Supplementary Information

Entrapment in Giant Polymersomes of an Inorganic Oscillatory Chemical Reaction and Resulting Chemo-Mechanical Coupling

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Materials and Methods:

Polybutadiene-b-polyethylene oxide (PB_{46}-b-PEO_{31}) supplied by Polymer Source, Canada. Sucrose, sulfuric acid, malonic acid, sodium bromate, 1,4 cyclohexadione (CHD), Tris(bipyridine)ruthenium(II) dichloride, [Fe(*o*-phen)$_3$]SO_4 (ferroin) aqueous solution 25 mM, chloroform and toluene was from Sigma Aldrich, USA. Water was always ultrapure 14 MΩ.

MA-BZ reaction recipe:

The final concentrations of the Belousov-Zabhotinsky (BZ) reaction components were: H_2SO_4 600 mM, malonic acid 70 mM, $NaBrO_3$ 100 mM, $Ru(bpy)_3$ 0.24 mM. For vesicle formation experiments we prepared 1 mL of the MA-BZ reaction solution and for potentiometer measurements we worked with a total volume of 10 mL.

CHD-BZ reaction recipe:

The final concentrations of the Belousov-Zabhotinsky (BZ) reaction components were: H_2SO_4 600 mM, CHD 0.111 mM, $NaBrO_3$ 246 mM, Ferroin 3.5 mM. For vesicle formation experiments we prepared 1 mL of the CHD-BZ reaction solution and for potentiometer measurements we worked with a total volume of 10mL.

Preparation of Vesicles:

A polymer stock solution with a concentration of 3mg/mL was prepared by solubilizing PB_{46}-b-PEO_{31} in either toluene or chloroform. The stock solution was kept under magnetic agitation for 2 hours and 360 rpm, which assured the complete solubilization of the polymer in either toluene or chloroform. A 100 mM sucrose stock solution was prepared in water.

Rapid evaporation method [1]: vesicles formed in the MA-BZ variant of the reaction.

1 mL of the polymer stock solution (3mg/mL in chloroform) was added to a 50 mL round bottom flask, and 1 mL of a freshly prepared MA-BZ reaction solution was added to it. The flask was then connected to a Rotavapor (Buck, Swiss), with a water bath previously heated to 40C, the pressure was adjusted to 50 mbar, and the chloroform was evaporated in 1 minute. The flask was removed from the Rotavapor. Immediately a 10 μL sample of the polymersome suspension was collected, placed in a glass slide with cover slip and observed in phase contrast microscopy with a Zeiss Achroplan 40x objective. Photographs were taken every 2 seconds for 90 minutes, using a Pixelink camera connected to an inverted microscope (Axio Observer A1 Zeiss, Germany).

Inverted emulsion method [2]: vesicles contain CHD-BZ reaction and are contained in a sucrose solution.

We followed the steps described by Patout et al. with a few modifications. Briefly, 500 μL of the polymer stock solution (3mg/mL) were placed in a polypropylene microcentrifuge tube and set aside to prepare the inverted emulsion/droplets. The inverted droplets were freshly prepared for each experiment and used immediately after preparation. The procedure consists of adding 5μL of the active CHD-BZ reaction mixture to the polymer in toluene, and vortexing for 30 seconds.

In a separate microcentrifuge tube, 30 μL of sucrose 100mM were initially added to the tube and immediately 30 μL of polymer dissolved in toluene were gently poured over the sucrose solution. The

