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Multiscale Approach for Tuning Communication among Chemical Oscillators Confined in Biomimetic Microcompartments

Federico Rossi,* Sandra Ristori,* and Ali Abou-Hassan*

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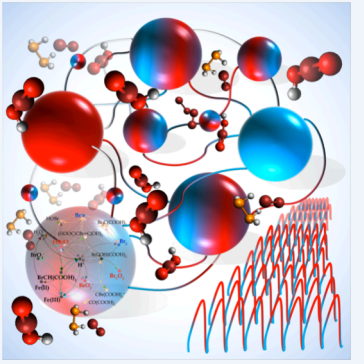
CONSPECTUS: Inspired by the biological world, new cross-border disciplines and technologies have emerged. Relevant examples include systems chemistry, which offers a bottom-up approach toward chemical complexity, and bio/chemical information and communication technology (bio/chemical ICT), which explores the conditions for propagating signals among individual microreactors separated by selectively permeable membranes. To fabricate specific arrays of microreactors, microfluidics has been demonstrated as an excellent method. In particular, droplet-based microfluidics is a powerful tool for encapsulating biological entities and chemical reagents in artificial microenvironments, mostly water-in-oil microdroplets. In these systems, the interfaces are liquid−liquid, and their physicochemical properties are key factors for tuning the coupling between molecular diffusion. Simple and double emulsions, where aqueous domains are in equilibrium with oil domains through boundary layers of amphiphilic molecules, are organized assemblies with high interfacial-area-to-volume ratios. These membranes can be engineered to obtain different surface charges, single- or multilayer stacking, and a variable degree of defects in molecular packing. Emulsions find application in many fields, including the food industry, pharmaceutics, and cosmetics. Furthermore, micro- and nanoemulsions can be used to model the propagation of chemical species through long distances, which is not only vital for cell signaling but also significant in molecular computing. Here we present in-depth research on the faceted world of solutions confined in restricted environments. In particular, we focused on the multiscale aspects of structure and dynamics from molecular to micro and macro levels. The Belousov−Zhabotinsky chemical reaction, known for its robustness and well-documented oscillatory behavior, was selected to represent a generic signal emitter/receiver confined within microenvironments separated by liquid−liquid interfaces. In this pulse generator, the temporal and spatial progressions are governed by periodic fluctuations in the concentration of chemical species, which act as activatory or inhibitory messengers over long distances. When organized into "colonies" or arrays, these micro-oscillators exhibit emergent dynamical behaviors at the population level. These behaviors can be finely tuned by manipulating the geometrical distribution of the oscillators and the properties of the interfaces at the nanoscale. By carefully selecting the membrane composition, it is possible to drive the system toward either in-phase, antiphase, or mixed synchronization regimes among individual oscillators, depending on messenger molecules. This relatively simple lab-scale model replicates some of the communication strategies commonly found in biological systems, particularly those based on the passive diffusion of chemical and electrical signals. It can help shed light on fundamental life processes and inspire new implementations in molecular computing and smart materials.



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- Torbensen, K.; Ristori, S.; Rossi, F.; Abou-Hassan, A. Tuning the Chemical Communication of Oscillating Microdroplets by Means of Membrane Composition. *J. Phys. Chem. C* **2017**, *121* (24), 13256−13264.[2] Oscillating simple emulsions with engineered liquid−liquid interfaces were arranged in arrays to characterize the communication among neighbors. An advanced modeling strategy was also introduced.
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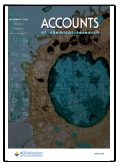

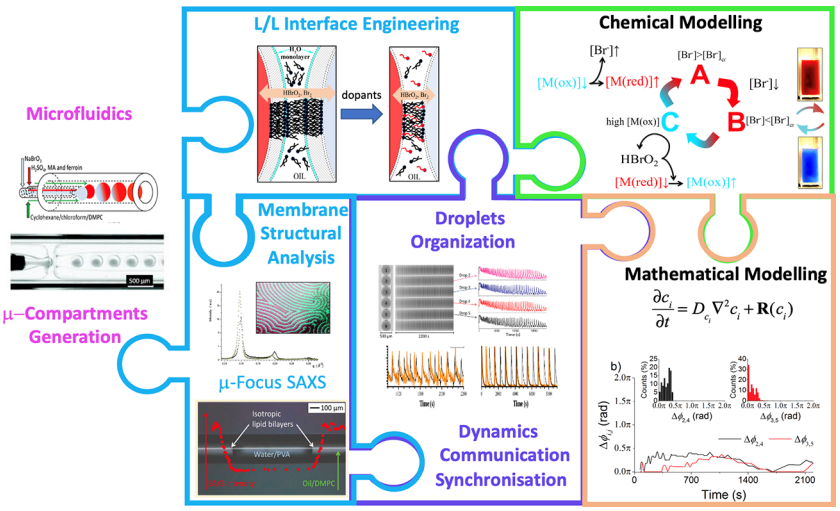

Figure 1. Sketch showing the multiscale puzzle-like approach adopted for the engineering of BZ phospholipid compartments with sustained oscillation for the study of their global macroscopic dynamics. This includes controlling the size of the compartments, the number of liquid−liquid interfaces, and their spatial organization using microfluidics; probing the phospholipid and dopants organization at nanointerfaces using μ-SAXS; and characterizing the interaction at the molecular level among BZ species and the assembled molecules at the interfaces by SAXS and different physicochemical methods. All experiments were supported at the molecular and global scales by chemical and mathematical modeling.

Scattering. *Langmuir* **2017**, *33* (36), 9100−9105.[3] An in-depth characterization of the liquid−liquid interfaces at the nanoscale was achieved through an advanced scattering technique.

- Budroni, M. A.; Torbensen, K.; Pantani, O. L.; Ristori, S.; Rossi, F.; Abou-Hassan, A. Microfluidic Compartmentalization of Diffusively Coupled Oscillators in Multisomes Induces a Novel Synchronization Scenario. *Chem. Commun.* **2020**, *56* (79), 11771−11774.[4] Stable BZ double emulsion arrays with sustained oscillations were engineered by microfluidics. A sharp synchronization through a period-halving transition was observed for the first time in a population of BZ micro-oscillators.
- Budroni, M. A.; Torbensen, K.; Ristori, S.; Abou-Hassan, A.; Rossi, F. Membrane Structure Drives Synchronization Patterns in Arrays of Diffusively Coupled Self-Oscillating Droplets. *J. Phys. Chem. Lett.* **2020**, *11*, 2014−2020.[5] The influence of the nanoscale organization at liquid−liquid interfaces on the macroscale oscillatory dynamics was clearly deciphered. An improved modeling strategy, which accounts for all the configurations explored, was introduced.

1. INTRODUCTION

The investigation of intricate networks of chemical reactions within confined environments, reminiscent of biological systems, has recently been gaining interest, particularly in the field of systems chemistry. This nascent discipline delves into the study of complex reactions and their emergent properties, thereby offering an integrated view of biochemical processes.[6−8] It underscores the connections and collective behavior of chemical systems, mirroring those observed in living organisms. A pivotal contribution to the advancement of systems chemistry has been the exploration of far-from-equilibrium chemical systems, which has primarily evolved by understanding chemical oscillations.[9,10] At the heart of this exploration lies the Belousov−Zhabotinsky (BZ) reaction, a classical example of a nonlinear chemical oscillator. Discovered in the 1950s, the BZ reaction has evolved from chemical curiosity into a model for studying complex dynamical systems, thanks to its rich array of oscillatory and wave phenomena, including chaotic behavior and Turing patterns.[11−13] In this reaction, an organic substrate, most commonly malonic acid, undergoes oxidation by bromate ions within an acidic medium, favored by the presence of a metal catalyst. Ferroin, a complex of phenanthroline and iron, is frequently used as a catalyst due to its ability to indicate the oxidative state of the reaction environment through a color change: it appears red in its reduced ferrous form, Fe(II), and becomes blue when oxidized to its ferric state, Fe(III).

