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Introduction to Droplet-Based Millifluidic Chemistry Using a Macroscopic-Droplet Generator

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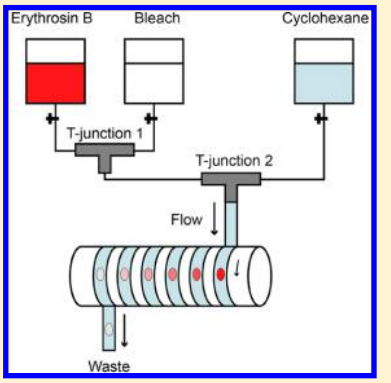
Supporting Information

ABSTRACT: This activity introduces droplet-based millifluidics and the usage of aqueous droplets as independent chemical microreactors for reaction-kinetics studies. Students build their own droplet generators from common macroscopic glassware and connecting parts to create trains of millimeter-sized droplets in which a redox reaction takes place. The activity allows students to work on reaction kinetics, hydrodynamics at low Reynolds numbers, and image analysis at the macroscopic scale.



KEYWORDS: *Second-Year Undergraduate, Microscale Lab, Kinetics, Hands-On Learning/Manipulatives, Physical Chemistry, Oxidation/Reduction, Laboratory Instruction*

INTRODUCTION

Micro- and millifluidics are now established research fields[1] and their concepts are perfusing the industry from high-end instrumentation (e.g., Sony flow cytometers) to mass-marketed cosmetic products (e.g., Capsum company) or new organic-synthesis methods (e.g., Syrris flow chemistry systems). Micro- and millifluidics are commonly defined as the ensemble of techniques and applications related to the manipulation of fluids at scales in the micrometer to millimeter range. Unlike in continuous-flow systems, droplet-based fluidic devices[1] involve the generation and manipulation of discrete droplets inside microdevices. This method can produce highly monodisperse droplets at rates of up to 10^3 droplets per second. Droplet-based microfluidics allows for independent control of each droplet, thus generating microreactors that can be individually transported, manipulated, and analyzed.[2]

In contrast to what is observed at larger scales, such as with the use of beakers or funnels for example, the effects of gravity and inertial forces on the droplets generated here are negligible on the fluid behavior. As a consequence, a simple operation such as mixing chemicals in each tiny reactor can become a real challenge.[3] Small-scale phenomena hence may appear as very counterintuitive to students learning chemistry. It is hence crucial to be able to introduce the most basic concepts and capabilities of microfluidics at the high school or undergraduate level.

Performing microfluidic experiments, however, necessitates the usage of microsystems, injection-control instruments, and microscopes,[4−9] which are not always available in undergraduate teaching laboratories. However, by scaling up the instrumentation a bit, it is possible to keep the same intellectual framework while increasing the ease both manipulation and observation. In the context of the preparation of an oral examination that takes place at the end of the second undergraduate year, we developed a simple, low-cost, and very demonstrative millifluidic experimental setup to measure the kinetics of a redox reaction.

AIM OF THE LABORATORY EXPERIMENT

The activity consists of producing dozens of independent millireactors containing the components of a redox reaction, leading to the discoloration of a food-coloring dye, E127.[10,11] The students build a millifluidic droplet generator using glassware and connecting devices commonly available in experimental-chemistry laboratories at university. Using a commercial hand-held color camera, students take pictures of the droplets over time, characterize their discoloration using a Python programming routine, and characterize the reaction kinetics within the droplets. Beyond the sole chemical concepts, this activity allows the introduction of millifluidic experiments, droplet-based chemistry, and the impact of low-Reynolds-number hydrodynamics on mixing.

