Electronic Supplementary Material (ESI) for Chemical Science

Electronic Supplementary Information

Chemical Communication Between Liposomes Encapsulating a Chemical Oscillatory Reaction

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A. Experimental details

All chemicals were purchased from Sigma Aldrich.

The microcapillary device has coaxial flow geometry. Capillaries were provided from Vitrocom, World Precision Instruments. The precise geometry of this device was described elsewhere.[1] In order to form the double emulsion, fluids were injected at different flow rates controlled by syringe pumps (Harvard Apparatus).

The BZ aqueous mixture was composed of a mixture of H_2SO_4 (300 mM), $NaBrO_3$ (120 mM), MA (30 mM) and ferroin (5 mM). The mixture was injected in the inner phase of the microfluidic device. DMPC was solublized in a mixture of chloroform: cyclohexane (40:60; v/v) and was injected in the middle phase. The external phase was an aqueous solution of PVA (7%, wt). An aqueous solution of $NaBrO_3$ (0.4 M) was used to recuperate the liposomes at the exit of the microfluidic device.

B. SECM investigations in aqueous-benzonitrile immiscible biphasic system.

Typically, the SECM tip (a 25 µm diameter microelectrode) is used to probe local fluxes of the electroactive redox mediator of the BZ system: ferroin, Fe^{II} or ferriin, Fe^{III} (see the ferriin reduction voltammogram in Fig S1). This flux is obtained from the measured variation of the tip current associated to the redox mediator transport and consumption at the tip. The biphasic system is composed of an upper organic phase: benzonitrile + 0.1 M NBu_4BF_4 placed on top of a lower aqueous phase containing 1 M H_2SO_4.

B.1. *Detection of oscillations in each phase.*

The aqueous phase contains all the BZ compounds, whereas the BENZ Phase contains only a few amount of ferroin (0.25mM). A reductive potential corresponding to the mass transfer limited reduction of ferriin (the oxidized form of ferroin) was applied at the SECM tip.

Electrode was held at about 200µm from the interface and chronoamperograms were recorded respectively in the aqueous and the organic phases.

B.2. *SECM inspection of interfacial electron transfer.*

In the case of ITIES, the upper organic phase contains the reduced form R1 of a redox mediator to probe charge transfers from feedback current measurements. When held at a sufficiently positive potential, in BENZ, the tip generates O1 from R1. The tip approaches the ITIES, the redox mediator R1 can be regenerated at the interface via a bimolecular reaction between O1 in the BENZ phase and a reducer, R2, in the aqueous phase according to:

$$O1_{(o)} + R2_{(w)} \rightarrow R1_{(o)} + O2_{(w)} \qquad (4)$$

The extent of (4) is obtained from approach curves relating, in dimensionless forms, the evolution of the tip current, i_T, with the tip-ITIES separation distance, d. When (4) is inoperative, i_T which describes the mass transport of R1 to the tip decreases from its infinite distance value, $i_{T,inf}$, with, d as a result of mass-transport hindrance by the interface (negative feedback limit, lowest curves in Figure 2 in the manuscript). When (4) operates, the R1 regenerated at the ITIES yields a feedback and in the vicinity of the ITIES, i_T values are higher than the negative feedback limit. The kinetics of the interfacial reaction can be evaluated from these approach curves according to existing analytic expressions[2] and using the diffusion coefficient of antracene D=5 10^{-6} cm^2 s^{-1}, estimated from cyclic voltammetry at the 25µm diameter tip.

The driving force of ET across the ITIES, $\Delta E_{1/2}$.[3,4] is related to the difference of formal potentials, ΔE°, between both redox couples, R1/O1 and R2/O2, and Δφ the relative value of the potential drop across the ITIES:

$$\Delta E_{1/2} = \Delta E° + \Delta\varphi$$

$\Delta E_{1/2}$ was estimated experimentally from the difference between the half-wave potentials obtained from voltammograms measured for each redox species in each phase with respect to the same reference electrode. When R2=ferroin, $\Delta E_{1/2}$ = 0.5 V, and from the knowledge of ΔE^0 the potential drop across the BENZ/aqueous interface is deduced, Δφ = 50 mV.

