# AN EXPERIMENTAL MODEL FOR MIMICKING BIOLOGICAL SYSTEMS: THE BELOUSOV–ZHABOTINSKY REACTION IN LIPID MEMBRANES

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#### ABSTRACT

Patterns and waves occur in many non-equilibrium processes. They are generated over a wide range of length scales and exhibit varying degrees of complexity. Ecosystem dynamics, self-organization at the cellular level and chemical oscillators are just three examples to illustrate the variety of systems where such phenomena occur. In this work we elucidate the mechanism through which patterns arise and propagate when the most famous example of a chemical oscillator (the Belousov–Zhabotinsky reaction) is carried out in a model biological environment, i.e. in the lamellar phase of several phospholipids that are relevant components of cell membranes. In particular, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) were chosen to mimic plasma membranes with different properties of bilayer fluidity and different chemical behaviour. Turing-like patterns and other complex structures were especially evidenced in the DPPC/water system in the range 18–40% w/w lipid content. The patterns obtained are discussed in terms of transport phenomena and coupling between independent oscillators. Interesting analogies are found with microemulsions, where the aqueous phase is confined in nanodroplets dispersed in a hydrophobic matrix.

Keywords: Belousov–Zhabotinsky reaction, biomimetic systems, cell membranes, lipid bilayers, morphogenesis, self-organizing systems.

### **1 INTRODUCTION**

Generation of patterns and shapes is a ubiquitous phenomenon in nature. It ranges from the microscopic level (e.g. self-organization in the cells) to the macroscopic level (e.g. the mantle of some animals) and it is transverse to living and non-living systems. At every level of complexity common features appear that seem to relate ecosystem dynamics to the development or to the organization of biological and chemical systems (the prey–predator model shares identical characteristics with chemical oscillators). Thus, understanding pattern formation is inherently related to understanding the notion of complexity in open systems [1, 2].

Stationary (or slowly varying) patterns, which occur in morphogenesis, and time-dependent patterns, which involve propagating waves, represent an important example of structures generated by a chemical or biochemical system. The Belousov–Zhabotinsky (BZ) reaction seems to present both types of patterns, depending on the specific operating conditions [3, 4].

The basic mechanism of morphogenesis, which is the differentiation of tissues starting from stem cells, was proposed 50 years ago by Turing [5], who pointed out that if morphogens obey a reaction–diffusion equation, they may undergo symmetry breaking transitions. This, in turn, generates spatially organized states, now simply known as 'Turing Structures', which may explain the initial stages of cell aggregation and development. In particular, if we deal with a generic reaction–diffusion system:

$$\frac{\partial C_i}{\partial t} = D_i \nabla^2 C_i + f_i(\dots, C_i, \dots, C_j, \dots),$$
(1)

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where  $C_i$  is the concentration of the *i*th species participating in the reaction,  $f_i$  represents the non-linear kinetic equation describing the reaction and  $D_i$  is the diffusion coefficient of the species *i*. Assuming that eqn (1) has an homogeneous steady-state solution,  $f_i(C_s) = 0$ , we consider the evolution of a small perturbation  $c_s$  around  $C_s$  and separate it in Fourier space,

$$c_s = \sum_k a_k \mathrm{e}^{\lambda_k t + i\bar{k}\cdot\bar{r}},\tag{2}$$

where  $\lambda_k$  is the growth rate of the mode with a wave vector  $\bar{k}$ . Substituting eqn (2) into eqn (1) and retaining only the linear term, we obtain an eigenvalue equation for  $\lambda_k$  for the linear operator

$$L_{ij} = F_{ij} - D_i k^2 \delta_{ij},\tag{3}$$

where  $F_{ij}$  is the Jacobian matrix  $(\partial f_i / \partial c_j)_{c=c_s}$  of the kinetic function  $f_i$ . Turing instability occurs when for a certain non-zero mode k, the real part of the eigenvalue  $\lambda_k$  of the operator (3) becomes positive so that the homogeneous steady state becomes unstable, and the system undergoes a transition from the homogeneous state to a patterned state.

Turing structures are characterized by a constant wavelength and a time-independent behaviour. They are generated when the diffusion coefficients of the morphogens (in the simplest case one activator and one inhibitor) are markedly different from one another.

Target patterns, where pulses are emitted periodically from the same leading centre (pacemaker), have been observed in a host of reaction–diffusion systems that are able to generate chemical oscillations. These systems occasionally produce more complex structures, e.g. outwardly rotating spirals [6].