In a bottom-up approach, numerous chemists now endeavor to construct complex systems designed to emulate self-organizing functions characteristic of life, such as replication, communication, and self-repair. These systems can be entirely formed by synthetic components or be a hybrid of synthetic and biological elements. Deciphering how basic chemical principles can evolve into intricate synchronized behaviors has proven highly effective in developing new technologies and their applications, which range from stimuli-responsive materials and drug delivery systems to medical devices.[14−17]

Here we endeavored to develop a network of independent chemical oscillators that exchange molecular signals by passive diffusion across semipermeable membranes, mirroring a common communication strategy in biological systems. Our cell-mimetic model features aqueous domains encased by amphiphilic molecular layers. Among amphiphilic molecules are phospholipids, composed of a large hydrophilic head and two hydrophobic tails, which are the basic modules of eukaryotic cell membranes. When dispersed in water, they self-assemble in bilayers that can be arranged as liposomes, cubic phases, etc. Focusing on the role of membranes, we explored how signals can be transmitted by cells according to a process historically attributed to integral proteins but is now recognized to involve lipids, sugars, and sterols.[18] The study of structural heterogeneities, such as transient pores[19] and compositional diversity,[20−24] is critical for understanding cell communication and signaling. Moreover, the transport of various molecular species across this network was designed to simulate activators, which favor specific processes and provide positive feedback,

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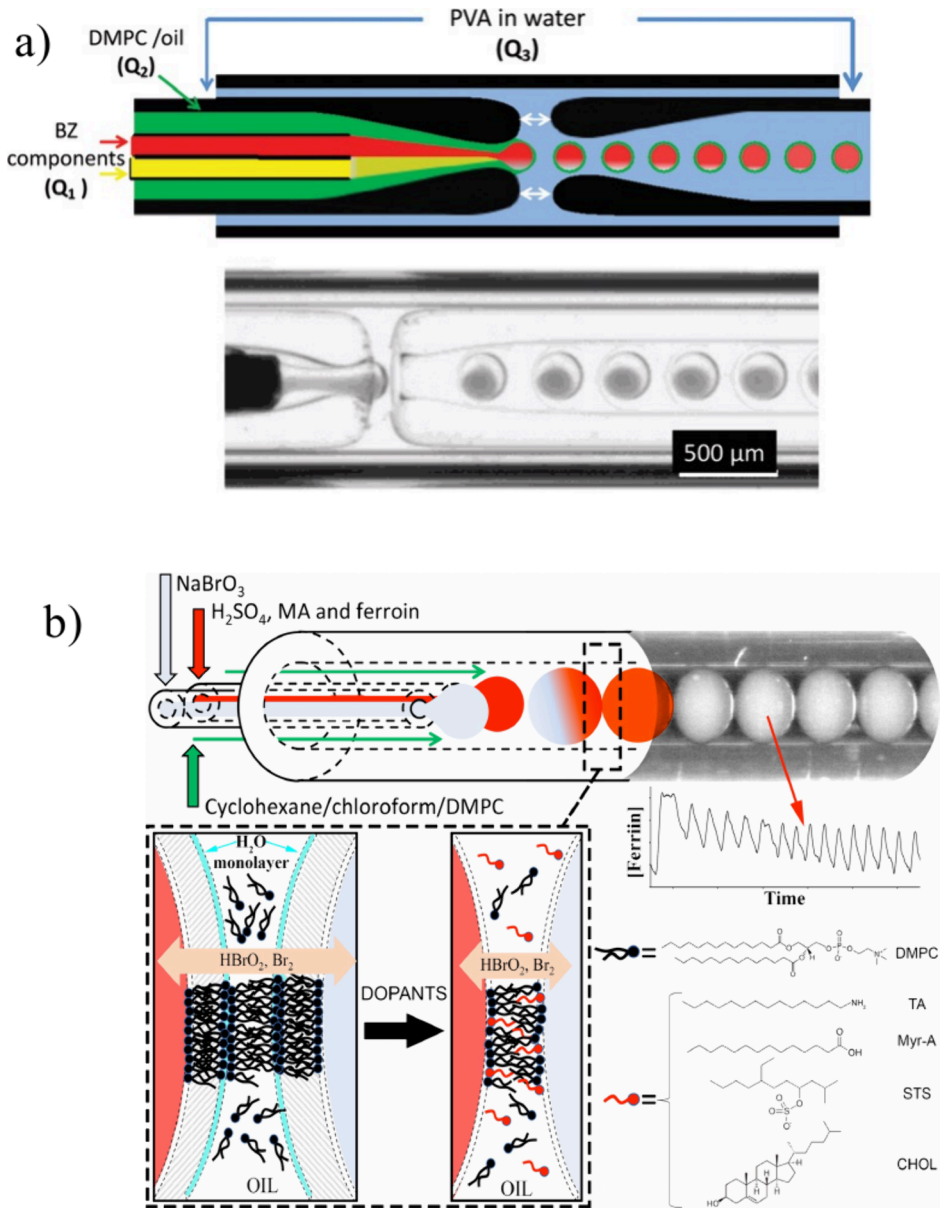

Figure 2. (a) Sketch of BZ/o/w DEs generation using a co-flow microfluidic device and an optical microscopy image of the same device during the real-time formation of BZ DEs. Reproduced with permission from ref 4. Copyright 2020 Royal Society of Chemistry. (b) Sketch of the coaxial flow microdevice during the formation of BZ/o emulsions and an image of their arrangement into 1D arrays in a PTFE tube for monitoring. The bottom part represents the two extreme membranes' arrangements obtained with pure DMPC (multilamellar) and with DMPC + other dopants (monolamellar). Reproduced from ref 5. Copyright 2020 American chemical Society.

and inhibitors, which function in a contrary manner. Examples of how single molecules acting as inhibitors can induce cell homeostasis have been reported,[25] and more in general, the transport of chemical species is a well-known mechanism though which a population of cells responds to temporal variations in the composition of the extracellular medium.[26] Indeed, the nature of membrane-mediated intercellular signals is pivotal in all scenarios requiring synchronous activities such as neural activity, cardiac contraction and expansion, calcium signaling, and quorum sensing.[27]

Inspired by the pioneer work at Brandeis University, which showcased populations of communicating BZ reactions in water-in-oil micro- and macroemulsions,[9,28−31] we developed a multiscale approach, detailed in Figure 1, to build systems where oscillators are encapsulated within phospholipid-based membranes and explored their structure and dynamics by operating interface engineering, droplet organization, and synchronization. This approach enabled us to investigate how membrane properties drive the selection of messenger molecules, thus influencing the type (activatory, inhibitory, or mixed) and strength of coupling. To generate solute-filled monodisperse micron-sized compartments, we exploited the advantages of flow in microfluidics, which is the technique of choice for engineering liquid−liquid interfaces in droplets,[32−34] emulsions,[35] liposomes,[1,36] and polymersomes.[37,38] In addition, microfluidics allows for the precise spatial organization of droplets to control and fine-tune the propagation of chemical messengers between adjacent compartments. Liquid−liquid interfaces were characterized at the molecular level by means of high-resolution small-angle X-ray scattering (SAXS)[39] to probe the thickness of

