	Experiment title: Structural Evolution Induced by a Chemical Oscillator in Biomimetic Systems	**Experiment number:** SC-1956
Beamline: ID2	**Date of experiment:** from: 26/04/2006 to: 28/04/2006	**Date of report:** 29/08/2006
Shifts: 6	**Local contact(s):** Emanuela Di Cola	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Sandra Ristori* (University of Florence)

Dr. Monique Dubois (CEA Saclay LIONS)

Prof. Irvin R. Epstein (Brandeis University)

Dr. Federico Rossi* (University of Siena)

Prof. Mauro Rustici* (University of Sassari)

Dr. Olivier Spalla* (CEA Saclay LIONS)

Dr Thomas Zemb (CEA Saclay LIONS)

Report:

Oscillating phenomena occur in a wide variety of fields, from simple physical and chemical systems to living organisms. A huge literature on chemical oscillators exists, but comparatively few studies have been performed in nanostructured systems of biological relevance. Moreover, to the best of our knowledge, no experiments so far have demonstrated a direct relationship between the pattern type and the host matrix structural features. This was mainly due to experimental difficulties, which in this case can be overcome by the high spatial and time resolution of a synchrotron beamline.
In the course of our experimental session, the Belousov-Zhabotinsky (BZ) oscillating reaction was carried out in the aqueous phases of two classes of nanoscopic structures: phospholipid membranes (DPPC and DPhPC) and water in oil reverse micelles (AOT/n-octane/water). In particular, among the membranes a very interesting example of model lipid for archaeobacteria membranes was used (DPhPC).

During the 6 shifts of the allocated beamtime 15 systems were analyzed, including references, and more than 1800 SAXS curves were recorded. The configuration used was: detector to sample distances 1.0 m and 3.0 m; beam energy 12 KeV.

The ample environment was a flat cell consisting of a 3cm diameter window in a 5 cm stainless steel support covered with mica sheets (6-10 μm thickness) separated by Teflon gaskets of various thickness (80-500 μm).
The WAXS camera was not set up, since too much time was necessary and only SAXS data were acquired. A digital CCD camcoder placed in line with the sample holder inside the experimental hutch allowed the recording of images.
After running tests on several samples the time limit of registration without appreciable beam damage was established to be 6-7 seconds. Samples were analyzed as a function of time and space.
Figure 1a) shos a typical image taken on the sample with (DPPC:DPhPC = 5:1) and total lipid content 30% w/w. For this sample the initial composition of the aqueous phase was: [malonic acid] = 0.11M, $[BrO_3^-]$=0.35M, $[Br^-] = 4.3 \cdot 10^{-2}$M, [ferroin] = $9.7 \cdot 10^{-3}$M, $[H_2SO_4]$ = 0.35M. Bright lines in the picture are chemical waves (λ_{mean} = 1.8 mm), moving from the edges to the centre of the reactor. The red dot shows the incidence position of the beam on the sample. Spiral structures are present near the edges. The dark spots are CO_2 bubbles produced in the course of the BZ reaction.
Figure 1b) shows the SAXS spectrum corresponding to the system in figure 1a) (red line), compared with its reference (black line). The latter spectrum was obtained by replacing the BZ solution with pure water. Similar images and spectra were obtained for samples containing only DPPC in the lipid bilayers. In all cases, the Bragg peaks of the lamellar phase were narrower in the presence of the BZ reactants, indicating that the spatial correlation was longer, i.e. the domains where the director of the lyotropic smectics is constant extend over a broader region in the lipid/BZ system than in the reference. Moreover, the lamellar periodicity was slightly reduced (by 3-4 Å) in the presence of the BZ reactants.
When recording SAXS curves at different points of a sample, marked intensity variations were evidenced for the lipid/BZ systems, but not for the corresponding references. Figure 2 reports the trend of the first Bragg peak intensity as a function of beam position along several linescans. Spatial variations were attributed to pattern formation occurring upon development of the oscillating reaction.