Recently, Epstein and co-workers found hybrid patterns that blend the properties of waves and Turing structures. In particular, these patterns arise when the BZ reaction is carried out in water/oil nanodroplets [4, 7–9]. They are probably due to the coherent coupling among randomly distributed domains, each of them representing an independent oscillator [7]. The simultaneous presence of propagating waves and stationary patterns was also found by our group when the BZ reaction was carried out in the anisotropic environment of a lipid/water binary system. However, a different coupling mechanism has been invoked to explain the onset of complex patterns in this system [10].

Lipids in water represent a good model for biological environments, since in living systems different aqueous domains are usually separated by a membrane with a single phospholipid bilayer as the basic structural unit. Moreover, there are numerous systems where several bilayers are stacked; examples include the myelin structure around nerve axons [11], the membranes in retinal rods [12] and the extracellular lipid matrix of the stratum corneum [13].

In this paper we describe the study of the BZ oscillator in the aqueous compartment of different phospholipid/water lamellar phases. All the lipids studied are among the major components of plasma membranes. In particular, we used both fluid and rigid bilayers to mimic different biological environments. In fact, a saturated amphiphile with 16 carbon hydrophobic tails [1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)] behaves as a rigid membrane domain at room temperature, whereas a lipid with chains of 14 carbon atoms [1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)] is known to be in a fluid state above 23–24°C. A fluid, long-chain phospholipid, such as 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), was also used as a complementary system.

### 2 EXPERIMENTAL

The BZ reaction belongs to a wider class of reactions known as 'oscillating chemical reactions' and involves the catalytic oxidation of an organic substrate with active methylenic hydrogens (e.g. those of malonic acid) by  $BrO_3^-$  in a strongly acidic solution [14, 15].



Figure 1: Structure of the phospholipids used in this work.

Experiments on the BZ system were carried out at  $25^{\circ}$ C, with the exception of those containing DMPC. The reactor was a glass Petri dish of 6 cm diameter. All reactants used for the oscillator were of analytical grade (Sigma) and were used without further purification. The following stock solutions were prepared: KBrO<sub>3</sub> 0.5 M (0.5 M H<sub>2</sub>SO<sub>4</sub>), malonic acid 0.5 M, ferroin 0.025 M and KBr 0.16 M. The phospholipids chosen as models of biological matrices were: DPPC, DOPC, DMPC (see Fig. 1 for the structures of the phospholipids). The phospholipids were purchased from Northern Lipids and used as received.

The BZ/lipid systems were prepared by mixing phospholipids with all the BZ reactants, except ferroin, in the following quantities: KBrO<sub>3</sub> 1.12 ml, malonic acid 0.56 ml, KBr 0.16 ml. All solutions were homogenized by vigorous stirring and warm–cold cycles, then ferroin was added and oscillations began. The total volume of each system was 2 ml. Photographs were taken with a high-resolution digital camera.

The structural properties of the BZ/DPPC and BZ/DMPC systems were investigated by smallangle X-ray scattering (SAXS) to verify that a lamellar structure was preserved in the presence of the BZ reactants. Results for the DPPC/BZ system showed that the typical smectic pattern of a lamellar stack was present for lipid content ranging from 7% up to 40% w/w [10].

Data collected on the DMPC/BZ system showed a more complex behaviour, and only a limited number of monophasic samples was found in the presence of the BZ reactants. In particular, the lipid concentration range between 12–13% and 30% by weight was macroscopically demixed and could not be used as a matrix for the BZ reaction. Samples with DMPC 10% w/w were therefore used to carry out the oscillating reaction.

Chemical decomposition of the lipids, which might occur in the reactive and acidic environment of the BZ solution, was checked and ruled out by high-resolution NMR (Bruker DRX 600 AVANCE). Data collected at different time intervals from sample preparations showed that no chemical decomposition took place for at least 5–6 h, either in the DPPC/BZ or DMPC/BZ system. Both SAXS and NMR measurements were performed on 'blank systems', i.e. the same solutions used for the BZ reaction but with the catalyst  $Fe(o-phen)_3$  being replaced by its inert analogue  $Zn(o-phen)_3$ . This was done to avoid complications that might arise from wave propagation during control experiments.

The BZ/DOPC system was not investigated for structural and chemical integrity since, from preliminary experiments, it was evident that the DOPC lipid matrix reacted with the BZ mixture.

However, some interesting aspects of this system will be discussed in the following sections in order to elucidate the communication mechanism between different aqueous domains.

