Synchronized Nano-Oscillators

DOI: 10.1002/ange.200802339

Diffusively Coupled Chemical Oscillators in a Microfluidic Assembly**

*Masahiro Toiya, Vladimir K. Vanag, and Irving R. Epstein**

From fireflies that synchronize their flashes with each other[1] to heart muscles contracting and relaxing in unison,[2] synchronized behavior of living cells or organisms is ubiquitous in nature.[3] Chemical reaction–diffusion systems can help us understand the mechanisms that underlie such synchronization. Coupled chemical oscillators have previously been studied in the laboratory with large reactors connected directly by small channels for controlled mass exchange of bulk solution.[4–7] In this case, coupling occurs via all species. In living systems, however, coupling often occurs through special signaling molecules, as in synaptic communication or chemotaxis.[8] Collections of neural oscillators can access a vast repertoire of coordinated behavior by utilizing a variety of topologies and modes of coupling, including gap junctions and synaptic links, which may be either excitatory or inhibitory, depending on the neurotransmitter involved.

To mimic such a fine level of communication in a chemical system, we need to do two things: a) reduce the size of each oscillator in order to bring the characteristic time of communication between diffusively coupled oscillators to or below the period of oscillation; and b) introduce a semipermeable membrane or other medium between the microoscillators to permit communication only via selected species. These goals can be achieved with the use of microfluidic devices. Our experimental system (Figure 1 a) is a linear array of tens of droplets of nanoliter volume containing aqueous ferroin-catalyzed Belousov–Zhabotinsky (BZ)[9] solution separated by octane drops in a glass capillary. The BZ reaction, in which the oxidation of malonic acid (MA) by bromate is catalyzed by a metal complex in acidic aqueous solution, is a well known chemical oscillator. Owing to the small spatial extent ($l_w = 100$–400 μm) of the BZ droplets, the characteristic time of diffusive mixing within a single droplet, l_w^2/D (5–80 s, D = diffusion constant of aqueous species), is smaller than the period of oscillation (180–300 s), and individual BZ droplets can be considered homogeneous. Bromine, an inhibitory intermediate of the BZ reaction, is quite hydrophobic and diffuses readily into hydrocarbons such as octane, thus mediating inhibitory interdroplet coupling. We have shown

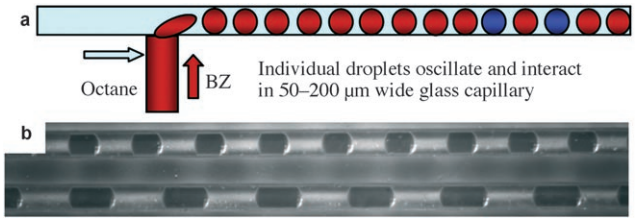

Figure 1. a) Schematic representation of the microfluidic device. Red droplets correspond to the reduced form of the catalyst (ferroin), blue droplets to the oxidized form (ferriin). A new method for fabricating such junctions is outlined in the Supporting Information. b) Snapshot of two capillaries with droplets. BZ droplets with convex surfaces are dark due to ferroin. Horizontal length of the frame and inner diameter (ID) of the capillary are 4.8 mm and 150 μm, respectively. BZ droplets were recorded by a CCD camera through a microscope with illumination by light passed through a 510 nm interference filter.

theoretically[10] that in such heterogeneous systems patterns analogous to the Turing patterns[11,12] found in homogeneous systems can emerge.

Without compartmentalization, the homogeneous BZ solution in a similar capillary exhibits trigger waves of excitation. Partitioning the medium into droplets dramatically changes this behavior. For BZ droplets (Figure 1 b) with $l_w > 400$ μm or oil droplets with length $l_O > 400$ μm, no discernible coherent patterns are seen. However if $l_w = 100$–400 μm and $l_O = 50$–400 μm, we observe stable anti-phase oscillations (Figure 2 a) at larger [MA] (greater than 100 mM) and Turing patterns (Figure 2 b) at smaller [MA] (less than 40 mM). At higher levels of [MA], initially in-phase arrays of droplets evolve to an anti-phase configuration within a few periods of oscillation (Movie in Supporting Information). For [MA] = 40 mM, the transition to the Turing regime goes through intermediate anti-phase oscillations. For slightly smaller [MA] (35 mM), initially in-phase droplets transform into Turing patterns almost immediately, without intermediate anti-phase oscillations. At small [MA], the behavior is rather sensitive to the size of droplets, with small drops reaching stationary state more rapidly than larger ones.