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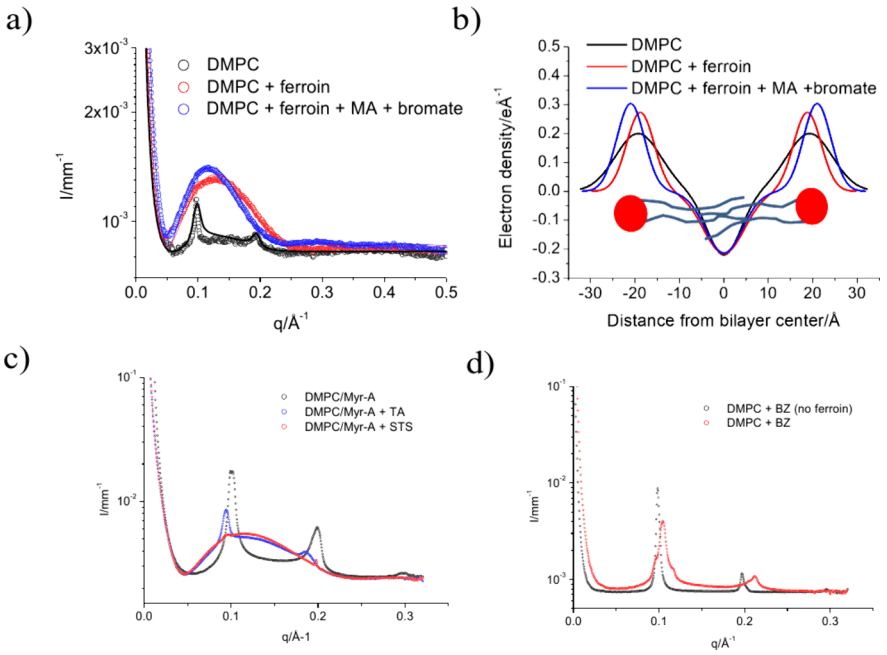

Figure 3. (a) SAXS intensity diagrams of DMPC liposomes with and without the addition of BZ reactants in water, showing the effect of lamellar reduction induced by ferroin. (b) Electron density profiles for the same systems. (c) SAXS intensity diagrams of liposomes with different bilayer compositions. (d) SAXS intensity diagrams of DMPC liposomes with ongoing BZ reaction. Adapted from ref 39. Copyright 2015 American Chemical Society.

different membranes and the incorporation of guest molecules.[19−24,40] For the investigation at larger scales, we used microfocus SAXS (μ-SAXS),[3] which allows one to describe the structural arrangement of lipid layers over distances of approximately 1−2 mm. Finally, structural and chemical data were integrated into a kinetic model to evaluate how nanoscale parameters can influence the overall dynamics of the network.

2. GENERATION OF COMPARTMENTS BY MICROFLUIDICS

To fabricate phospholipid-limited domains encapsulating the BZ reaction, DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine) was chosen as the main component since, at room temperature, it forms fluid bilayers with high resistance to the harsh oxidizing and acidic environment, typical of the BZ reaction.[41−43] Flow-focused geometries in microfluidics allowed us to generate monodisperse micron-sized compartments by tuning the flow and other physicochemical conditions, such as lipid concentration, viscosity of the outer and inner phase, and nature of the organic layer.[44] In this way, it was relatively simple to adjust the frequency of the compartments' generation, their size, and the distance between different liquid−liquid interfaces.[36]

Our first challenge was to encapsulate the BZ reaction inside giant DMPC liposomes. For this purpose, a co-flow microfluidic device assembled from glass capillaries, initially described by Utada et al.[45] and modified by our group,[36] was used (Figure 1a). The process of liposome preparation started by forming water/oil/water double emulsions (w/o/w DEs) as templates. Upon subsequent evaporation of the oil phase, closed lipid layers were obtained. The mixture of BZ reactants was injected in the inner phase, while the middle phase consisted of a small DMPC fraction dissolved in the chloroform−cyclohexane mixture used as volatile organic solvents. To avoid starting the BZ reaction before droplet formation, two disjointed tubes were focused inside the inner capillary, thus separating sodium bromate from other BZ reactants. This setup allowed us to precisely trigger the BZ reaction in space and time. The outer water phase in all our studies was a solution of poly(vinyl alcohol) (PVA), which played the role of a viscosity modulator and increased the stability of the interfaces, as demonstrated by μ-SAXS studies (see below). PVA proved to be an appropriate choice, as it showed chemical inertness toward BZ and its generated species. At the confluence point in the microfluidic system, inner drops containing the BZ reaction were formed in the dripping regime from the small injection tube, while the middle oil stream containing the drops was flow-focused by the outer continuous phase. Hydrodynamically focused inner and middle fluid streams broke up at the orifice of the outer capillary, forming monodisperse BZ DEs drops with 300 mm diameters with a thin layer of organic solvent at the entrance and exit of the collection capillary tube. At the outlet, double emulsions were either left to freely float in a Petri dish for evaporation or continuously parked inside capillaries to form 1D arrays. To better understand the role of water/oil interface in the communication dynamics, we then studied the partition of BZ species and the role of membrane lamellarity by elaborating water/oil emulsions. For this we used a biphasic flow-focusing microfluidic system (Figure 2b). We observed that at the water/oil interface, DMPC self-assembled into different structures,[3] including bilayers when two compartments were in contact, and we took advantage of this evidence for tuning the passage of chemical information. Lamellarity changes were induced by inserting dopant molecules such as myristic acid (Myr-A), sodium tetradecyl sulfate (STS), and tetradecylamine (TA) in the bilayers (Figure 2b, bottom panel). These amphiphiles have the same tail length as DMPC but a different packing parameter and surface charge. So, they can induce the transition to monolamellar structures and interact with different BZ intermediates, modulating the chemical information. Cholesterol (CHOL) was also inserted