tube was closed and allowed to rest for 30 minutes (equilibration time, required to form a monolayer of polymer in the water/toluene interface, with hydrophilic head groups oriented towards water and hydrophobic tails oriented towards toluene). Then, 50 μL of the inverted droplets formed using 5μL of freshly prepared BZ reaction in 500 μL of polymer in toluene were poured over this doubly layered system, forming a third layer. This microcentrifuge tube was immediately centrifuged for 10 minutes at 2000 rcf., at 20 C. After centrifugation, one could visually observe the pellet that had already formed at the bottom of the microcentrifuge tube together, with a clear layer of toluene over the aqueous solution. The pellet was then separated from the toluene by pipetting, and toluene discarded. To remove traces of toluene the microcentrifuge tube was placed in the oven at 50 C for 10 minutes. Immediately after that, 10 μL of the polymersome were collected, placed in a glass slide with a cover slip and observed in phase contrast microscopy with a Zeiss Achroplan 40x objective. Photographs were taken every 2 seconds for 90 minutes, using a Pixelink camera connected to an inverted microscope (Axio Observer A1 Zeiss, Germany).

For vesicles formed in water, the procedure described above was followed with modifications: sucrose was substituted for 40 μL of water, and then 40 μL of polymer in toluene stock solution were poured over it. The inverted droplets were formed using 5 μL of water instead of the BZ reaction. The other steps were the same.

For vesicles entrapping sulfuric acid 600 mM, the procedure described for preparing vesicles containing a CHD-BZ reaction in 100 mM sucrose was also followed in this case, although with some modifications: The inverted droplets were formed using 5 μL of 600 mM sulfuric acid instead of the recipe for the case of BZ reaction entrapment. Pictures were obtained using an Evolve 512 (Photometrics, USA) camera. The remaining steps were the same.

Potentiometric measurements:

The BZ reaction was prepared in a 10 mL beaker. The BZ reaction was stirred for 30 seconds at 360 rpm, and then the magnetic stirrer was turned off. All data were collected with no agitation. The redox potential of the BZ reaction was measured using an Ag|AgCl (filled with KCl 3M) micro redox electrode (Microelectrodes, USA). The Ag|AgCl electrode was connected to a Benchtop meter (Sper Scientific, USA) itself connected to a Dell computer. The software HandHeld (Sper Scientific, USA) was used to collect the data, which were subsequently exported and analyzed using Excel.

Supplementary Figures:

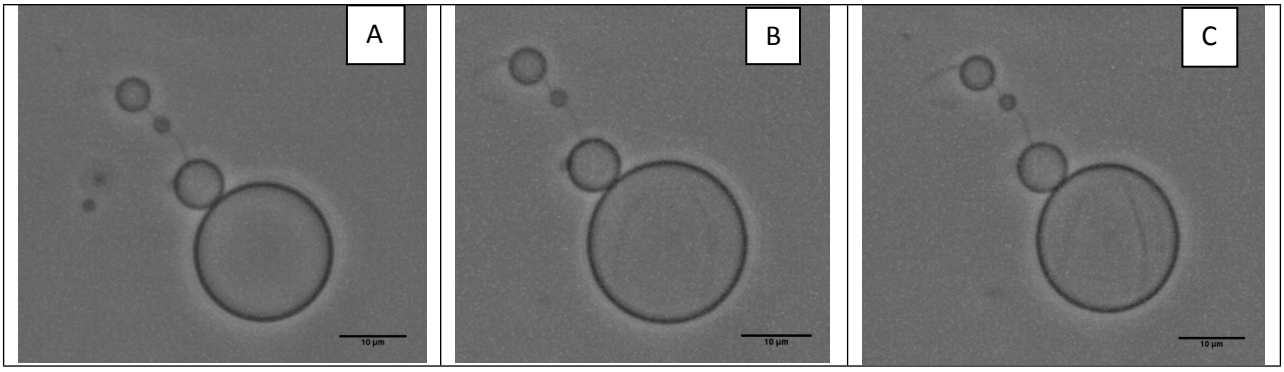

Figure S1: Polybutadiene-b-polyethylene oxide polymersomes (PB_{46}-PEO_{31}) in pure water. The photographs represent the process that polymersomes go through during an elapsed time of 93 minutes. The micrograph in panel A. was taken 2 minutes after the start of the experiment; Panel B shows the time evolution 41 minutes after the start; And panel C was taken 93 minutes after the initiation of the experiment. Initially, the larger vesicle has a mean size of about 19 μm, and after 40 minutes it increases by approximately 2μm in size and, finally, after 90 minutes the process of shrinking described in the text starts to occur. Scale bar 10μm.