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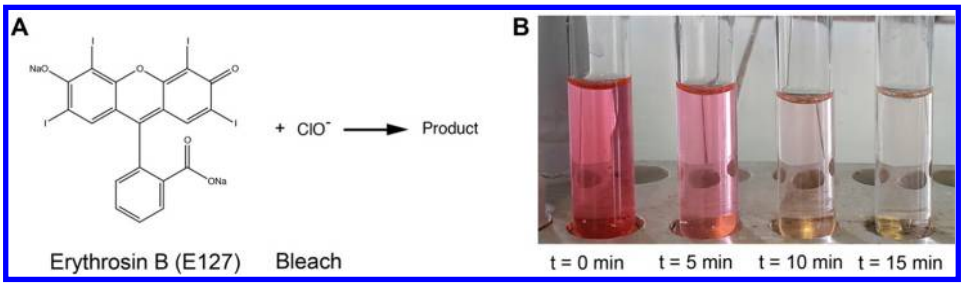

Figure 1. (A) Scheme of the redox reaction of the food-coloring compound erythrosin B with the hypochlorite oxidizing reagent, ClO^-. (B) Time evolution of the color of the reacting mixture ($[E]_0 = 3.2 \times 10^{-7}$ mol·L^{-1}, $[ClO^-]_0 = 27 \times 10^{-2}$ mol·L^{-1}) in a test tube. The original pink color of the solution disappears after several minutes because of the redox reaction that takes place.