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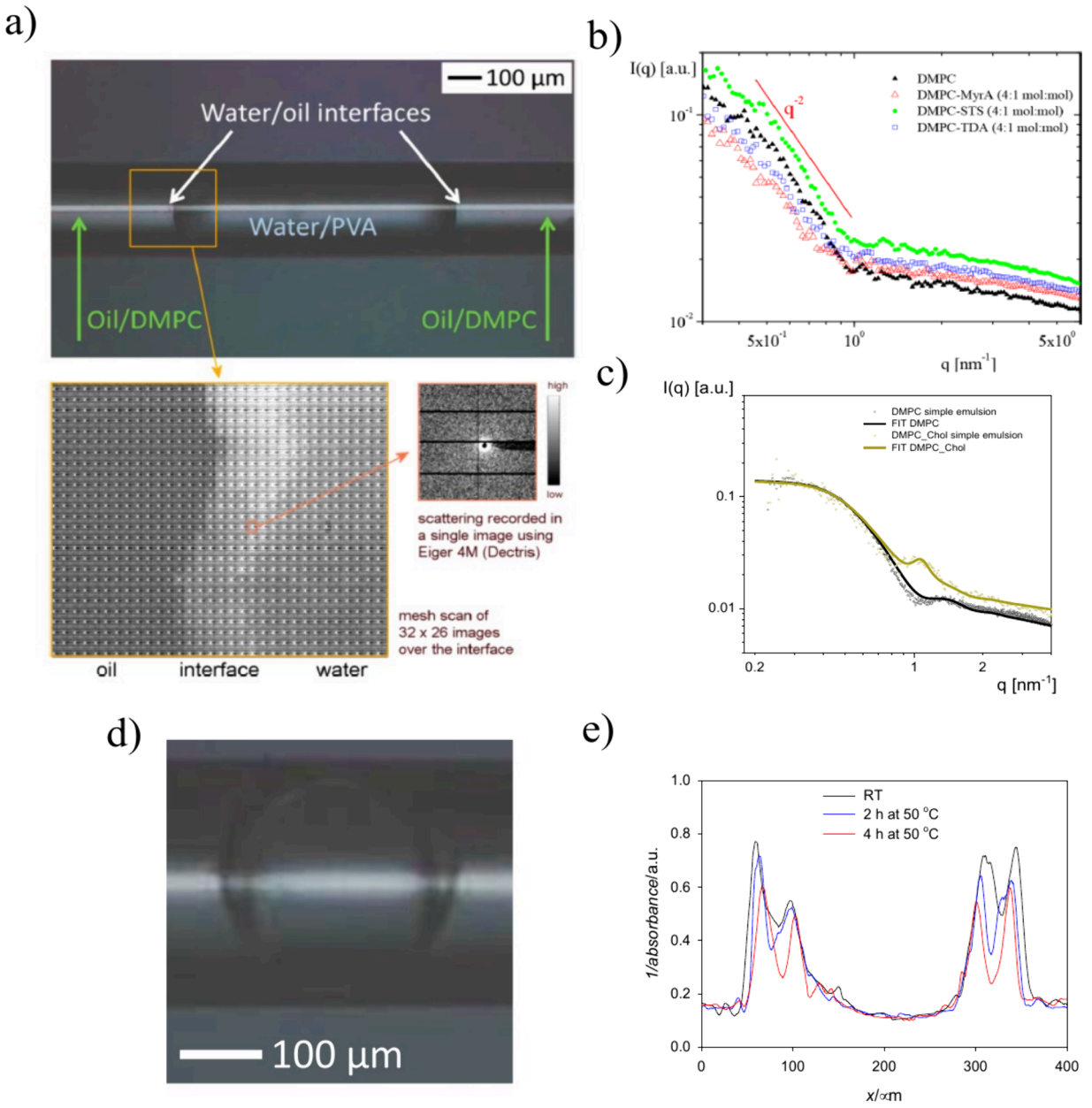

Figure 4. (a) w/o emulsion droplet of an aqueous solution of PVA (2%, w/w) in a mixture of chloroform−cyclohexane (1:2, v/v) containing DMPC (1% w/w) in a glass capillary and a two-dimensional reconstruction of the droplet interface by imaging diffraction (bottom). (b) Scattering intensity profiles of pure and mixed lipid layers. (c) SAXS experimental intensity diagrams and best fit with the bilayer form factor for pure DMPC and DMPC−CHOL systems (4:1 mol/mol), evidencing the correlation peak, which indicates increased ordering within the bilayer stacks. (d) Optical image of the w/o/w DEs taken at the exit of the microfluidic device at room temperature. (e) Horizontal scanning of the SAXS intensity at $q = 0.74\ nm^{-1}$ along the capillary in the zone where a droplet is present, taken at room temperature and after moderate heating. (a−c) Adapted from ref 3. Copyright 2017 American Chemical Society. (d, e) Adapted from ref 49. Available under a CC BY-NC 3.0 license. Copyright 2019 I. Clemente, K. Torbensen, E. Di Cola, F. Rossi, S. Ristori, A. Abou-Hassan.

in the bilayers for its stiff hydrophobic core, which is known to induce defects and increase the rigidity of fluid membranes. Moreover, CHOL is able to interact with brominated BZ intermediates, influencing the communication between droplets.[39]

3. INTERFACE ENGINEERING AND STRUCTURAL ANALYSIS

Engineering stable and communicating interfaces requires fine-tuning the physicochemical properties of the boundary membranes, such as the lamellarity, permeability, and stability. We adjusted these properties by varying the composition of the phospholipid layer, a task made possible by the versatile nature of amphiphilic molecules.[20−22,40] The surface charge of solution liposomes with the same composition was assessed by zeta potential measurements. SAXS intensity diagrams recorded in water and in the presence of BZ reactants showed that these latter species brought small changes to the structure of DMPC bilayers with the exception of ferroin, which was able to reduce the liposome lamellarity by being adsorbed on the surface. Charged dopants introduced in the DMPC matrix also altered the structural configuration of liposomes, shifting from a

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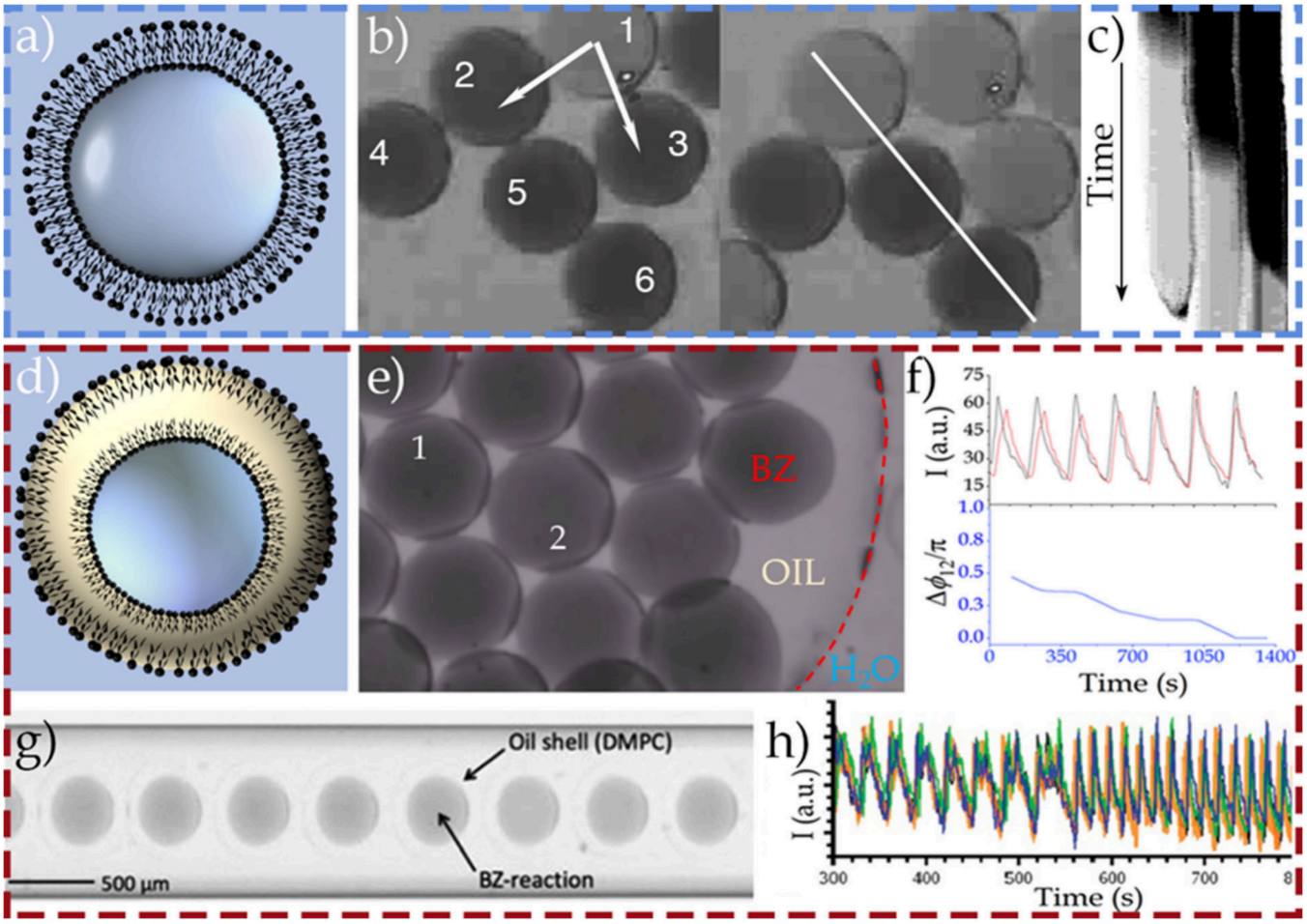