### **3 RESULTS**

In this section an extensive analysis of the DPPC/BZ system will be presented. In fact, this system was the most stable over a wide interval of lipid concentration as has been outlined above. However, interesting results were also obtained for the DMPC/BZ and DOPC/BZ systems.

### 3.1 DPPC

All experiments in the lamellar phase of this phospholipid were carried out at  $25^{\circ}$ C. When the DPPC percentage varied between 7 and 10% w/w, no significant differences were observed with respect to the BZ reaction performed in bulk water (Fig. 2). The same travelling waves and pacemaker structures were visible both in the upper and in the lower surface of the reactor.

When the lipid content was increased, new and peculiar behaviour could be evidenced. Figure 3a shows the system with DPPC 18% w/w. The stationary complex structure coexisted in different zones of the Petri dish. In particular, labyrinthine Turing-like patterns were found in the centre (Fig. 3b) of the reactor and inwardly rotating spirals were found at the edge (Fig. 3c).

When the lipid content reached 25% w/w, inwardly rotating spirals (Fig. 4a) coexisted with spiral-like structures (Fig. 4b) and striped standing waves (Fig. 4c).

In the systems with 18 and 25% lipid content, structures were typically formed at the bottom of the Petri dish, and waves became visible on the upper surface only after 10–15 min. No complete synchronization was observed between the patterns developed on opposite sides. Moreover, all chemical waves started at the edge of the dish, except for a few cases in which pacemaker structures appeared in the middle of the reactor and slowly diffused toward the centre. This behaviour was probably due to the zero flux boundary conditions. After 8–10 min from the initial development of patterns, labyrinthine structures or standing striped waves appeared, and persisted till the end of the reaction. Inwardly rotating spirals appeared 1–2 min after the beginning of the experiment and persisted for the whole course of the reaction.



Figure 2: Travelling waves and pacemaker structures formed by the BZ reaction in pure water (a) and in the BZ/DPPC system with 10% w/w lipid content. (b) No significant differences were found for the structures observed in the two media.

For lipid concentrations higher than 40% w/w, none of the systems presented any type of interesting structures, and only a few blue filled circular spots that slowly formed the uniform reduced steady state (typical of the end of the process) were observed (light grey colour in Fig. 5).

# 3.2 DMPC

The internal fluidity of the bilayer varies with temperature, chain length and degree of saturation of lipids. Once the lipid type and composition that generate the bilayer are fixed, only the temperature is a controlling parameter for 'chain melting' [16]. In the case of pure DMPC, the transition from a gel-like state (in which chains are fully extended and tightly packed) to a liquid-crystalline state (in which chains are highly mobile and disordered) occurs at  $23^{\circ}$ C.

This value is particularly suited to investigate the influence of bilayer fluidity on the BZ system, because it is well known that the kinetic and the overall behaviour of an oscillating reaction are deeply



Figure 3: (a) The BZ/DPPC system with 18% w/w lipid content. (b) Stationary Turing-like labyrinthine structure. (c) Inwardly rotating spirals. All the photographs refer to the lower surface of the reactor. Horizontal bar scales = 1 mm.



Figure 4: The BZ/DPPC system with 25% w/w lipid content. (a) Striped standing waves, (b) inwardly rotating spirals and (c) spiral-like structures generated from the collision between travelling waves and spirals. Horizontal bar scales = 1 mm.



Figure 5: The DPPC/BZ system with 40% w/w lipid content. This system is not able to form any kind of structure.



Figure 6: The DMPC/BZ system with 10% w/w lipid content. Experiment performed at (a) 18°C and (b) 30°C.

influenced by temperature changes. Thus, a lamellar phase containing DPPC (chain melting point at  $42^{\circ}$ C) or another lipid with a gel/liquid crystal transition far from room temperature would not be suitable for our purposes.

Figure 6a shows the patterns obtained in the DMPC/BZ system with 10% w/w lipid content, performed at a controlled temperature of 18°C. In these conditions, the structures obtained were similar to those observed in the DPPC/BZ system at 10% w/w lipid content and did not differ from those found in pure water in the sense that no interesting structure or stationary patterns were observed.

When the temperature was increased up to 30°C, interesting structures were not found again, even if the propagating wave fronts were appreciably different from those observed at 18°C (Fig. 6b). In addition, in the DMPC/BZ system at 30°C wave fronts were generally less compact. This probably reflected the more disordered state of the lipid chains.