To establish whether bromine is responsible for communication between the BZ droplets, surfactant Span 80 (sorbitan mono-oleate) at concentrations of 5% was added to the octane. In separate experiments, it was found that Span 80, which possesses an unsaturated double bond in its hydrocarbon tail, reacts with bromine in octane in less than 1 s. The water-insoluble Span 80 thus acts as a trap for bromine, removing it from the octane. When Span 80 is added to the droplet system, inhibiting the communication between BZ droplets, individual droplets oscillate independently. If we initiate the system (see Experimental Section) with all droplets in the same phase, in-phase oscillations persist.

[*] Dr. M. Toiya, Prof. V. K. Vanag, Prof. I. R. Epstein
Department of Chemistry and Volen Center for Complex Systems
MS015, Brandeis University, 415 South St., Waltham, MA 02454 (USA)
Fax: (+1) 781-736-2516
E-mail: epstein@brandeis.edu
Homepage: http://hopf.chem.brandeis.edu/

[**] This work was supported by the National Science Foundation (CHE-0615507) and the Defense Advanced Research Projects Agency. We thank Milos Dolnik and Anatol Zhabotinsky for helpful comments and suggestions.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200802339.

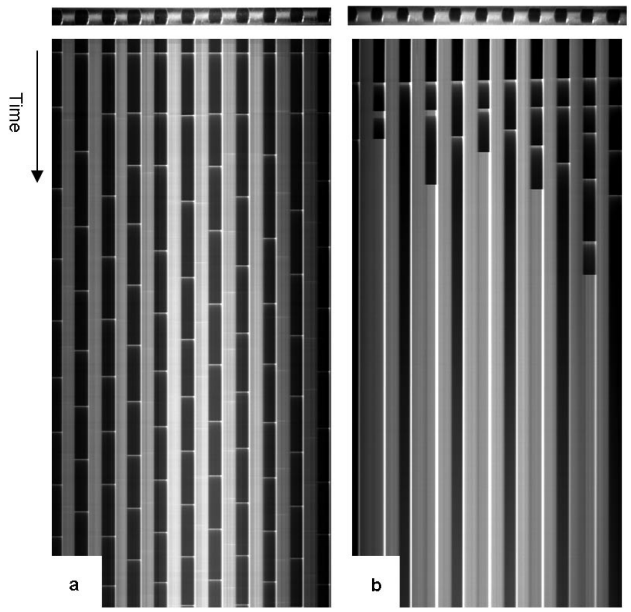

Figure 2. Space–time plots showing a) anti-phase oscillations with spikes of oxidation of ferroin seen as light horizontal lines across BZ-droplets and b) stationary Turing structures with alternating oxidized and reduced states evolving from an initial oscillatory state. Horizontal lengths of the frame and the capillary ID are 4.8 mm and 150 μm, respectively; the total times for (a) and (b) are 5200 s and 10800 s, respectively. Patterns extend to the left and right of the segments shown.