Figure 5. (a–c) GUV configuration. (a) Structure of a liposome. (b) Propagation of an oxidation pulse through the lipid membrane. (c) ST plot representing the dynamics of pulse transmission along the white line in (b). Adapted with permission from ref 1. Copyright 2014 Royal Society of Chemistry. (d–h) Multisome and double emulsion configurations. (d) Structure of a w/o/w DE. In the case of multiple water domains in the oil pool, the structure is known as a multisome. (e) Multisome arrangement. (f) Evolution toward synchronized regime of the period and of the phase difference for droplets 1 and 2 in (e). Adapted with permission from ref 51. Copyright 2015 Old City Publishing. (g) DE arrangement. (h) Evolution toward synchronicity through a period-halving transition of the period (~600 s) for the four central droplets in the array in (g). Adapted with permission from ref 4. Copyright 2020 Royal Society of Chemistry.

multilamellar arrangement of pure DMPC to oligolamellar structures with the inclusion of Myr-A and TA. This transformation culminated in a monolamellar arrangement when STS was added. In SAXS profiles, the presence of stacked bilayers was clearly evidenced by the onset of sharp quasi-Bragg peaks, which were superimposed to broader signals of the bilayer form factors. Figure 3 shows the SAXS intensity and electron density profiles for representative systems.[3,39]

Moving further to systems with a higher degree of complexity with respect to liposomes, w/o emulsions were considered. These are formed by aqueous droplets dispersed in an oil continuous phase, and similarly to liposomes, they are well suited to study the crossing of chemical species between liquid regions with different hydrophilic character. High-resolution (nanometer or molecular level) studies are generally difficult in emulsions due to the complexity and short-term permanency of these assemblies. However, the kinetics of droplet evolution is a subject of utmost importance since it governs the system stability with respect to coalescence.[23,24]

By using microfluidic methods, we prepared w/o emulsions stabilized by a layer of DMPC and other intercalating amphiphiles. The structural characteristics of the liquid–liquid interface were probed with μ-SAXS by scanning across the boundary between water and oil domains. We thus proved that the chosen lipids formed a stable and versatile limiting film of ~100 μm thickness (Figure 4a). In all DMPC pure and mixed systems, the SAXS intensity diagrams at intermediate q showed the q^{-2} decay typical of flat objects, indicating that in the interfacial region, the basic units were the lipid bilayer (Figure 4b). This hypothesis was confirmed by fitting the experimental curves with the form factor of uncorrelated bilayers (Figure 4c). From the thickness values of these bilayers (8.6–9.0 nm), marked swelling by oil molecules could be evidenced with respect to simple DMPC bilayers (typical thickness = 4.8–6.0 nm).[22] The internal arrangement of the interfacial film was tuned by adding cholesterol, which was able to trigger interbilayer correlation. Indeed, when cholesterol was intercalated in the bilayers at 20% mol/mol, the scattering profile could be reproduced by adding a correlation peak. In summary, the experimental evidence reported here indicated that in the interfacial layer of simple emulsions, phospholipids were arranged in disordered bilayers, as it happens for L_3 sponge phases[46] or in large monolamellar vesicles.[47] The addition of cholesterol increased the bilayer rigidity, resulting in partial

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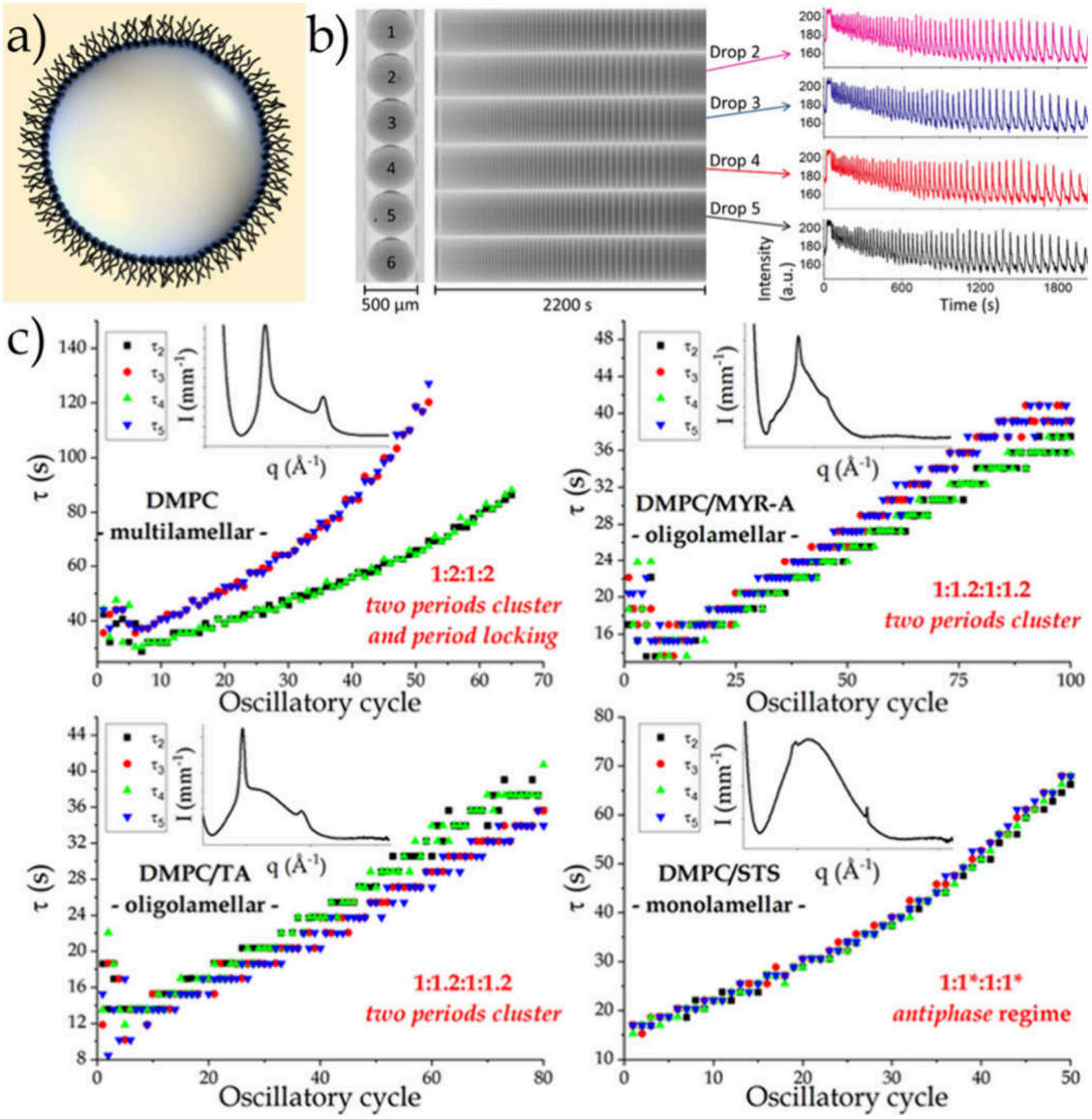

Figure 6. Simple emulsion configuration. (a) Structure of a w/o emulsion. (b) Procedure for time series extraction from ST plots in an array of micro-oscillators; dark areas correspond to Fe(II), and bright spikes correspond to Fe(III). (c) Synchronization patterns depending on the composition and nanostructure of the membranes: moving from a multilamellar to monolamellar configuration, the behavior of adjacent droplets progressively shifts from a phase-uncorrelated scenario (different τ with a defined ratio) toward a perfectly antiphase synchronization (π phase-shift, equal τ), where τ_i is the oscillation period of single droplets in the array. Adapted from ref 2. Copyright 2017 American Chemical Society.

ordering and interbilayer correlation.[48] On the contrary, amphiphilic dopants, such as Myr-A, STS, and TA, did not induce marked modifications to the DMPC layers surrounding the emulsion droplets.