In the case of interfacial ET between the anthracene radical cation and malonic acid, the observed feedback is consistent with the exergonic MA oxidation by different electrogenerated oxidant such as $Ru(bpy)_3^{3+}$ of comparable E^0.[5] The k_{het} value is comparable to that measured for the SECM mediated ITIES oxidation of 60 mM aqueous oxalic acid (HOOC-COOH) phase by RuL_3^{3+} in BENZ phase[6] and suggests that both organic acids have comparable interfacial electron transfer, ET, driving force $\Delta E_{1/2}$ = -0.17 V. From the measured $\Delta\varphi$ of 50 mV, the E^0 of malonic acid is about 1.47 V vs Ag/AgCl.

The effect of temperature, T, on the charge transfer process has been studied from approach curve both in the presence and absence of lipids. The change in temperature is not associated with a change in diffusion coefficient. Indeed, the tip current for the steady-state current oxidation of the different probes in each phase (anthracene and ferroin respectively in BENZ and water) did not change appreciably. In the absence of lipids, approach curves recorded at the BENZ/water interface successively at 15°C and 21°C are identical (<10% difference) for the Anthracene / Ferroin charge transfer. In the presence of DMPC, the change in temperature is associated with a change in feedback as measured on the approach curve experiments presented in Figure 2B. Therefore, the effect of temperature, T, on the SECM approach curves illustrates a change on the interfacial charge transfer rate constant, k_{het}. It can be related to the thermodynamics of this charge transfer process, $\Delta E_{1/2}$, according to an activation-driving force relationship. A Butler Volmer relationship, in a linearized form of Marcus equation, can be used:[7]

$$\ln k_{het} = \text{const} - \alpha F/RT\ \Delta E_{1/2} \qquad (5)$$

with α the apparent charge transfer coefficient, typically lower than 0.5 in various ITIES systems, F the Faraday's constant, R the gas constant.

The predicted temperature dependence of k_{het} yields by differentiation of (5):

$$dk_{het}/k_{het} = \alpha F/RT\ \Delta E_{1/2}\ dT/T \qquad (6)$$

at the BENZ/water interface depicted here a change in T from 17 to 21°C ($\Delta T/T = 0.014$) should be associated to a predicted change of k_{het} of the order of $\Delta k_{het}/k_{het} = 0.13$. It is in agreement with the experimentally negligible variation in the absence of DMPC. The applicability of Marcus equation at the ITIES has been demonstrated in a number of cases and particularly the temperature dependence effect on k_{het} was investigated at the 1,2-dichloroethane/water interface.[8] This work reported also a slight temperature dependence in agreement with (6) and our observation at the BENZ/water interface. In the presence of DMPC, the temperature dependence (2 fold variation) is much stronger than expected according to the above equation, and this can be ascribed to a phase transition affecting the bilayer barrier properties.

B.3. *Ferroin partitioning.*

Ferroin partitioning between an aqueous and the organic BENZ phase is evidenced from the progressive coloration of this later. A partition coefficient is calculated from the plateau current of ferroin oxidation recorded at the microelectrode in both phases when equilibrated. The partition coefficient $P_{BENZ/H2O} = 0.33$ is given using $i_T = 4nFCDa$, with D the diffusion coefficient of ferroin $D_{H2O} = 7.10^{-6}$ cm² s^{-1} in water and $D_{BENZ} = 3.10^{-6}$ cm² s^{-1} in benzonitrile with n=1. The equilibrium partition coefficient is approximately unchanged in the presence of 100µM DMPC in the BENZ phase.

B.4. *Voltammogram of ferriin reduction.*

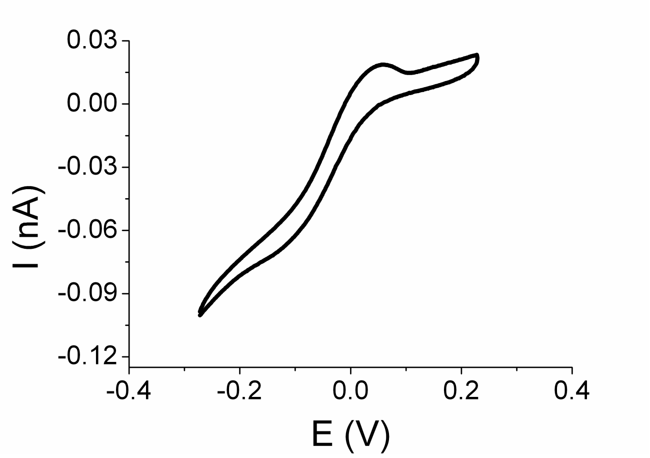

Figure S1: Cyclic voltammogram recorded in a solution containing 1M H_2SO_4 + 60 mM Malonic acid + 2 mM Ferroin + 180 mM BrO_3^- showing the reduction of Ferriin (FeIII) to Ferroin.

