (1995), AAAS. (f) Courtesy of Geoclassics.com. (g) Ref. 48, reproduced by permission of the Association for Computing Machinery. (h) Courtesy of M. Bishop, Niagara Cave, Minnesota.

1.3.1 Animate Systems

The idea that RD phenomena are essential to the functioning of living organisms seems quite intuitive – indeed, it would be rather hard to envision how any organism could operate without moving its constituents around and using them in various (bio)chemical reactions. Surprisingly, however, rigorous evidence that links RD to living systems is relatively fresh and dates back only to the discoveries of Alan Turing[3] in the 1940s and Boris Belousov[4] in the 1950s. Turing recognized that an initially uniform mixture containing diffusing, reactive activator and inhibitor species can spontaneously break symmetry and give rise to stationary concentration variations (i.e., to spatially extended patterns; Figure 1.3(a)). Belousov, on the other hand, discovered a class of systems in which nonlinear coupling between reactions and diffusion gives rise to chemical oscillations in time and/or in space (the latter, in the form of chemical waves; Figure 1.3(b)).

While at first sight these findings might not seem directly relevant to living species, it turns out that Turing's and Belousov's systems contain the essential 'ingredients' – nonlinear coupling and feedback loops – whose various combinations provide a versatile basis for regulatory processes in cells, tissues, organisms and even organism assemblies. For instance, Turing-like, instability-mediated processes can differentiate initially uniform chemical mixtures into regions of distinct composition/function and can thus underlie organism development; chemical oscillations can serve as clocks synchronizing biological events, and the waves can transmit chemical signals. The examples below illustrate how these elements are integrated into biological systems operating at various length scales.

A great variety of regulatory processes inside of cells rely on calcium signals mediated by oscillations or chemical waves. The temporal oscillations in Ca^{2+} concentration are a consequence of a complex RD mechanism (Figure 1.4), in which an external 'signal' first binds to a surface receptor and then triggers the synthesis of inositol-1,4,5-triphosphate (*IP3*) messenger. Subsequently, this

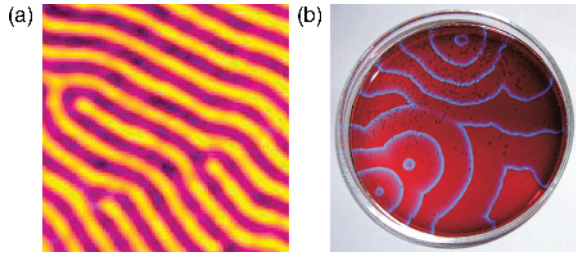

Figure 1.3 (a) Turing pattern formed by CIMA reaction. (b) Traveling waves in the Belousov–Zhabotinsky chemical system. (Image credits: (a) Courtesy of J. Boissonade, CRPP Bordeaux. (b) Courtesy of I. Epstein, Brandeis University. Reproduced by permission of the Royal Society of Chemistry.)

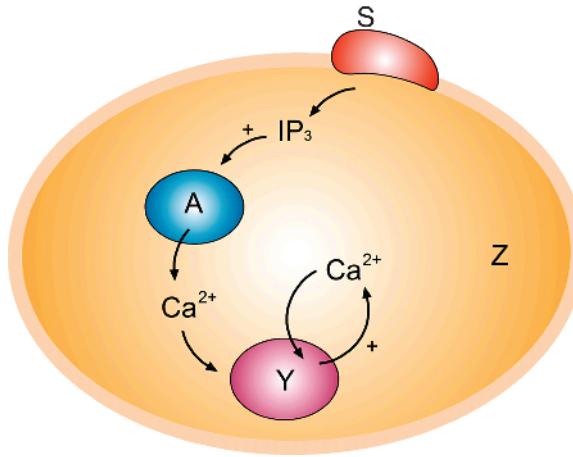

Figure 1.4 Schematic representation of a RD process controlling intracellular oscillations of Ca^{2+}