μ-SAXS was used to scan, at the micron/submicron scale, the water compartments of w/o/w DEs trapped in a glass capillary.[49] Figure 4d,e shows the optical image of droplets and the spatial trend of the SAXS intensity along the horizontal direction taken at a fixed q-value, where no peaks were present (q = 0.74 nm^{-1}). A higher scattering intensity was obtained in the layer where more phospholipids were present. As can be seen in Figure 4e, after keeping the sample at 50 °C for several hours, a small shrink of the boundary region and of the whole droplet occurred, indicating that solvent evaporation from the oil swollen interfacial region was slow and hindered by intertwined disordered bilayers. Fitting of the SAXS diagrams showed that in double emulsions, the lipid arrangement was similar to simple emulsions, with more polydispersity and less marked effect due to cholesterol insertion. Notably, the shrinkage of the interface did not bring any ordering of the bilayers, though a small increase in their spatial correlation was observed.

4. SYNCHRONIZATION DYNAMICS AS A FUNCTION OF THE ORGANIZATION OF THE DROPLETS

Having secured the possibility to successfully engineer with microfluidics monodisperse phospholipid-stabilized microcompartments with sustained BZ oscillations and define the properties of interfaces, the next step was to organize BZ microcompartments into different spatial configurations to study their chemical communication and global dynamics. For this purpose, we used well-established tools of nonlinear dynamics.[50] Time series data were extrapolated from experimental snapshots or video recordings, and space-time (ST) plots were created to follow the actual state of the droplets array

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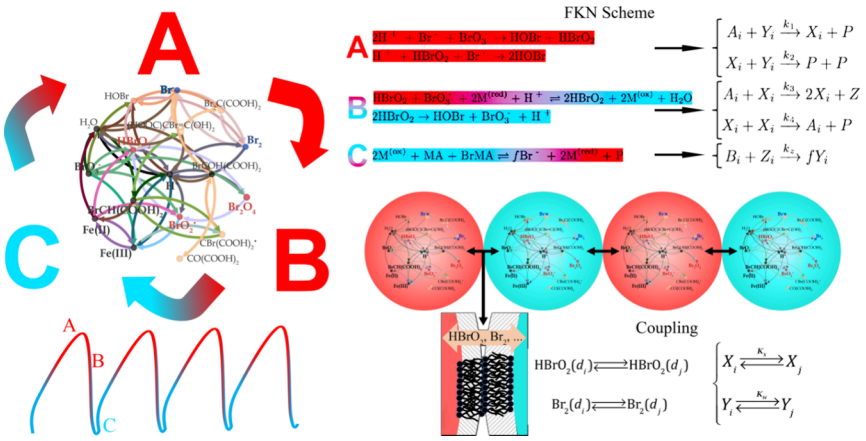

Figure 7. Description of the oscillatory and coupling mechanism. In process A, the inhibitor Br^- is slowly depleted to produce the autocatalytic species $HBrO_2$; when $[Br^-]$ decreases below a certain threshold (determined by the experimental conditions), process B becomes dominant, and a fast production of the activator $HBrO_2$ determines the oxidation of the catalyst (the solution's color changes from red to blue). Finally, when [Fe(III)] is high, process C starts to regenerate the reduced form of the catalyst (the solution's color goes back to red), and the increase of $[Br^-]$ resets the cycle to start oscillations over. $A_i = [BrO_3^-]_i$, $B_i = [CHBr(COOH)_2]_i$, $X_i = [HBrO_2]_i$, $Y_i = [Br^-]_i$, $[Br_2]_i$, and $Z_i = [Fe(III)]_i$. Micro-oscillators are coupled through the exchange of intermediates penetrating the confining membranes. Typical values for the kinetic parameters used in the simulations are k_1 (M^{-1} s^{-1}) = 0.245, k_2 (M^{-1} s^{-1}) = 1.05×10^6, k_3 (M^{-1} s^{-1}) = 14.7, k_4 (M^{-1} s^{-1}) = 1.05×10^3, k_z (M^{-1} s^{-1}) = 1, and f = 0.5. Reliable values for coupling parameters range between 10^{-4} and 10^{-3} s^{-1} for k_x and between 10^{-3} and 10^{-1} s^{-1} for k_w.

at a given time and monitor each droplet's oscillatory behavior (see an example of this procedure in Figure 6b). This approach permits us to assess the collective dynamics of the network by examining the oscillation period (τ_i) and the phase difference ($\Delta\varphi_{ij}$) among the coupled oscillators.

In scenarios where water is the dispersing medium, a synchronized in-phase behavior was consistently observed across all experimental setups, indicating that activatory coupling predominates as the mode of communication (refer to the Modeling and Synchronization Mechanism section for further details).[1,51,52] Figure 5b depicts a cluster of liposomes, also known as giant unilamellar vesicles (GUVs), which were templated from DEs gathered in an open Petri dish to allow for the evaporation of the oil phase. The GUV configuration supported pulse transmission through the propagation of oxidation waves (Figure 5c). Electrochemical investigations[1,53] and specific experiments with cholesterol intercalated into the membranes[39] revealed that oxybromine species, including the activator $HBrO_2$, were responsible for the transmission of the chemical signal. The same type of coupling was observed in oscillatory populations of multisomes obtained when simple BZ/oil emulsions were collected at the exit of the microfluidic channel in a covered Petri dish (Figure 5e).[51] Once we established the nature of the coupling in GUV experiments, in multisomes we investigated the long-term oscillating behavior of the network to find a general tendency to in-phase synchronization (Figure 5f).

Although multisomes are a simple model to investigate the effect of the membrane on the passage of chemical information, the role of the external water phase is mainly to act as a sink for polar intermediates. However, in nature, multicompartmentalization is omnipresent, and the passage of chemical information also occurs through aqueous phases. To mimic this, we encapsulated the BZ reaction inside linear arrays of BZ/o/w DEs[4] (Figure 5g) produced through the coaxial flow-focusing microfluidic system described in section 2. Similar to the multisome configuration, activatory coupling emerged as the predominant mode of communication, leading to the progressive synchronization of all micro-oscillators in an in-phase manner after a period-halving transition (Figure 5h).