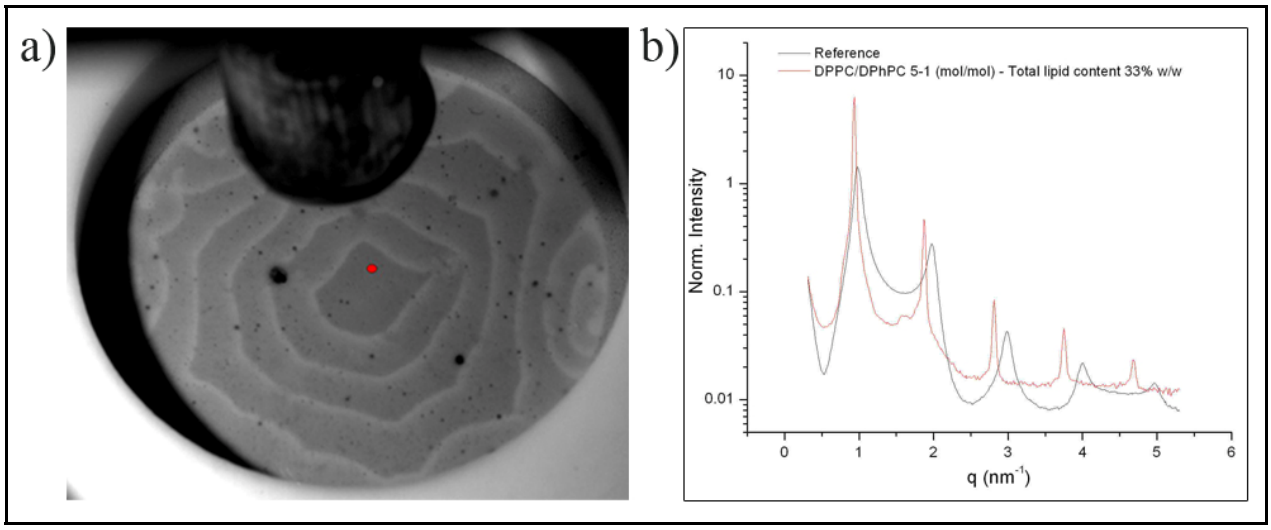

Figure 1. In a) the diameter of the imaged region is 29 mm. The size of the red dot is arbitrary.

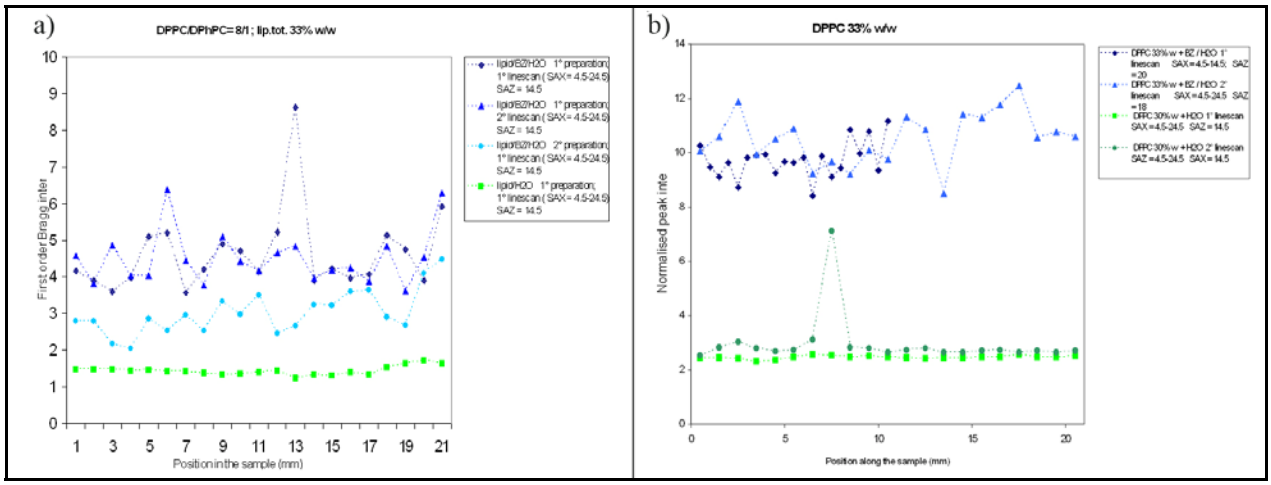

Figure 2

Figure 3a) shows typical stationary Turing patterns (λ_{mean} = 0.3 mm), which occur in the BZ/microemulsion system. The sample in the picture had the following composition: [malonic acid] = 0.03194M, [BrO_3^-] = 0.02619 M, [ferroin] = 0.0006M, [H_2SO_4] = 0.02927M, $[H_2O]_{total}$=7.985M, [AOT]=0.53M. Based on the water/surfactant molar ratio (ω = [H_2O]/ [AOT] = 7.985/0.53 = 15), the aqueous phase is expected to be confined in spherical droplets with radius ~ 25-26 Å.