messenger causes the release of Ca^{2+} from the so-called *IP3* sensitive store, *A*, whose calcium influx into a cytosol (*Z*) activates an insensitive store *Y*. The net diffusion into and out of *Y* is regulated by a positive feedback loop regulated by calcium concentration in the *Z* region. Ultimately, this mechanism causes and controls rhythmic variations in the concentration of Ca^{2+} ions within the cell. These oscillations, for example, increase the efficiency of gene expression, where the oscillating signals enable transcription at Ca^{2+} levels lower than for steady-concentration inputs. In addition, changes in the oscillation frequency allow entrainment and activation of only specific targets on which Ca^{2+} acts, thereby improving the specificity of gene expression.[5] When calcium signals propagate through space (Figure 1.2(a)) in the form of chemical waves,[6,7] the steep transient concentration gradients of Ca^{2+} interact with various types of calcium binding sites (e.g., calcium pumps like ATPase;[8] buffers like calbindin, calsequestrin and calretinin;[9] enzymes like phospholipases[10] and calmodulin[11]) and give rise to complex RD systems synchronizing intracellular and intercellular events as diverse as secretion from pancreatic cells, coordination of ciliary beating in bacteria or wound healing.[12]

Many aspects of cellular metabolism and energetics also rely on RD. For example, glucose-induced oscillations help coordinate the all-important process of glycolysis (i.e., breaking up sugars to make high-energy ATP molecules), induce NADH and proton waves and can regulate other metabolic pathways.[13]

RD also facilitates efficient 'communication' between ATP generation (mitochondria) and ATP consumption sites (e.g., cell nucleus and membrane metabolic 'sensors'), which is essential for normal functioning of a cell.[14,15] In order to ferry ATP timely to ATP-deficient sites, nature has developed a sophisticated RD system of spatially distributed enzymes, collectively known as a 'phosphoryl wires'

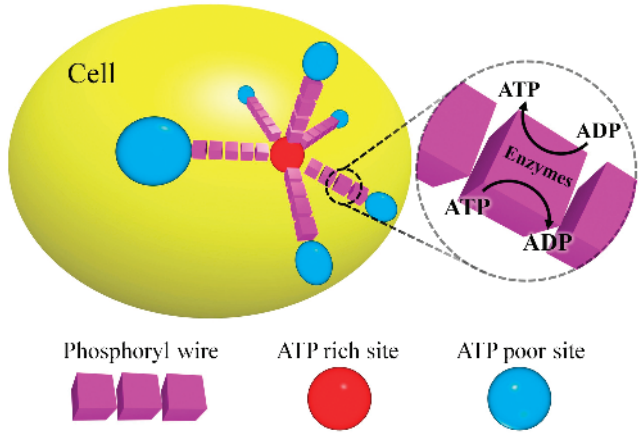

Figure 1.5 Cellular transport of ATP along 'phosphoryl wires' (purple) from an ATP generation site (red) to ATP consumption sites (blue). The panel on the right magnifies one unit of the wire. This unit comprises of a pair of enzymes: the first enzymes hydrolyzes ATP to ADP and generates chemical energy that triggers the reverse, ADP-to-ATP reaction on the second enzyme. The regenerated ATP diffuses to the next unit of the wire and the cycle repeats

(Figure 1.5). These enzymes hydrolyze ATP to ADP at one catalytic site while generating ATP from ADP at a neighboring site. The newly generated ATP then diffuses to another nearby enzyme and the process iterates along the wire. In this way, the ATP is 'pushed' along the wire in a series of domino-like moves called 'flux-waves'. Overall, ATP is delivered to a desired location rapidly, in a time significantly shorter than would be expected for a random, purely diffusive transport through the same distance.[14–16]

Finally, cells use RD to build and dynamically maintain their dynamic 'bones' called microtubules (cf. Figure 1.2(b)), which are constantly growing (at the so called plus-ends pointing toward the cell's periphery) and shrinking (at the minus-ends near centrosome). The balance between these processes depends on the local supply of monomeric tubulin components and a variety of auxiliary microtubule-binding proteins and GTP.[17]

As we have already mentioned in the context of calcium waves, RD can span more than a single cell. In some cases, such long-range processes can have severe consequences to our health. For instance, if RD waves of electrical excitation in the heart's myocardiac tissue propagate as spirals (Figure 1.6),[18] they can lead to life-threatening reentrant cardiac arrhythmias such as ventricular tachycardia and fibrillation.[19] Another prominent example is that of periodically firing neurons synchronized through RD-like coupling,[20] which can extend over whole regions of the brain and propagate in the form of the so-called spreading depressions – that is, waves of potassium efflux followed by sodium influx.[21] These waves temporarily shut down neuronal activity in the affected regions and can cause migraines and peculiar visual disturbances ('fortifications').[21]