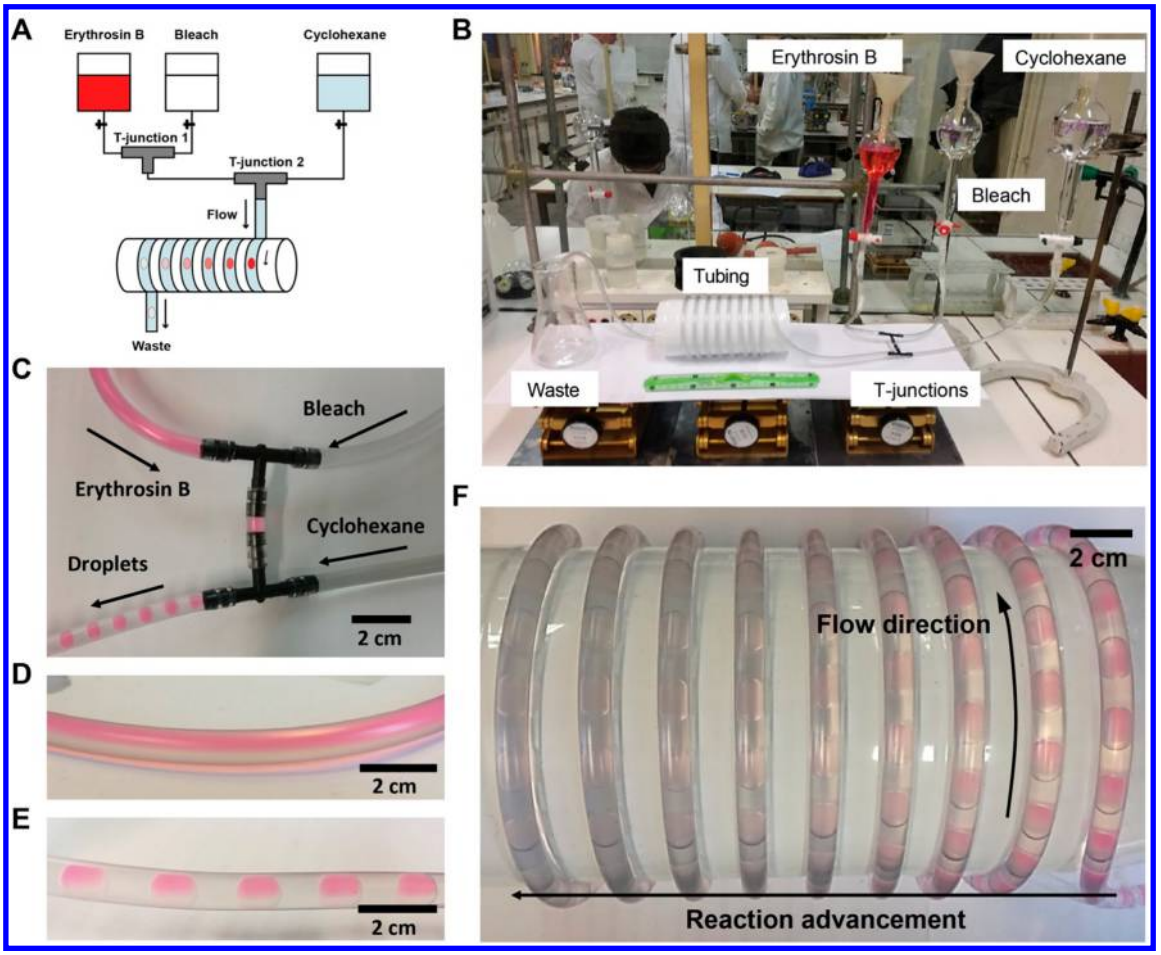

Figure 2. (A) Schematic representation of the experimental setup, composed of three separating funnels, two T-junctions, several pieces of flexible tubing, a cylinder to loop the tubing around, and a waste Erlenmeyer flask. The funnels contain, respectively, the dye solution (erythrosine), the hypochlorite solution (bleach), and the continuous phase (cyclohexane). (B) Representative picture of the experimental setup. (C) Representative picture of the two T-junctions, the second one being used as a droplet generator. (D) Enlarged view of the tubing connected to the outlet of the first T-junction. (E) Enlarged view of the droplets formed after the second T-junction containing the reactants. (F) Picture of the droplets flowing within the tubing looping around the cylinder. The color of the droplets disappears as a consequence of the redox reaction taking place in each microcompartment. The arrow indicates the flowing direction.

SAFETY HAZARDS

Students should wear eye protection, lab coats, and gloves. The dye should be handled carefully to avoid spills on the balance or benchtop. Sodium hypochlorite (household bleach) is corrosive and should be handled with care. Because food-coloring dyes are strongly colored, small quantities are used, and the nontoxic, aqueous solutions can be safely disposed down the drain. The diluted solutions of sodium hypochlorite can also be safely disposed in the drain after dilution with water. Cyclohexane must be disposed in an organic-waste container.

PRELIMINARY EXPERIMENT

To conduct this activity, we selected a simple redox reaction involving a food-coloring molecule, erythrosin B (commercially known as E127), and bleach (ClO^-) as the oxidizing agent.[12,13] The reaction scheme is summarized in Figure 1A. In a test tube, the erythrosin B solution is originally characterized by a strong

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pink color, as a consequence of its absorption spectrum (λ_{max} = 526 nm), as shown in Figure S1. After the erythrosin B solution is mixed with the bleach solution, oxidation takes place, and the mixture becomes colorless in several minutes, as shown in Figure 1B. According to the literature,[12] the reaction kinetics can be written as

$$v = k[E]^1[ClO^-]^1 \quad (1)$$

In this equation, v is the speed of the reaction ($mol{\cdot}L^{-1}{\cdot}s^{-1}$), and k is its kinetic constant. [E] and [ClO^-] are, respectively, the E127 and bleach concentrations ($mol{\cdot}L^{-1}$). The kinetics of the reaction has a partial order of 1 for each reactant in solution.