C. DSC measurements

DSC runs were recorded with a DSC Q2000 apparatus (TA Instruments) calibrated with indium and the obtained data were elaborated with the Q Series software, version 5.2.4.

A weighted amount of samples (10-20 mg) was inserted in aluminum pans and sealed with the aid of a Tzero press. The temperature ramp was 8-38 °C with 2 °C/min scan rate. All samples were equilibrated at 5° for 30' before starting the measurement.

Ferroin was not included in the samples containing the BZ mixture to prevent gas release in the sealed pans. PVA and BZ reactants were at the same concentration as in the microfluidics experiments.

The DSC curves (Figure S2) obtained for DMPC in the presence of PVA and BZ reactants exhibited marked broadening and an upward shift of the gel/liquid crystal transition by 4-5°C with respect to the value recorded in pure water. This meant that the order of lipid molecules in the bilayer was modified by the ions, neutral molecules and PVA dissolved in the water phase. Therefore, some species were able to breach the polar head region. However, the transition was not suppressed, indicating that the ordering of the lipid chains was still significant in the bilayer.

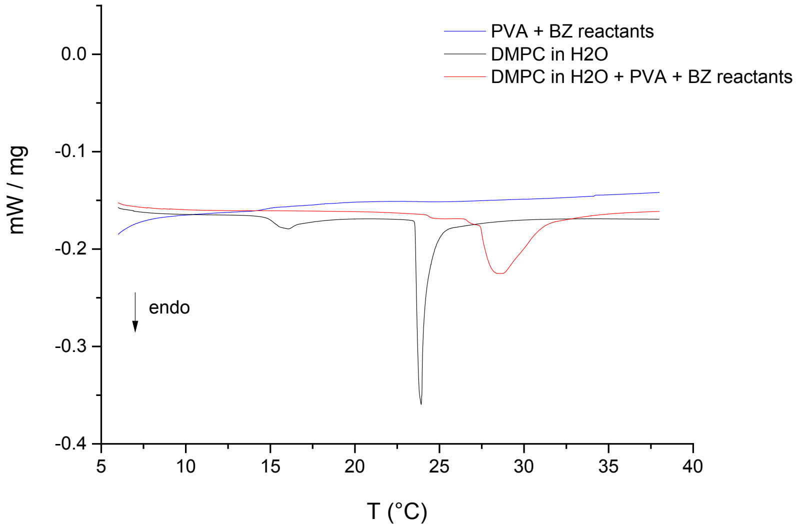

Figure S2: DSC curves of DMPC in pure water (10% w/w) and in the presence of PVA + BZ reactants. The reference curve without DMPC shows that no thermal event occurred in the T range investigated.

D. Simulations

Figure S3 shows a sketch of the physical system, where the droplets are approximated to chemically homogeneous spherical microreactors. Here two droplets are in touch and an impulse is passing from the droplet 1 to the droplet 2. For the sake of simplicity we assumed a unidirectional flow of bromous acid from one droplet to the other

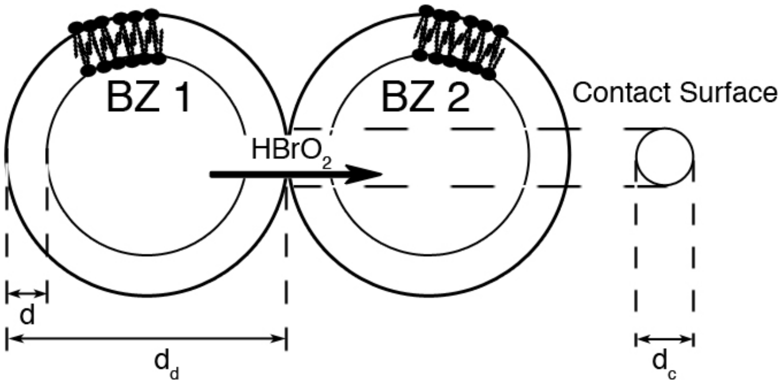

Figure S3: sketch of two touching droplets. *d* represents the thickness of the lipid bilayer surrounding a droplet. d_d is the total droplet diameter and d_c is the diameter of the contact surface between two droplets, which has been approximated to a circle.