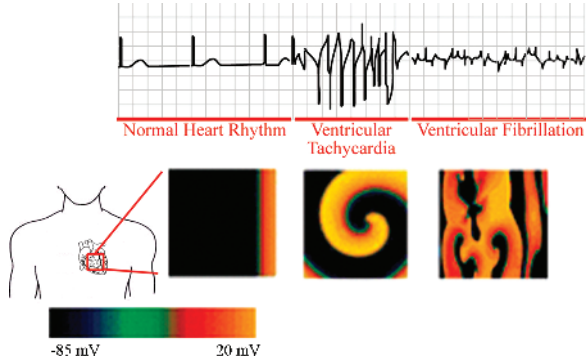

Figure 1.6 The top panel shows a representative ECG recording following the transition from a normal heart rhythm to ventricular fibrillation, an arrhythmia that can lead to sudden cardiac death. The bottom panel shows computer-generated images of RD electrical-activity waves involved in the transition. Left: a single electrical wave produced by the heart's natural pacemaker spreads throughout the heart and induces a contraction. These waves normally occur about once every 0.8 s. Middle: a spiral wave with a period of about 0.2 s can produce fast oscillations characteristic of an arrhythmia called tachycardia, which often directly precedes the onset of fibrillation. Right: multiple spiral waves produced by the breakup of a spiral wave can lead to fast, irregular oscillations characteristic of fibrillation. (Images courtesy of the Center for Arrhythmia Research at Hofstra.)

In organism development, RD is thought to mediate the directed growth of limbs. This process has been postulated[23–25] to involve transforming growth factor (TGFβ), which stimulates production of fibronectin (a 'cell-sticky' protein) and formation of fibronectin prepatterns (nodes) linking cells together into precartilageous nodules. The nodules, in turn, actively recruit more cells from the surrounding area and inhibit the lateral formation of other foci of condensation and potential limb growth.

RD is sometimes used to coordinate collective development or defense/survival strategies of organism populations. For example, starved amoebic slime molds (e.g., *Dictyostelium discoideum*) emit spiral waves of cAMP that cause their aggregation into time-dependent spatial patterns.[26] Similarly, initially homogeneous bacterial cultures grown under insufficient nutrient conditions form stationary, nonequilibrium patterns (Figure 1.2(c)) to minimize the effects of environmental stress.[27–29]

Lastly, some biological RD processes give rise to patterns of amazing aesthetic appeal. Skin patterns emerging through Turing-like mechanisms in marine angelfish *Pomacanthus*,[30] zebras (Figure 1.2(d)), giraffes or tigers[31,32] are but a few examples.

1.3.2 Inanimate Systems

While living systems use complex RD schemes mostly for regulatory/signaling purposes, inanimate creations employ RD based on simple, inorganic chemistries

to build spatially extended structures. Many natural minerals have textures characterized by compositional zoning (examples include plagioclase, garnet, augite or zebra spa rock)[33–38] with alternating layers composed of different types of precipitates. An interesting example of two-mineral deposition is the alternation of defect-rich chalcedony and defect-poor quartz observed in iris agates (Figure 1.2(e)).[39] Interestingly, the striking similarity to Liesegang rings[40–43] created in 'artificial' RD systems suggests that banding of mineral textures is governed by similar (Ostwald–Liesegang[44] or two-salt Liesegang[45]) mechanisms. Cave stalactites (Figure 1.2(h)) owe their shapes to RD processes[33] involving (i) hydrodynamics of a thin layer of water carrying Ca^{2+} and H^{+} ions and flowing down the stalactite, (ii) calcium carbonate reactions and (iii) diffusive transport of carbon dioxide. Formation of a stalactite is a consequence of the locally varying thickness of the fluid layer controlling the transport of CO_2 and the precipitation rate of $CaCO_3$. RD-driven dendritic structures (Figure 1.2(f)) appear on surfaces of limestone.[46] These dendrites are deposits of hydrous iron or manganese oxides formed when supersaturated solutions of iron or manganese diffuse through the limestone and precipitate at the surface on exposure to air. The structure of these mineral dendrites can be successfully described in terms of simple redox RD equations.[47] Finally, RD has been invoked to explain the formation and pigmentation of intricate seashells,[48] such as those shown in Figure 1.2(g).