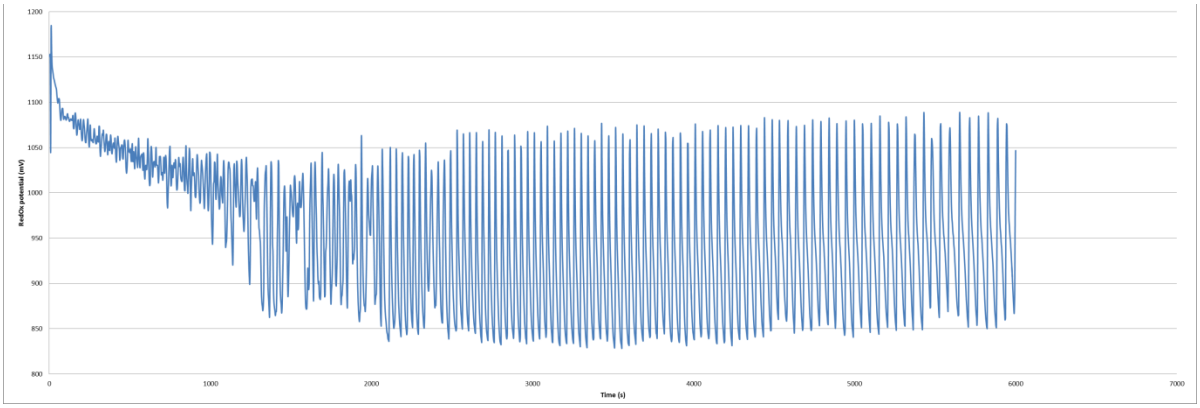

Figure S2: Time series of the RedOx potential for our BZ reaction run as an unstirred system. The time series display the evolution of the reaction immediately after the addition of the rhutenium catalyst to the system. The recipe was: H_2SO_4 600 mM, malonic acid 70 mM, $NaBrO_3$ 100 mM, $Ru(bpy)_3$ 0.24 mM. The RedOx potential was measured with an Ag|AgCl (filled with KCl 3M) micro RedOx electrode and the temperature was 19C. It is clearly seen that the recipe used for the BZ reaction required an induction time of 2532 seconds, before the onset of regular oscillatory behavior sets in.