Finally, to specifically understand the influence of nanoscale modifications of membranes on the global behavior of the networks, we configurated the oscillators in a simple emulsion array, where the DMPC-stabilized droplets were dispersed in the oil phase.[5,32] Figure 6 shows an example of six BZ oscillators, obtained using biphasic microfluidics, leveraging fluid flow to strategically position the droplets within a Teflon tube, which was subsequently sealed at both ends to maintain the arrangement. By eliminating the aqueous phase as the medium for communication, the system experienced a notable decrease in the diffusivities of polar intermediates, consequently making Br_2 the predominant messenger species.[52] This alteration in the chemical messaging landscape led to a pronounced shift toward an inhibitory coupling scenario and revealed new types of synchronization patterns. Interestingly, we demonstrated that the nature of patterns depends on the nanoscopic properties of the membrane lamellarity. As outlined in section 2, proximity among droplets in a 1D array configuration leads to the formation of a spatially extended bilayer at the contact points between their membranes. This extended bilayer acts as a crucial diffusive pathway, regulating chemical communication between adjacent droplets. DMPC tends to form multilamellar membranes, while the addition of dopants progressively decreased the lamellarity in the order Myr-A, TA, and STS, with DMPC/STS membranes being monolamellar (see insets in Figure 6).[3,49] In the case of the DMPC/STS arrangement, the strong inhibitory coupling induces antiphase oscillations with alternate droplets synchronized in-phase, while adjacent oscillators present a phase lock close to π (scenario 1:1*:1:1*, where "*" indicates the π phase-shift among successive oscillators); when the number of bilayers increased (DMPC, DMPC/TA, DMPC/Myr-A), the coupling strength decreased, and the new dominant dynamical behavior was a two-period clustering and/or 1:a:1:a period locking ($\Delta\varphi = \varphi_i - a\varphi_j$, $1 < a < 2$ and $a = \tau_i/\tau_j$), a regime representing a novel scenario in the study of coupled chemical oscillators without external feedback.

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Acc. Chem. Res. 2024, 57, 2607−2619

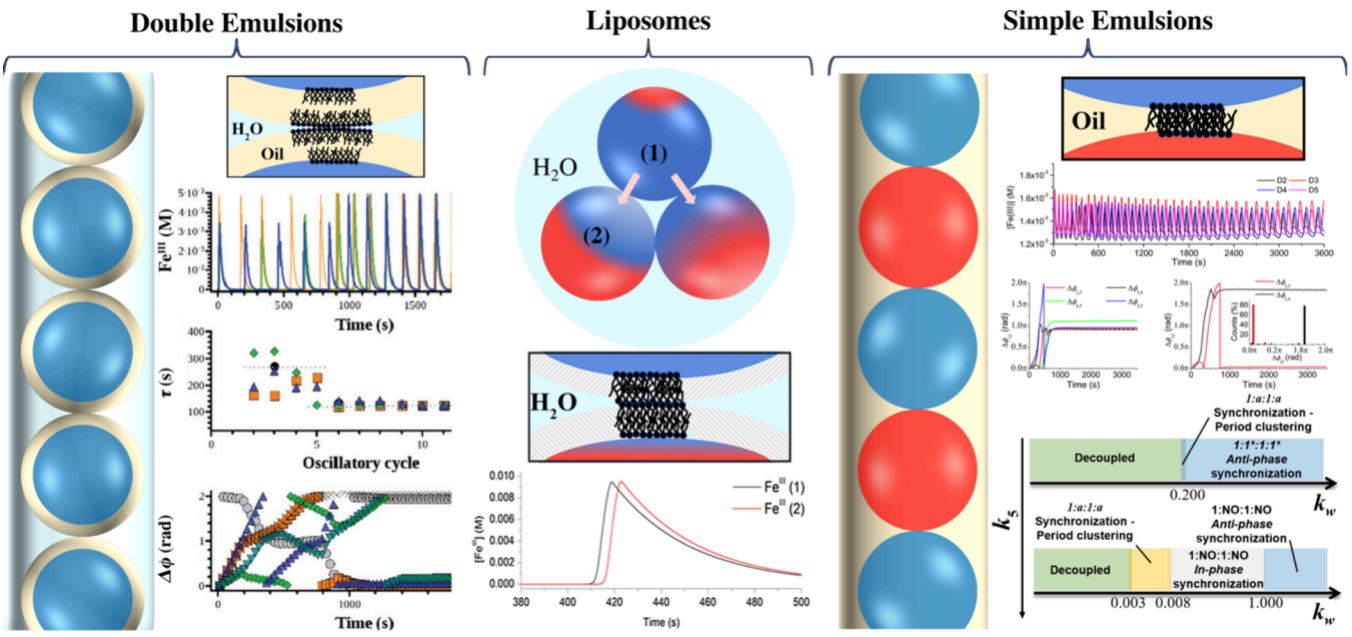

Figure 8. Simulations of the experimental configurations explored in our biomimetic systems: in-phase synchronization ($\Delta\varphi_{ij} = 0, 2\pi$) through period-halving in DEs. Adapted with permission from ref 4. Copyright 2020 Royal Society of Chemistry. Pulse transmission in liposomes. Antiphase synchronization ($\Delta\varphi_{ij} = \pi$) and phase diagram mapping the dynamical behavior in simple emulsion networks as a function of the coupling strength. Adapted from ref 5. Copyright 2020 American Chemical Society.

5. MODELING AND SYNCHRONIZATION MECHANISM

Several models have been sketched to reproduce the oscillatory kinetics of the BZ reaction, most of them developed from the seminal Field–Koros–Noyes (FKN) scheme.[54] Figure 7 reports a basic sketch of a ferroin-catalyzed BZ system, where bromomalonic acid, $CHBr(COOH)_2$, is the starting substrate, BrO_3^- is the oxidizer, and the couple Fe(III)–Fe(II) is the catalyst. Among the several intermediates, a key role is played by the autocatalytic species $HBrO_2$ (bromous acid) and its related species Br_2O_4 and $BrO_2^\bullet$, which act as activators (promote oscillations), and by the inhibitory intermediates (damp oscillations) Br^- and Br_2. Bromine is a precursor of the primary inhibitor Br^- through its reaction with H_2O and organic species,[55] and it is typically neglected in bulk aqueous systems. However, in heterogeneous media comprising nonpolar phases, bromine becomes a critical messenger molecule.[30] Similar to the dynamics observed in the predator–prey system, the oscillatory behavior in the BZ reaction is driven by the interplay between activator and inhibitor species, which is evidenced by periodic changes in the catalyst's color. In homogeneous systems, the BZ reaction can be described using a series of coupled ordinary differential equations (ODEs) that are based on the FKN mechanism. The complexity of the model can vary; it might encompass all elementary steps for detailed mechanistic insights or be simplified to a version with five variables (see Figure 7). Moreover, more streamlined nondimensional models, such as the well-known Oregonator,[56] further reduce the system to just two variables. To investigate the behavior of networks of BZ/micro-oscillators and get insights on the coupling mechanisms, we mostly modeled each oscillator with ODEs derived from the simplified mechanism reported in Figure 7.[57,58] The oscillatory dynamics can be reproduced in each compartment i by integrating the ODE systems $d[C]_i/dt = r([C]_i)$, where C generically stands for BZ intermediates and $r([C]_i)$ represents the set of reaction rates involving C. The coupling among oscillators takes place through the exchange of lipophilic intermediates ($HBrO_2$, Br_2, Br_2O_4, and $BrO_2^\bullet$) between nearest-neighbor compartments (i, j), according to their membrane permeability (P_i). P_i can be accounted for in the transfer constants $k_{x,w} = P_i A_c/V_d$, where V_d is the droplet volume and A_c is the contact surface area between two droplets. Therefore, the ODEs describing the changes in the concentrations of C in the oscillators' network read

$$\frac{d[C]_i}{dt} = r([C]_i) + k_{x,w} \sum_j ([C]_i - [C]_j) \tag{5.1}$$

The set of eqs 5.1 provides a flexible computational tool to simulate and describe the oscillatory dynamics of all the experimental arrangements of BZ oscillators coupled through the exchange of several intermediate species. In a few cases, where coupling took place only through the exchange of the activator $HBrO_2$, a simpler Oregonator model has been used.[1]

The dynamical behavior of the global network of oscillators is driven by the local coupling among two or more touching droplets. In general, the exchange of pure activatory signals promote in-phase coupling, resulting in coordinated pulsations of all the network's elements, while the exchange of inhibitory molecules drives the system to antiphase oscillations (1:1*:1:1*). Between the two limit regimes, a series of intermediate behaviors can be found, depending on the relative coupling strength determined by the experimental conditions. The relative importance of the two possible coupling schemes is tuned by parameters k_x (activatory) and k_w (inhibitory), both depending on the membrane permeability, composition, and lamellarity;[5] the chemical influence of the dopants was accounted for by changing the kinetic constant k_5, thus influencing the rate of regeneration of the reduced form of the catalyst and of the Br^- ions.[5] By integrating the numerical model (eq 5.1), we could reproduce all the experimental behaviors shown in Figures 5 and 6, including period clustering and phase locking, as reported in Figure 8.