1.4 RD IN SCIENCE AND TECHNOLOGY

The range of tasks for which nature uses RD in so many creative ways is really impressive. RD appears to be not only a very flexible but also a 'convenient' way of manipulating matter at small-scales – once RD is set in motion, it builds and controls its creations spontaneously, without any external guidance, and apparently without much effort. From a practical perspective, this sounds very appealing, and one might expect that hosts of smart scientists and engineers all over the world are working on mimicking nature's ability to make technologically important structures in this nature-inspired way. After all, would it not be great if we could just set up a desired micro-/nanofabrication process and have nature do all the tedious work for us (while we pursue one of our multiple hobbies)?

Probably yes, but for the time being it is more of a Huxley-type vision. In reality, until very recently, there have been virtually no applications of RD either in micro- or in nanoscience. Worse still, a significant part of research on RD is focused on how to avoid it! For example, engineers are striving to eliminate oxidation waves emerging via a RD mechanism on catalytic converters in automobiles and in catalytic packed-bed reactors (Figure 1.7). Such waves introduce highly nonlinear – and potentially even chaotic[49] – temperature and concentration variations that are challenging to design around, problematic to control and can drastically affect automobile emissions.[50] In catalytic packed-bed reactors, RD nonlinearities introduce hot zones,[51] concentration waves[52] and unsteady-state temperature

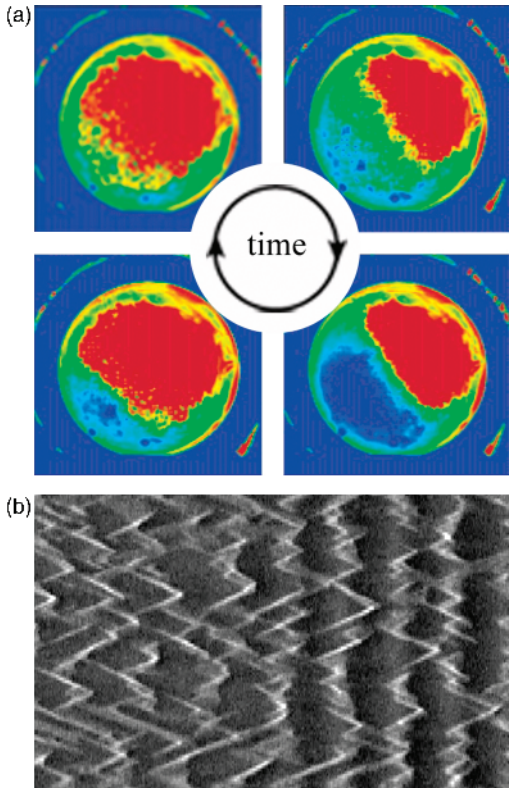

Figure 1.7 Reaction–diffusion in catalytic systems (a) Periodic temperature variations on the top surface of a packed-bed reactor (times are 40 s, 2 min, 4 min and 25 min starting in the upper left-hand corner and moving clockwise). (b) Feedback-induced transition from chemical turbulence to homogeneous oscillations in the catalytic oxidation of CO on Pt(110); this photo was taken between 85 and 125 s with homogeneous oscillations occurring around 425 s. (Image credits: (a) Ref. 51 © 2004 American Chemical Society. (b) Ref. 68 © 2003 American Physical Society.)

profiles[53] that can prevent the system from attaining optimal performance. These phenomena have significant impacts for industry (economic), as well as for the environment (societal).

There are several reasons why RD has not yet found its rightful place in modern technology. First, RD is difficult to bring under experimental control, especially at small scales. As we will see in the chapters to come, RD phenomena can be very sensitive to experimental conditions and to environmental disturbances. In some cases (albeit, rare), changing the dimensions of a RD system by few thousandths of a millimeter (cf. Chapter 9) can change the entire nature of the process this system supports. No wonder that working with such finicky phenomena has not yet become the bread-and-butter of scientists or engineers, who are probably accustomed to more robust systems. Second, even if this and other practical issues were resolved, RD would still present many conceptual challenges since the nonlinearities it involves often make the relationship between a system's ingredients and its final structure/function rather counterintuitive. It takes some skill – and often some serious computing power – to see how and why the various feedback loops and