Figure 7: The BZ reactants mixed with DOPC. (a) The red (dark grey in the picture)–blue (light grey in the picture) colour change took place in about 30s through the development of some spots in the solution. (b) The uniform blue (light grey in the picture) oxidized state typical of the absence of oscillation.

# 3.3 DOPC

When the BZ reactants were added to DOPC, the colour of the catalyst suddenly changed from ferroin red (reduced state) to ferriin blue (oxidized state) without forming any kind of waves (Fig. 7) and without further variation. This behaviour indicates that the lipid substrate interferes with the oscillating mechanism of the BZ reaction. The only possible explanation for such a different behaviour with respect to saturated lipids (DPPC and DMPC) is that the DOPC double bonds react with the oxidizing species in solution, preventing catalyst regeneration.

#### 4 DISCUSSION

The experimental results reported in this paper showed that the anisotropic environment of lipid/water lamellar phases is able to generate stationary patterns, which extends earlier studies carried out in water nanodroplets. Our findings strengthen the relevance of spatial symmetry that breaks processes in heterogeneous media into the biological self-organization theories. In particular, two aspects deserve further discussion: (i) the mechanisms of coupling and synchronization of different aqueous domains, and (ii) the influence of lipid concentration on the global behaviour of the BZ system.

The occurrence of pore-like defects in lipid membranes has been widely established. This explains the diffusion of small molecules through water compartments that should be, in principle, separated by a lipid barrier. In fact, although permeability is low enough to maintain high gradients of ions and polar solutes, a significant flux of small neutral molecules such as water, urea and simple monocarboxylic acids has been measured [17, 18] and explained theoretically [17, 19, 20]. In our system, several radicals and reactive intermediates, such as Br<sup>•</sup>, BrO<sup>•</sup><sub>2</sub>, Br<sub>2</sub> and HBrO<sub>2</sub>, could play this function and allow the communication between independent oscillators. The case of the DOPC/water system is significant in this respect, since it shows that 'active' species do cross the barrier of the hydrophobic chains and react with double bonds located more than 10 Å apart from the polar surface of the lipid bilayer.

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Comparing different types of bilayers, as we have done in this paper, it appears that more numerous and complex patterns are obtained in the DPPC/BZ system at room temperature, i.e. when the lipid chains are in the gel state. This is apparently in contrast with the higher permeability of fluid membranes with respect to rigid bilayers. However, it should be kept in mind that neither the free coupling of oscillators, which occurs in bulk solution, nor the slightly restricted coupling, which occurs in percolated microemulsions [4], are effective in producing stationary patterns. This seems to indicate that only restricted communication among oscillators, which are located in different water domains, can generate certain types of patterns, such as Turing structures or inwardly rotating spirals.

Patterns observed during the development of some bacterial colonies are strongly dependent on the concentration of cells [21, 22]. A similar behaviour was observed in the BZ/DPPC system where the substrate was the lipid/water matrix. In particular, increasing DPPC concentration from 10% w/w to 18% w/w and higher brought the system to a different spatial organization. As illustrated in the previous section, labyrinthine patterns were found at 18% w/w lipid content and were replaced by striped standing waves at 25% w/w. Inwardly rotating spirals were present in systems containing DPPC  $\geq$  18% w/w, but wavelengths varied with lipid content. Thus, the phospholipid concentration seems to affect both the communication among oscillators and the diffusivity of the reactive species. In particular, the upper and lower surfaces of the reactor showed identical patterns only at low DPPC and DMPC concentrations (7–10% w/w), whereas at higher lipid content no efficient synchronization between the two surfaces was observed. However, the specific influence of the system composition on the communication between different oscillators and on the diffusivity of chemical species is not well understood yet, and further investigation is required to clarify this point.

### **5** CONCLUSION

Understanding complex biological processes may draw useful hints from simple models which are able to resume their principal characteristics. The BZ reaction–diffusion system is a good candidate to catch key features of pattern and structure generation, in both homogeneous and heterogeneous media.

In this paper, we studied wave propagation and patterns obtained when the BZ reaction-diffusion system is confined in different lipid/water systems, as models of biological membranes. The chemical nature of the lipids used and the physical properties (e.g. fluidity) of the corresponding bilayer membranes were important features to define the type and number of patterns. The lipid concentration also acted as a control parameter. A range of lipid (and consequently water) content was evidenced at which complex patterns were present. The most interesting system in this respect was the DPPC/water system, where Turing structures and inwardly rotating spirals arose and fell down between 18 and 40% w/w lipid content.

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