EXPERIMENT

The macroscopic millifluidic setup is composed of three separating funnels with Teflon stopcocks that serve as reservoirs for the erythrosin B solution; the bleach solution; and the flowing solvent carrier, which in our case is cyclohexane, as shown in Figure 2A. A detailed description of the materials and chemicals necessary to understand the experiment is shown in Figure 2B. The flow rates of the flowing solutions are set by the hydrostatic pressure difference between the reservoirs and the waste container and can be finely adjusted with the stopcocks. Using PVC tubing (circular cross-section, inner diameter of 4 mm) for the fluidic connections, one T-junction connector is used to create the reacting mixture by flowing the erythrosin B solution and the bleach solution, as shown in Figure 2C. The outflowing solution is then injected at the inlet of a second T-junction, which acts as a droplet generator with cyclohexane as the continuous phase. The choice of the tubing material is an important parameter of the experiment, as the cyclohexane should favorably wet the inner wall of the tubing to avoid droplet breakage during the experiment.[14] Before reaching the waste container, the droplets flow over a tubing distance of ca. 250 cm, which is generated by rolling the tubing around a cylinder, to ease both the manipulation and observation, as shown in Figure 2F. The experimental setup allows the creation of a discrete set of dozens of droplets, each containing a mixture of the dye and bleach solutions. The average flowing velocity is on the order of 5 $mm{\cdot}s^{-1}$, and each single droplet flows for around 8 min within the rolled outflowing tubing while the redox reaction takes place. According to the test-tube reaction shown in Figure 1B, this is sufficient to observe a strong discoloration of the solution.

RESULTS

We can calculate the Reynolds number associated with the droplets, and by hypothesizing that both the viscosities and the densities of the dye and bleach solutions are similar to those of water, we get

$$Re = \frac{\rho L V}{\mu} = \frac{10^3 \times 0.004 \times 0.005}{10^{-3}} = 20 \quad (2)$$

In this equation, ρ ($kg{\cdot}m^{-3}$) is the density, L (m) is the diameter of the flexible tubing, V ($m{\cdot}s^{-1}$) corresponds to the flow rate, and μ ($kg{\cdot}m^{-1}{\cdot}s^{-1}$) is the dynamic viscosity of water.

Although the Re is not as small as what can be achieved using droplet generators with microfluidic dimensions,[15] the Re is low enough to observe that as shown in Figure 2D, the bleach and dye solutions flow along each other after the mixing step, and as shown in Figure 2E, the reactive solutions can be encapsulated in the droplets without the occurrence of any convective mixing.[16] The mixing state of the droplets can be adjusted by using the three different stopcocks that will alter the relative flow rates of the flowing liquids.

At the exit of the droplet-generating T-junction, the droplets act as independent microreactors that move at the same speed and are identical in composition. The kinetics of the reaction can thus be quantified either by following one of the droplets over time or by taking a picture of several droplets simultaneously that have different positions along the cylinder and hence the tubing. Following this second method, we used a smartphone color camera to record a picture similar to the one shown in Figure 2F. A simple Python script was developed for image analysis after manual cropping of the droplets. The routine converts each droplet picture in a black and white image and averages its pixel intensities over the area of the droplet. A schematic representation of the image-analysis workflow is summarized in Figure S2, and a copy of the Python code is reported in the Supporting Information.

To illustrate the possibility of measuring kinetic data in the experiment, the apparent kinetic constant, $k_{app}=k[ClO^-]^{\alpha}$, was measured for five different initial ClO^- concentrations and was large enough to consider them constant over the timecourse of the reaction. The erythrosin B dye concentration was equal to 3.2×10^{-7} $mol{\cdot}L^{-1}$, and Figure S3A shows that the partial order of the reaction with respect to the dye is 1. As shown in Figure S3B, by plotting $\ln(k_{app})$ as a function of $\ln([ClO^-])$, we measure that the partial order with respect to [ClO^-] is 1, and the value of the kinetic constant, k, is 0.009 ± 0.001 $mol{\cdot}L^{-1}{\cdot}s^{-1}$.

PEDAGOGICAL CONSIDERATIONS

We have built in this document a droplet-based millifluidic experimental setup allowing the kinetic quantification of the redox reaction of a dye, erythrosine B, with bleach. As compared with existing protocols available in the teaching literature aimed at introducing microfluidic concepts,[4−9] the full setup can be built from materials and chemicals widely available in chemistry laboratories for undergraduate students. The setup does not require the acquisition of specific and expensive instruments such as pressure regulators and microfabricated devices.