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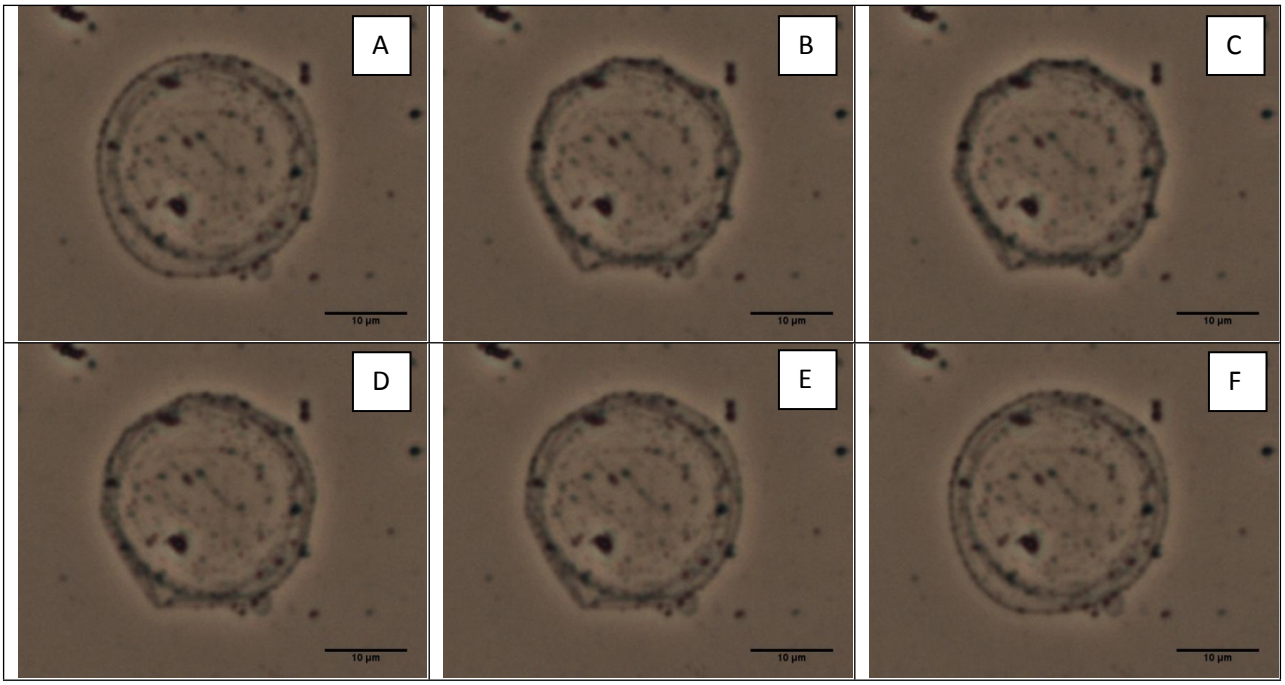

Figure S3: Polybutadiene-b-polyethylene oxide polymersome (PB_{46}-b-PEO_{31}) containing MA-BZ reaction (H_2SO_4 600 mM, malonic acid 70 mM, $NaBrO_3$ 100 mM, Ru(bpy)3 0.24 Mm) prepared using rapid evaporation method. BZ reaction is present inside as well as outside the polymersomes. The micrographs shown in panels A to F demonstrate coupling betweenthe MA-BZ reaction and the PB_{46}-b-PEO_{31} polymersome membrane. Panels above represent the evolution of a particular vesicle. The time in seconds after vesicles formation and the respective vesicle diameter sizes are: A (1024 s; 27.8 um); B

(1028 s, 25.7µm); C (1048 s, 25.0 µm); D (1060 s, 25.6 µm); E (1076 s, 26.3 µm); and F (1092 s, 27.5 µm). Scale bar 10 µm.

Stability of a polymersome entrapping a 600 mM sulfuric acid solution.

Figures S4 and S5 illustrate vesicles formed in 100 mM sucrose and entrapping a 600 mM sulfuric acid. This experiment was carried out to evalute the stability of the vesicle membrane to acidic conditions, and by performed observing the contrast or shape changes through time in order to identify possible leakage of the internal medium. We prepared a batch of vesicles and collected a sample that was observed during 2 hours under phase contrast microscopy. As soon as we finished the first sample observation, 8100 seconds after vesicle formation, a second sample was collected and observed for 2 hours.

Figure S4, panels A to E, shows the time evolution for a 10 µm diameter vesicle obtained from the first sampling of our polymersomes stock suspension. The intensity of contrast and the vesicle size remained unchanged and no membrane displacement was observed during the course of the experiment.