In order to explore the dynamics of the two touching droplets we performed some simulations by using a three variables *Oregonator* model:[9,10]

$$\begin{aligned}
\varepsilon \frac{dx_i}{d\tau} &= qy_i - x_i y_i + x_i(1-x_i) \\
\varepsilon' \frac{dy_i}{d\tau} &= -qy_i - x_i y_i + f z_i \\
\frac{dz_i}{d\tau} &= x_i - z_i
\end{aligned} \tag{1}$$

where x_i, y_i and z_i represent the dimensionless concentration of $HBrO_2$, Br^- and Fe^{III} in droplet *i*, respectively; $\varepsilon = 0.02$, $\varepsilon' = 3.97 \times 10^{-5}$ and $q = 2.86 \times 10^{-5}$ are dimensionless parameters related to the initial concentrations of the reagents and to the reaction rate constants, $f = 3.5$ is a stoichiometric factor which account for the Br^- regeneration. The *Oregonator* model can't give a satisfactory quantitative description of the ferroin catalysed BZ reaction, however it can catch the basic features of our system and allows to have a rough estimation of the space-time scales involved in the transmission of the chemical pulses. Depending on the value of the parameters, the model can be oscillatory or excitable. When excitable, like in this case, the system has a stable steady state and in case of small perturbations quickly returns to its initial concentrations, but perturbations that exceed a threshold first grow before the system relaxes back to its original state.

In our model, the value of the dimensionless numerical parameters was calculated in accordance with our experimental conditions, namely $[BrO_3^-] = 120$ mM, $[MA] = 30$ mM, $[H_2SO_4] = 300$ mM and $[Fe^{II}] = 5$ mM.

As the first step we calculated the minimal concentration of the activator that should cross the membrane from droplet 1 to excite the system in droplet 2 ($[HBrO_2]_{ex} = 1.1 \times 10^{-6}$ M). Fig. S4 shows an example of the excitability of the system, two signals were triggered at a short distance one from the other, with the first at $\tau = 5$ M.T.U. (1 model time unit corresponds to 33 s in experiments) having a concentration just below the excitation threshold, namely $[HBrO_2] = 1 \times 10^{-6}$ M (1.2×10^{-3} in model units M.U.) and the second at $\tau = 10$ M.T.U. is $[HBrO_2]_{ex} = 1.3 \times 10^{-3}$ M.U., moreover we also calculated the concentration of the residual $HBrO_2$ in the transmitting droplet after the oxidation of the catalyst ($[HBrO_2]_{tr} = 7.4 \times 10^{-5}$ M).

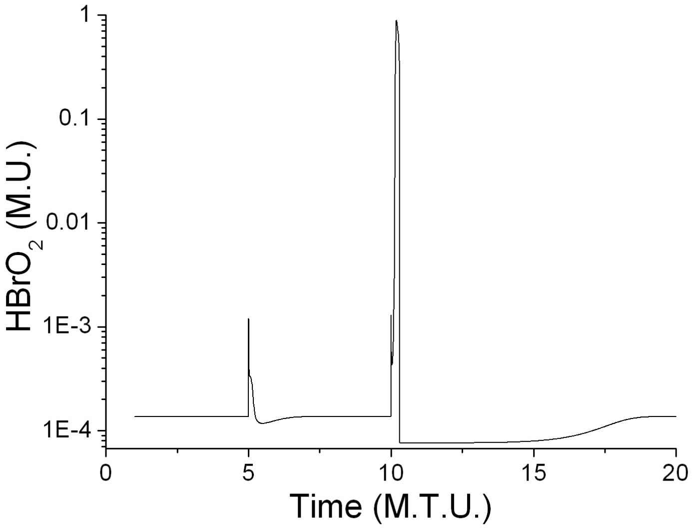

Figure S4: Example of the excitability of the system. At $\tau = 5$ M.T.U. $[HBrO_2] = 1.2 \times 10^{-3}$ M.U., at $\tau = 5$ M.T.U. $[HBrO_2] = 1.3 \times 10^{-3}$ M.U.