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Acc. Chem. Res. 2024, 57, 2607−2619

We also used our model to theoretically investigate different coupling approaches among individual oscillators from a theoretical point of view, aiming to more accurately mimic the communication patterns of biological systems. For example, the model was enhanced to account for the effects of delayed feedback on the collective synchronization of oscillator groups[55] to replicate the inherent time delays resulting from spatial distance and from the mechanisms of signal transmission. Interestingly, we found how varying the delayed feedback leads to changes in synchronization patterns, showing a direct transition from antiphase to in-phase synchronization and back to the initial antiphase scheme as the time delay increases from zero to the natural oscillation period of the uncoupled oscillators. These findings mirrored the dynamic shifts seen in the coordination and movement of limbs, which are believed to stem from the communication delays between the central nervous system and the cells of peripheral limbs.[59,60]

6. SUMMARY AND FUTURE OUTLOOK

Over the past decade, we have been contributing effectively to the field of complex systems through a multiscale perspective, using methodologies derived from the physicochemical investigation of nanostructures, as well as from nonlinear dynamics. This interdisciplinary approach allowed us to gain a good understanding of the engineering, interfacial properties, and dynamics of BZ cell-like compartments. By harnessing the advantages of microfluidics, scattering techniques, interfacial engineering, and theoretical and simulation approaches, we succeeded in piecing together a puzzle-like strategy to overcome the harsh chemical conditions of the BZ reaction and formulate stable monodisperse BZ compartments with different liquid−liquid interfaces. We were thus able to evaluate the role of lipid self-assembly at the interface in facilitating the transmembrane crossing by chemical messengers. Consequently, by controlling the membrane composition and the arrangement of the cell-like compartments, we successfully tuned the communication properties of the entire oscillatory network to drive the system toward stable dynamical attractors. In general, configurations where water is the dispersing medium, such as liposomes, multisomes, or DEs, favor pulse and wave transmission and a global activatory coupling with a final in-phase regime. On the contrary, when BZ droplets are dispersed in oil, such as in simple emulsions, an inhibitory coupling regime, dominated by antiphase oscillations, represents the final attractor of the system.

The lesson learned from the BZ networks allows one to imagine sophisticated systems where compartmentalized (bio)-chemical reactions, for example enzymatic sets, can cross-talk and self-organize to deliver complex signals to biological systems.[61−63] Synthetic or semisynthetic networks could simulate brain-like functionalities such as learning, memory, and pattern recognition and be programmed to interact with living cells for applications in precision medicine or to develop novel computing architectures where computation is achieved not through silicon-based hardware but through biochemically inspired systems.[64−66]

To date, we have relied mostly on microfluidics to organize the oscillating compartments. It would be interesting to explore the possibility of manipulating and organizing these compartments using magnetic fields,[67] potentially employing magnets or magnetic tweezers to build multidimensional structures on demand.[68] This may lead to new dynamics and synchronization scenarios not yet reported. However, integrating magnetic nanoparticles into the system poses a significant challenge due to the chemical conditions of the BZ reaction, which could lead to rapid degradation of the particles. Developing methods to stabilize these nanoparticles or engineering robust nanoparticle coatings that can withstand such harsh environments will be crucial for advancing this innovative direction in the field of dynamic chemical networks.

AUTHOR INFORMATION

Corresponding Authors

Federico Rossi − *Department of Physical Science, Earth and Environment, University of Siena, 53100 Siena, Siena, Italy;* orcid.org/0000-0002-1854-532X; Email: federico.rossi2@unisi.it

Sandra Ristori − *Department of Chemistry & CSGI, University of Florence, 50019 Sesto Fiorentino, Firenze, Italy;* orcid.org/0000-0003-0708-3956; Email: sandra.ristori@unifi.it

Ali Abou-Hassan − *Sorbonne Université, CNRS, PHysico-chimie des Electrolytes et Nanosystèmes InterfaciauX (PHENIX), F-75005 Paris, France; Institut Universitaire de France (IUF), 75231 Paris, France;* orcid.org/0000-0002-9070-1024; Email: ali.abou_hassan@sorbonne-universite.fr

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.accounts.4c00232

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. F.R.: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, writing—original draft, and writing—review and editing. S.R.: conceptualization, formal analysis, investigation, methodology, project administration, writing—original draft, and writing—review and editing. A.A.-H.: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, writing−original draft, and writing−review and editing. CRediT: **Federico Rossi** conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, visualization, writing-original draft, writing-review & editing; **Sandra Ristori** conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, visualization, writing-original draft, writing-review & editing; **Ali Abou-Hassan** conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, visualization, writing-original draft, writing-review & editing.

Notes

The authors declare no competing financial interest.

Biographies

Federico Rossi is an associate professor of physical chemistry in the Department of Physical Science, Earth and Environment at the University of Siena (Italy). He was a Marie Curie fellow at the Department of Chemistry at Brandeis University (USA) and an assistant professor at the University of Salerno (Italy). His current research interests include the study of chemical systems with complex kinetics (oscillating, excitable, etc.) coupled with transport phenomena

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and the analysis of networks of oscillating droplets and their applications to systems and environmental chemistry.

Sandra Ristori is an associate professor of physical chemistry at the University of Florence, Italy, and a member of the Center for Colloid and Surface Science (Italy). She completed her thesis in 1992, having developed a Ph.D. project in collaboration with the PCM laboratory at the CEA, Grenoble. She then continued her training in the structural characterization of nanosystems at the University of Montpellier and at the Max Planck Institute for Colloids and Interfaces, Potsdam. Her current scientific interests are the design and development of nanocarriers for drug delivery, both for biomedical purposes and for sustainable plant treatments. She is currently teaching courses of physical chemistry for the life sciences and nanosystems for biotechnology.

Ali Abou-Hassan is a full professor of physical chemistry at Sorbonne University and a junior member of the Institut Universitaire de France. He completed his thesis in 2009 at Pierre and Marie Curie University. In 2009−2010, he worked as a postdoc with Profs. Dayang Wang and Helmut Möhwald at Max Planck Institute for Colloids and Interfaces, Germany. His current scientific interests are the elaboration of optimized nanostructures for different applications including in nanomedicine and the study of their structure−properties−function relationship in different media (mimetic or biological ones) using a combination of physical chemistry, microfluidics, and materials science approaches. He also uses the same approaches for engineering and studying out-of-equilibrium reactions as those encountered in nature.

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■ DEDICATION

This Account is dedicated to Prof. Vladimir Vanag (1954−2023), whose pioneering contributions have profoundly shaped our research.

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