Being macroscopic, the experiment can be advantageously used as demonstration in a classroom and can serve to introduce microfluidic concepts such as low-Reynolds-number hydrodynamics, time−distance equivalence, compartmentalization, and more general droplet-based microfluidic concepts and their applications.

The laboratory experiment should be, in the most favorable configuration, carried out by students at the undergraduate level with at least a basic knowledge of chemical kinetics, redox reactions, hydrodynamics, and interfacial tension. By choosing in an appropriate manner a specific aspect of the experiment or the experiment as a whole, we believe that high-school and graduate students might also be interested in working on the setup.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.8b00876.

Chemicals and instrumentation, safety hazards, time required, instructor's and students' notes, UV−visible-

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absorption spectrum of erythrosin B in water, image recording and analysis, and quantitative results of the kinetics experiments (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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SUPPLEMENTARY INFORMATIONS

Introduction to Droplet-Based Millifluidic Chemistry Using a Macroscopic-Droplet Generator

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Contents

1. Chemicals

Erythrosin B (CAS# 568-63-8, 2′,4′,5′,7′-Tetraiodofluorescein disodium salt) and cyclohexane (CAS# 110-82-7) were purchased by Sigma-Aldrich (L'Isle d'Abeau, France). Commercial bleach (Javel Lacroix) was purchased at a local supermarket. Its concentration in sodium hypochlorite (CAS# 7681-

52-8) is equal to 0.634 $mol \cdot L^{-1}$. All chemicals were used as received without further purification. Aqueous, diluted solution were prepared using demineralized water.

2. Equipment and instrumentation

PVC tubing (OD = 6 mm, ID = 4 mm, ref.: B005JYLABK) and T-junctions (OD = 4 mm, ref. : B01KZNJGSS) were purchased by Amazon.com. Other parts (Pyrex separating funnels with Teflon stopcocks, stands, erlenmeyers, etc.) are commonly available in chemistry laboratories dedicated to teaching.

3. Safety hazards

Students should wear eye protection, lab coats, and gloves. The dye should be handled carefully to avoid spills on the balance or bench top. Sodium hypochlorite (household bleach) is corrosive and should be handled with care. Since food coloring dyes are strongly colored, small quantities are used. Their nontoxic, aqueous solutions can be safely disposed of down the drain. The diluted solutions of sodium hypochlorite can also be safely disposed of in the drain after dilution with water. Cyclohexane must be disposed in an organic waste container.

4. Time required

The millifluidics experimental setup described in the document is easy to build and most of the experimental work related to the reaction kinetics can be achieved within a 3 h teaching session. However, the activity can be focused on several different aspects that could lengthen the duration, for the benefit of the students:

(1) Before performing the redox reaction and its kinetic analysis, one can ask the student to study the droplet formation mechanism and build a phase diagram of the mixing state of the droplets as a function of the relative flow rates of the different fluids. For this specific part, bleach is not necessary and can be replaced by water as the mixing only is analyzed.

(2) The image analysis can be done using already written ImageJ macros or Python routines, or one can ask the students to build these codes themselves.

Considering all these scientific aspects (hydrodynamics, chemistry, analysis), the activity could go up to 3 teaching sessions, the first two taking place in the chemistry lab, the last one taking place in a computing classroom or at home.

5. Instructor's notes

(1) Determination of the partial orders of the reaction kinetics of erythrosin B with bleach is dependent on several parameters such as the ionic strength of the solution[1]. We hence recommend the instructor to perform these reactions in bulk conditions with various initial concentrations and characterize their kinetics before giving the activity to students.

(2) The choice of the tubing material is an important parameter of the experiment as the cyclohexane should wet favorably the inner wall of the tubing to lubricate the droplet displacement and avoid their breaking during the experiment.