Considering that small polar molecules, such as $HBrO_2$, can cross phospholipids bilayers through passive diffusion, we calculated the time needed to reach $[HBrO_2]_{ex}$ in the excitable droplet by integrating an adapted form of the Fick's flux equation[11]

$$\frac{d[HBrO_2]}{dt} = \frac{P_m A_c}{V_d}\left([HBrO_2]_{tr} - [HBrO_2]\right) \quad (2)$$

where V_d is the droplet volume (1.1×10^{-5} cm^3), A_c is the contact surface area between two droplets ($\pi d_c^2/4 = 3.2 \times 10^{-5}$ cm^2) and P_m is the permeability constant of $HBrO_2$ (2×10^{-3} cm/s). The values for V_d and A_c where determined from our experiments, while the value for P_m was arbitrarily chosen, but it is in line with those for other organic and inorganic acid (HCOOH, HNO_3, HF, etc.) determined for egg-PC bilayers.[12] However, by integrating the diffusion equation (2) for a range of P_m within a factor of 5, we obtained a delay time in the interval 2 – 9 s, in excellent agreement with the timescale of our experiments.

Further simulations were performed by including the contribution of the diffusion of $HBrO_2$ into the FKN kinetic skeleton.[13] The diffusion process between two homogeneous droplets can be modelled by mass action kinetics

$$HBrO_2(d1) \xrightarrow{k_c} HBrO_2(d2) \tag{3}$$

where $k_c(s^{-1}) = P_m A_c / V_d$. Figure S5 shows the numerical integration of the coupled oscillators; in this case we integrated the dimensional form of the kinetic scheme, in order to have a direct comparison with the experimental results. At time t = 400 s an excitatory pulse of $HBrO_2$ (1.1×10^{-6} M) has been triggered in droplet 1. After a delay time of about 4 s, the concentration of the autocatalytic species begins to increase in droplet 2 with the consequent oxidation of the catalyst.

However, to have a better picture of the two communicating droplets, we are developing a model that takes into account spatial effects.

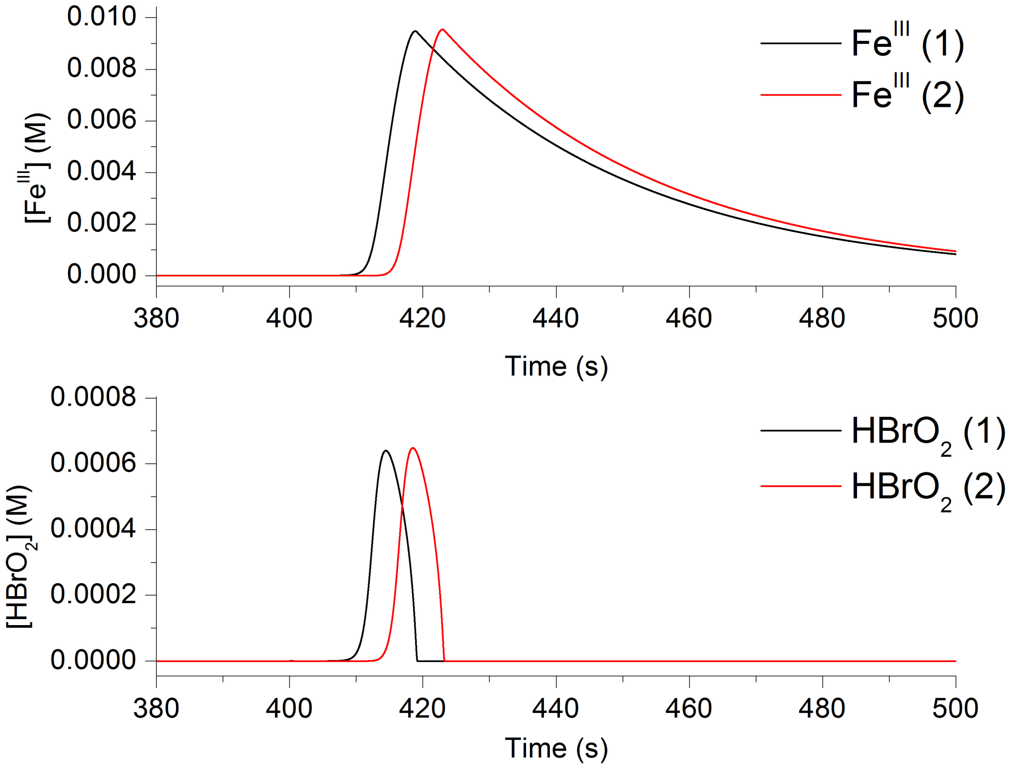

Figure S5: Signal transmission between two droplets. At t = 400 s a signal has been triggered in droplet 1 ($[HBrO_2]_{ex} = 1.1 \times 10^{-6}$ M). After about 4 s the signal reaches droplet 2 and causes the production of the autocatalytic species and the consequent oxidation of the ferroin.

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