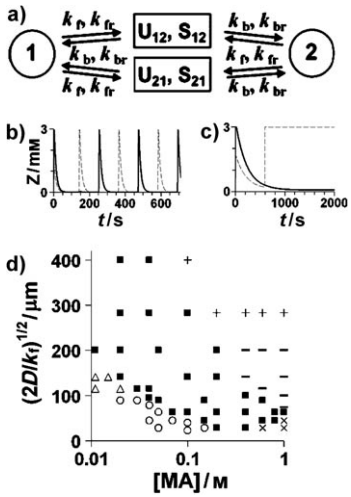

Figure 3. a) Configuration of two coupled BZ oscillators used in simulations. b) Typical anti-phase oscillations. c) Typical Turing mode; bold and dashed lines in (b) and (c) represent z in two neighboring BZ droplets. d) Parametric diagram in [MA]–k_f plane for two oscillators whose initial phases are slightly shifted ($k_b = k_f/P_U$, $k_{br} = k_{fr}/P_S$; partition coefficients $P_U = 20$, $P_S = 1$, $[H^+] \equiv h = 0.2$ M, $[BrO_3^-] \equiv A = 0.3$ M, $z + c = 3$ mM); $D = 2 \times 10^{-5}$ cm^2 s^{-1} is used for conversion of k_f into a characteristic length $L = (2D/k_f)^{1/2}$. Symbols: ○ and △: Turing mode for $k_{fr} = 0$ and for both $k_{fr} = 0$ and $k_{fr} = k_f$, respectively; ■: anti-phase oscillations; −: unstable in-phase oscillations transforming into anti-phase oscillations for larger number (four or six) of coupled oscillators; ×: stable in-phase oscillations (for $k_{fr} = 0$); Turing mode marked by ○ is replaced by in-phase oscillations (×) for $k_{fr} = k_f$; +: weak communication (initial phases of oscillators change very slightly after ten periods of oscillations). Subscripts "12" and "21" refer to U ($[Br_2]$) and S ($[BrO_2^{\bullet}]$) in octane droplets.

Seeking to understand our experimental results in more depth, we performed a series of computer simulations to ascertain whether bromine is the single, or at least the major, signaling molecule, and whether other patterns may be found at smaller droplet sizes inaccessible in our experiments. The BZ reaction generates a second, excitatory intermediate, $BrO_2^{\bullet}$, which is also capable of diffusing in the oil phase. The model, which is described in detail in the Supporting Information, is based on the Field–Körös–Noyes (FKN) mechanism[13] of the BZ reaction and employs seven concentration variables to describe the aqueous phase and two more, corresponding to $[Br_2]$ and $[BrO_2^{\bullet}]$, for the oil phase. The concentrations of the major reactants, H^+, MA, BrMA, and BrO_3^- which are significantly larger than those of the variable species, are taken to be fixed in a given experiment. The model contains $9n$ variables for n coupled oscillators. In addition to the initial concentrations, key parameters in the model include the coupling constants, k_f and k_{fr}, which characterize the strength of coupling mediated by Br_2 and $BrO_2^{\bullet}$, respectively. We simulated arrays with two (Figure 3a), four, and six coupled oscillators to investigate how the behavior of the system depends on the number of oscillators.

The two stable modes found in the experiments, anti-phase oscillations and Turing patterns, are seen in the model at large and small [MA], respectively, and are shown in Figure 3b and c. Note that if $k_f = 0$, that is, coupling via Br_2 is absent, neither the anti-phase oscillations nor the Turing mode occurs, so Br_2 is an essential "messenger" for these two regimes. At higher [MA] (greater than 0.2 M), where the anti-phase mode dominates, the results of the simulation are nearly independent of the presence of $BrO_2^{\bullet}$, while at lower [MA] (less than 0.1 M) and at large k_f (greater than 0.5 s^{-1}), the Turing mode dominates at $k_{fr} = 0$ and the in-phase mode dominates at $k_{fr} = k_f$ (Figure 3d). Since at $k_{fr} = k_f$ the Turing mode is found only at [MA] < 20 mM, while in our experiments we found the Turing mode at larger [MA] (40 mM), we infer that $BrO_2^{\bullet}$ plays a minor, if any, role in communication between the BZ droplets.