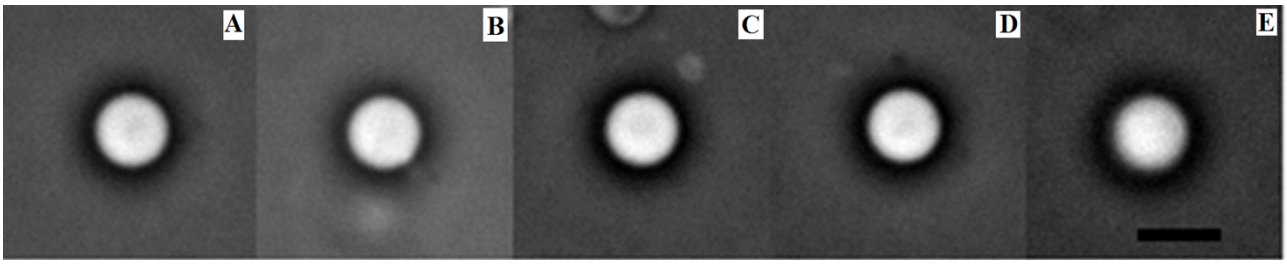

Figure S4: Polybutadiene-b-polyethylene oxide polymersome (PB_{46}-b-PEO_{31}) containing H_2SO_4 600 mM, prepared using inverted method in sucrose solution 100 mM as imaged by phase contrast microscopy. The micrographs shown in panels A to E illustrate the time evolution of the vesicles clusters. The times are: A) 900s; B) 1694s, C) 2488s, D) 3282s and E) 4076s. Scale bar 10 µm.

2 hours later a second sampling from our polymersome stock suspension was observed under phase contrast microscopy. The results are presented in Figure S5. Panels A to E show a 15 µm diameter vesicle, and we can clearly see that there was no membrane displacement or change in contrast during the course of this experiment.

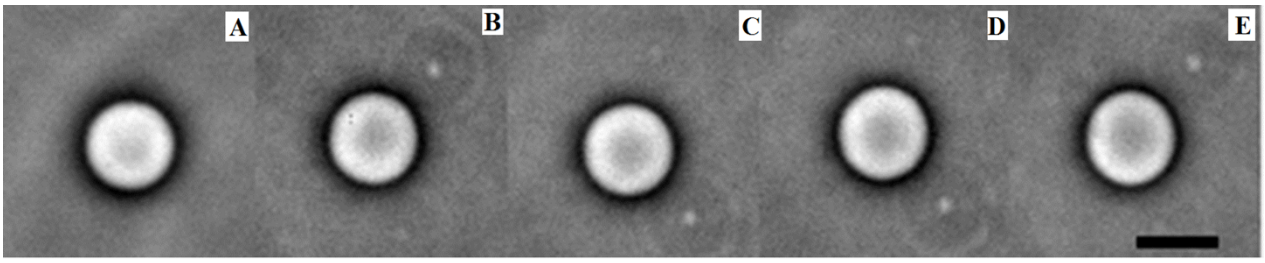

Figure S5: Polybutadiene-b-polyethylene oxide polymersome (PB_{46}-b-PEO_{31}) containing H_2SO_4 600 mM, prepared using inverted method in sucrose solution 100 mM as imaged by phase contrast microscopy. The micrographs shown in panels A to E illustrate the time evolution of the vesicles clusters. The times are: A) 8100s; B) 8812s, C) 9538s, D) 10182s and E) 10956s. Scale bar 10 μm.

It follows from these experiments that our polymersomes are pH resistant and that indeed they can endure the 2 hours observation under microscopy just as in our MA-BZ or CHD-BZ reaction experiments.

Videos:

PolW1.avi: PB_{46}-b-PEO_{31} polymersomes prepared in pure water.

PolBZ1.avi: PB_{46}-b-PEO_{31} polymersome prepared in MA-BZ reaction.

PolBZ2.avi: PB_{46}-b-PEO_{31} polymersome prepared in MA-BZ reaction

PolBZ3.avi: PB_{46}-b-PEO_{31} polymersomes prepared in CHD-BZ reaction

References:

1. **A. Moscho, O. Orwar, D. T. Chiu, B. P. Modi and R. N. Zare, *Proc Natl Acad Sci U S A*, 1996, 93, 11443-11447.**
2. **S. Pautot, B. J. Frisken and D. A. Weitz, *Langmuir*, 2003, 19, 2870-2879.**