(3) For these experiments, we used a bleach solution sold in French supermarkets, composed of sodium hypochlorite. This reagent can be replaced by the pure product or a solution purchased by chemical suppliers like Sigma-Aldrich.

(4) Depending on the flow rate conditions during the millifluidic experiment, droplets can be homogeneously mixed or biphasic, which may change the final kinetic quantitation of the redox reaction. These experimental conditions and their influence on the final results should be checked before giving the activity to students.

(5) For the quantitation of the color of the droplets, we chose to use Python as the programming language as it is open source and now widely used at the undergraduate level in STEM. Before running the activity, make sure that the installed Python distribution provides image analysis and plotting librairies. Fiji/ImageJ macros can also be used for the image analysis and quantitation. It is up to the instructor to decide whether students have to build their programming routine themselves or use a routine built beforehand.

6. Students' notes

(1) The experimental setup is relatively easy to build, but a good comprehension of the control of the flow rates of the different liquids is necessary before starting the actual kinetic analysis of the reaction. We advise the students to "play" with the height of the separating funnels and the stopcocks to have an idea of the parameters that will lead to droplets regularly generated with a stable reactant mixture at the exit of the second T-junction.

(2) When taking pictures, please pay attention to the existence of flares or uneven light conditions that may alter the image analysis.

(3) Please quantify the flow rate of the droplets inside the tubing and estimate their transit time. These results can be compared to the time of the reaction that has been measured independently in bulk conditions with the spectrophotometer.

(4) Are the quantitation methods used for the cuvettes (spectrophotometry) and the droplets (colorimetry) similar or different?

7. UV-Visible spectrum of Erythrosin B in water

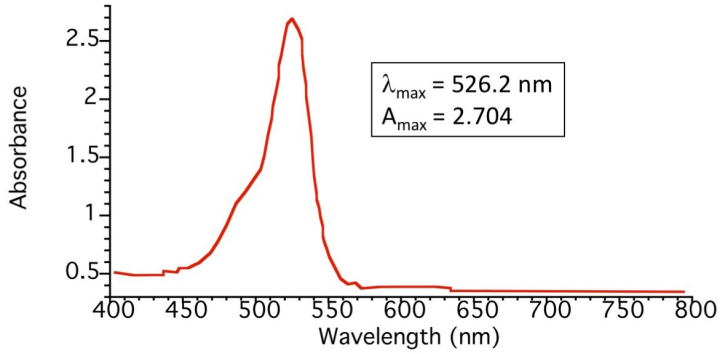

Figure S 1: Visible absorption spectrum of Erythrosin B in water (Concentration = $3{,}2\times10^{-7}$ mol·L^{-1}). The spectrum shows an absorption maximum at λ_{max}=526.2 nm.

8. Image recording and analysis

Pictures of the droplets were recorded using a smartphone camera (Huawei P9 and P20). Image analysis was performed using an in-house Python script (ver. 3.4.2, distribution: Pyzo).

The image analysis workflow described in Figure S 2 follows these< steps :

(1) Recording of a JPG picture of the experimental setup recorded with a smartphone camera or another type of digital camera available in the laboratory

(2) Uploading the pictures to a personal computer

(3) Manually cropping and individualization of the droplets of interest, using a image analysis software such as Fiji/ImageJ[2] (open-source).

(4) Running of a handmade Python script executing the following steps :

   a. :Conversion of the cropped RGB images of the droplets in BW pictures

   b. Measurement of the average intensity per droplet, i.e. the sum of the intensities of all the pixels with a non-zero value divided by the area of these pixels.

(5) Computed value are either analyzed in Python or using and external plotting and data analysis software as MS Excel, SciDAVIS (Open-source), etc.