For many sets of parameter values, two or more modes are simultaneously stable, and the mode obtained depends upon the initial conditions. Simulations with four and six oscillators, however, reveal that, when they coexist, the in-phase mode is always less stable than the Turing or anti-phase modes. Stable in-phase behavior is found only at large $k_{fr} = k_f$, corresponding to very small droplet lengths (less than 100 μm). With such small droplets, we also find several more exotic regimes, some of which are illustrated in the Supporting Information.

Chemical nano-oscillators diffusively coupled by known signaling species may provide useful analogs for biological processes. The microfluidic BZ–octane system employed here is convenient in that we are able to identify the inhibitor bromine as the main messenger species, and the production and function of bromine in the overall BZ process are well characterized. By choosing the fundamental oscillator and the scavenger species added to the connecting medium, it should be possible to build systems with controllable degrees of

inhibitory or excitatory coupling. Microfluidic technology makes it possible to construct two- as well as three-dimensional arrays of coupled oscillators. The possibility of developing computational devices by combining oscillatory chemical reactions with droplet-based microfluidic techniques has recently been suggested.[14]

Experimental Section

BZ mixture: The aqueous reaction mixture contains H_2SO_4 (80 mM), $NaBrO_3$ (0.288 M), and ferroin (3 mM). In addition, for experiments on the oscillatory mode we add NaBr (10 mM) and MA (0.64 M), while for experiments on Turing patterns [NaBr] = 0, [MA] = 30–50 mM. To make the BZ reaction photosensitive, we add a small amount (0.4 mM) of $[Ru(bpy)_3]$ (bpy = bipyridine).[15]

Microfluidics: The BZ solution and octane are driven by syringe pumps into a microfluidic junction at the entrance to the capillary as shown in Figure 1a. The sizes of and separation between the BZ droplets depend upon the junction size and the flow rates with which the two components are injected into the system.[16] Before the start of each experiment, we put the BZ micro-oscillators into the in-phase mode by illuminating the capillary with a strong 450 nm light. Further technical details are given in the Supporting Information.

Received: May 19, 2008
Published online: August 29, 2008

Keywords: Belousov–Zhabotinsky reaction · coupled oscillators · microfluidics · oscillatory reactions · synchronization

[1] A. Moiseff, J. Copeland, *J. Insect Behav.* **2000**, *13*, 597.
[2] L. Glass, *Nature* **2001**, *410*, 277.
[3] A. Pikovsky, M. Rosenblum, J. Kurths, *Synchronization: A Universal Concept in Nonlinear Science*, Cambridge University Press, Cambridge, **2001**.
[4] M. Marek, I. Stuchl, *Biophys. Chem.* **1975**, *3*, 241.
[5] M. Dolnik, M. Marek, *J. Phys. Chem.* **1988**, *92*, 2452.
[6] G. Dechert, K. P. Zeyer, D. Lebender, F. W. Schneider, *J. Phys. Chem.* **1996**, *100*, 19043.
[7] M. F. Crowley, I. R. Epstein, *J. Phys. Chem.* **1989**, *93*, 2496.
[8] H. C. Berg, *E. Coli in Motion*, Springer, New York, **2004**.
[9] A. M. Zhabotinsky, *Proc. Acad. Sci. USSR Chem. Sect.* **1964**, *157*, 4.
[10] V. K. Vanag, I. R. Epstein, *J. Chem. Phys.* **2003**, *119*, 7297.
[11] A. M. Turing, *Philos. Trans. R. Soc. London Ser. B* **1952**, *237*, 37.
[12] V. Castets, E. Dulos, J. Boissonade, P. De Kepper, *Phys. Rev. Lett.* **1990**, *64*, 2953.
[13] R. J. Field, R. M. Noyes, E. Körös, *J. Am. Chem. Soc.* **1972**, *94*, 8649.
[14] I. R. Epstein, *Science* **2007**, *315*, 775.
[15] V. Gáspár, G. Bazsa, M. T. Beck, *Z. Phys. Chem.* **1983**, *264*, 43.
[16] T. Ward, M. Faivre, M. Abkarian, H. A. Stone, *Electrophoresis* **2005**, *26*, 3716.