autocatalytic steps involved in RD give rise to a particular structure/function that ultimately emerges. It is even harder to reverse-engineer a problem and choose the ingredients in such a way that a RD process would evolve these ingredients into a desired architecture. Third, there appear to be some 'sociological' barriers related to the interdisciplinarity of RD systems. Although RD phenomena are inherently linked to chemistry, relatively few chemists feel comfortable with coupled partial differential equations, Hopf bifurcations or instabilities. These aspects of RD are more familiar and interesting to physicists – few of them, however, are intimate with or interested in such mundane things as solubility products of the participating chemicals, ionic strengths of the solutions used, or the kinetics of the reactions involved. Finally, materials engineers who are often the avant-garde of micro- and nanotechnology, focus – quite understandably – on currently practical and economical techniques, and not on some futuristic schemes requiring basic researches. Given this state of affairs, it becomes apparent that implementing RD means making all these scientists talk to one another and cross the historical boundaries of traditional disciplines. As many readers have probably experienced in their own academic or industrial careers, it is not always a trivial task.

And yet, at least the author of this book believes that RD has a bright future – especially in micro- and nanotechnology. As we will learn shortly, RD can be controlled experimentally with astonishing precision and by sometimes surprisingly simple means. It can be made predictable and it can build structures for which current fabrication methods do not offer viable solutions. It can be made robust, economical and even interesting to students from different backgrounds. The following are some additional arguments.

(i) Micro- and nanoscales are just right for RD-based fabrication. Since the times required for molecules to diffuse through a given distance scale with a square of this distance (cf. Chapter 2), the smaller the dimensions of a RD system, the more rapid the fabrication process. With a typical diffusion coefficient in a (soft) medium supporting an RD process being $\sim 10^{-5}\,\mathrm{cm}^2\,\mathrm{s}^{-1}$, RD can build a $10\,\mu\mathrm{m}$ structure in about one-tenth of a second. To build a millimeter-sized structure, RD would have to toil for 1000 seconds, and making an object 1 cm across would keep it busy for 100 000 seconds. For RD, smaller is better.

(ii) RD can be initiated at large, easy-to-control scales and still generate structures with significantly *smaller* dimensions, down to the nanoscale. Liesegang rings can be much thinner that the droplet of the outer electrolyte from which they originate (cf. Figure 1.1), arms of growing dendrites are minuscule in comparison with the dimensions of the whole structure, and the characteristic length of the pattern created through the Turing mechanism is usually much smaller than the dimensions of the system containing the reactants.

(iii) The emergence of small structures can be *programmed* by the initial conditions of an RD process – that is, by the initial concentrations of the chemicals and by their spatial locations. In particular, several chemical reactions can be started simultaneously, each performing an independent task to enable parallel fabrication. No other micro- or nanofabrication method can do this.

(iv) RD can produce patterns encoding spatially continuous concentration variations (*gradients*). This capability is especially important in the context of surface micro-patterning – methods currently in use (photolithography,[54,55] printing[56,57]) modify substrates only at the locations to which a modifying agent (whether a chemical[58,59] or radiation[60,61]) is delivered, and they do so to produce 'binary' patterns. In contrast, RD can evolve chemicals from their initial (patterned) locations in the plane of the substrate and deposit them onto this substrate at quantities proportional to their local concentrations. Gradient-patterned surfaces are of great interest in cell motility assays,[62,63] biomaterials[64,65] and optics.[66,67]

(v) RD processes can be *coupled* to chemical reactions modifying the material properties of the medium in which they occur. In this way, RD can transform initially uniform materials into composite structures, and can selectively modify either their bulk structure or surface topographies. Moreover, the possibility of coupling RD to other processes occurring in the environment can provide a basis for new types of sensing/detection schemes. The inherent nonlinearity of RD equations implies their high sensitivity to parameter changes, and suggests that they can *amplify* small 'signals' (e.g., molecular-scale changes) influencing RD dynamics into, ideally, macroscopic visual patterns. While this idea might sound somewhat fanciful, we point out that chameleons have realized it long time ago and use it routinely to this day to change their skin colors. In Chapter 9 you will see how we, the humans, can learn something from these smart reptiles.

The rest of the book is about realizing at least parts of the ambitious vision outlined above. We will start with the very basics of reaction and diffusion, and then gradually build in new elements and classes of phenomena. Although by the end of our story we will be able to synthesize several types of RD systems rationally and flexibly, we do not forget that even the most advanced of our creations are still no match for the complex RD machinery biology uses. What we do hope for is that this book inspires some creative readers to narrow this gap and ultimately match biological complexity in human-designed RD.

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