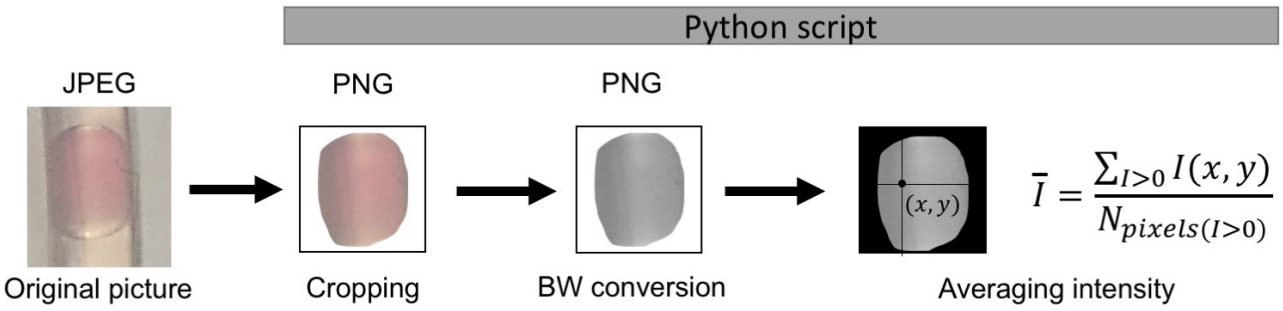

Figure S 2: Schematic representation of the image analysis and quantitation workflow. Starting from a JPG picture of the experimental setup recorded with a smartphone camera, we first manually crop droplets areas. Then, using a Python script, we convert the RGB images in a BW pictures and we measure the average intensity per droplet, considering solely the pixels with a non-zero value.

A copy of the Python code is available here : https://github.com/FattaccioliLab/Codes-Macros/

9. Quantitative analysis of the kinetics experiments

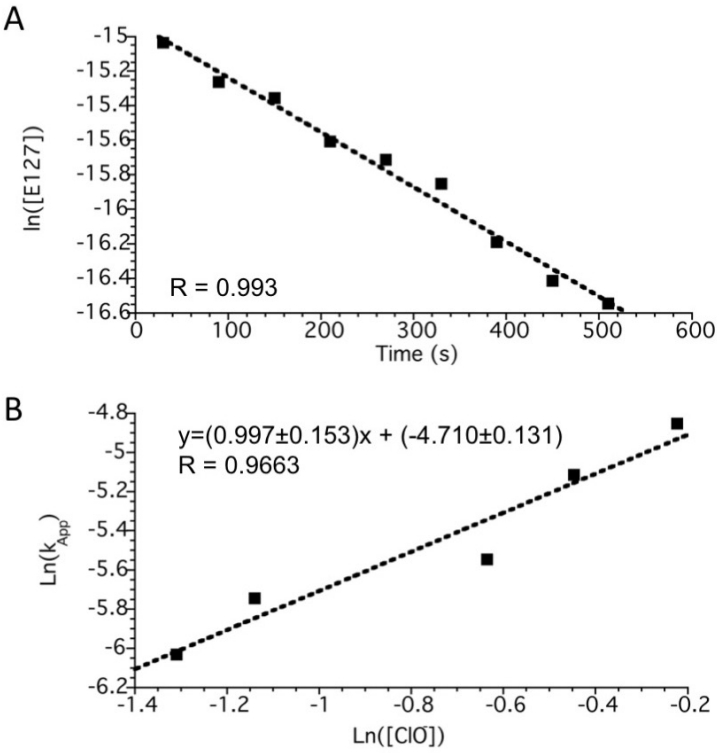

Figure S 3: (A) Plot of ln([E127]) as a function of time for [E127] = 3.2×10^{-7} mol·L^{-1} and [ClO$^-$] = 0.32 mol·L^{-1}. Data are fitted by a linear equation. (B) Plot of ln(k_{app}) = f(ln([ClO$^-$]) for a concentration of Erythrosin B to 3.2×10^{-7} mol·L^{-1}. Data are fitted by a linear equation (slope = 0.997 ± 0.153, intercept = -4.710 ± 0.131).

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