As in the case of BZ/lipid systems, marked intensity variations were observed during data acquisition at different points of the linescan. Figure 3b) exihibits the oscillations of the main peak normalized intensity along the linescan (SAX 4.5-24.5, SAZ 14.5). The inset of figure 3b) shows a comparison between two microemulsion systems with the same [H_2O]/[AOT] ratio, one loaded with BZ reactants (red line), the other a blank (black line).

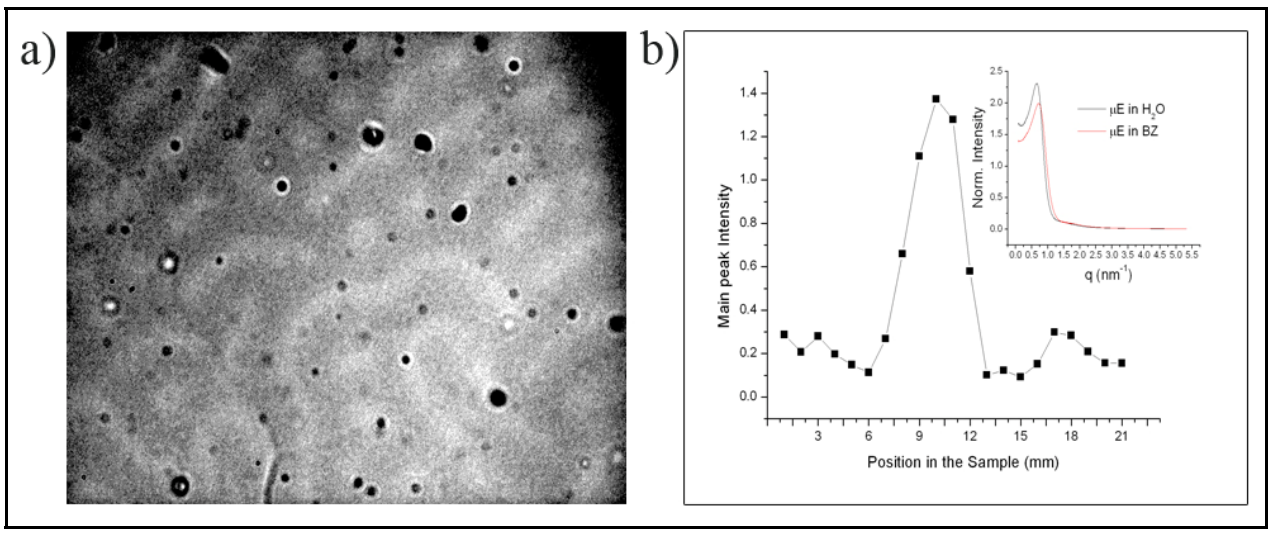

Figure 3. The horizontal size of the image in a) is 2.5 mm

In conclusion, the above described experiments enabled us to define some limitations in our experimental setup, as well as to suggest several promising improvements. The former were primarily CO_2 bubble production, partial mica deterioration over long experimental periods and certain lacks in the video recording system. Solutions to such technical problems are already identified: substitution of malonic acid with different organic substrates, use of glass windows and better performing video apparatus.
Nevertheless, we obtained encouraging evidence that SAXS investigation can provide valuable insights about the interactions between chemical oscillators and the host matrix.

Abbreviations: DPPC = 1,2-Dipalmytoyl-*sn*-Glycero-3-Phosphocholine; DPhPC = 1,2-Diphytanoyl-*sn*-Glycero-3-Phosphocholine, AOT = bis(2-ethylhexyl) sulfosuccinate, sodium salt.