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Why can artificial membranes be fabricated so rapidly in microfluidics?†

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Droplet interface bilayers are a convenient tool to produce and explore lipid membrane properties. We discuss why their formation time in microfluidics can be three to six orders of magnitude faster compared to conventional bulk settings.

Because of their biological significance, there has recently been great interest in the formation and the exploration of artificial lipid bilayers in microfluidic settings.[1–3] Creating well controlled bilayers *in vitro* is a long-lasting challenge and a variety of different techniques have been developed over the years to create free standing or supported bilayers.[4] A recent technique to generate free standing bilayers is to first create individual aqueous droplets in an external oil phase whereas the membrane forming lipids are either dissolved in the aqueous or in the oily phase.[1,5] Over time, the dissolved lipids self-assemble into a lipid monolayer at the oil–water interface. In a second step two such fully covered interfaces are brought into contact and the oil between two lipid monolayers drains away leaving behind a lipid bilayer between the aqueous droplets (droplet interface bilayer). In this method, the time needed to stabilize an oil–water interface is dominated by the fairly slow diffusion of lipids to the interface meaning that two droplets cannot be brought into contact directly after formation but have to be "pre-stabilized" in the oily phase to obtain sufficient surface coverage. Depending on the exact procedure, the required waiting time for interface stabilization can vary from a few milliseconds to tens of minutes.[5]

In this article, we discuss the stabilization time for two typical microfluidic situations: the automatic, continuous and fast formation of bilayers in microfluidic channels, and the formation of individual bilayers in a microfluidic cross. Comparing the formation time in different geometries, we derive the reasons leading to the very fast membrane formation in microfluidics.

Microfluidic devices are made from Sylgard 184 (Dow Chemical) using a standard soft lithography technique[6] and are bonded to glass slides. The liquid flows are volume controlled using syringe pumps and monitored using an inverted fluorescence microscope (Olympus, IX81).

The ultra-fast formation of stable asymmetric membranes can be impressively demonstrated in a microfluidic setting by the *in situ* formation of densely packed emulsion droplets using a step emulsification device[7] as shown in Fig. 1. The continuous oil phase consists of 2.5 wt% 1,2-diphytanoyl-*sn-glycero*-3-phosphocholine (DPhPC) in squalane (mixture A). Squalane does not swell PDMS, and is further immiscible in lipid bilayers, resulting in solvent free bilayers.[4] The liquid injected from top consists of an aq. solution (Millipore™) with 0.5 wt% of the red fluorescing lipid 1,2-dipalmitoyl-*sn-glycero*-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) solved by sonication (mixture B). Similarly, the liquid injected from below consists of an aq. solution of 0.45 wt% of the fluorescent lipid 1,2-dioleoyl-*sn-glycero*-3-phosphoethanolamine-*N*-(carboxy fluorescein) (mixture C).§ All lipid concentrations are well above the CMC. Droplet pairs were generated at frequencies of about 10 Hz at a large dispersed phase volume fraction exceeding 0.9, *i.e.* the ratio of dispersed to total liquid volume. According to the production frequency, each droplet interface is brought into contact with

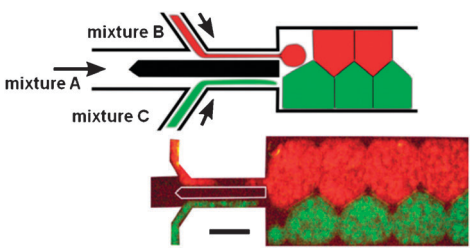

Fig. 1 Formation of asymmetric bilayers in microfluidics. The continuous phase is injected from the left consisting of the oil–lipid mixture A, the dispersed aqueous phases consist of lipid mixtures B and C injected from the side channels. Top: schematic of a double step-emulsification device[7] for the synchronized production of droplet pairs. Bottom: overlay of two fluorescence images showing the formation of a dense arrangement of droplets. Height of the channel is 100 μm, scale bar denotes 100 μm.

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the previously generated droplet ~50 ms after its generation, forming stable membranes in a foam-like topology.

In the shown example three types of lipid bilayers are automatically formed: two symmetric bilayers of lipid AB : AB and AC : AC are formed within the top and bottom rows, respectively, and asymmetric bilayers of lipids AB : AC are formed between the droplets of the top and bottom rows. The existence of membrane formation was demonstrated by measuring the capacitance of the membranes, as described below.

Because of the ample possibilities to explore membrane properties in microfluidics, it is of particular interest to explore why lipid membranes that take several ten minutes for stabilization in static bulk settings can be stabilized within milliseconds in microfluidic environments. In a static setting the coverage of a droplet interface with a surfactant depends on the concentration and diffusion of the surfactant into the interface.[8] At sufficiently high surfactant concentration the surfactant might be present in the solvent in micellar form.

To estimate the time needed for interface stabilization we measure the time dependent variation of the surface tension of a water droplet immersed in hexadecane containing 2 wt% of the phospholipid 1,2-dioleoyl-*sn-glycero*-3-phosphocholine (DOPC) using the pendant drop method (OCA20, Data Physics). Such a measured droplet is shown in the inset of Fig. 2, whereas the symbols show the time evolution of the interfacial tension. For the measurements displayed as circles and triangles, DOPC was dissolved in the oil, respectively, in the water phase. In both scenarios, the lipid molecules, which are present in micellar form, reach the interface by diffusion where they open up to spread, thus reducing the surface tension.[8] Immediately after the droplet is formed in the oil phase, a quick decrease in the interfacial tension is observed. This fast decrease is followed by a very gradual reduction thereafter reaching an interfacial tension of about 18 mN m^{-1} after about 17 min.

Applying a gentle flow of continuous phase around the droplet the transport of micelles to the water/oil interface is accelerated and the time evolution of the interfacial tension is enhanced dramatically as shown in Fig. 2. About 130 s after creating a water droplet, the additional flow of hexadecane containing DOPC was applied for 10 s at a volumetric flow rate of 250 μL s^{-1} around the water droplet. The flow around the droplet results in a sudden decrease of the interfacial tension of the droplet (green squares). Subsequent to this sharp decrease there is only a slight reduction of the surface tension with further waiting time indicating that the equilibrium coverage of the droplet with lipid molecules has been reached. This plateau value at about 5 mN m^{-1} is more than three times lower than that of the static droplets after ~17 min, such that the surface coverage of the droplets is expected to stabilize two touching droplets efficiently against coalescence. So with a surrounding flow, the surface coverage of a droplet with the surfactant does not only depend on concentration and diffusion of surfactant and micelles into the interface but also on the local flow field transporting the (micellar) surfactant to the interface. The typical time scale for this advective transport is given by the transport length L divided by the flow velocity U of the surfactant containing liquid, $t_{adv} \sim L/U$. For longer distances the advection time can be orders of magnitude faster than the typical time scale for surfactant diffusion $t_{diff} \sim \rho L^2/\eta$ which is additionally dependent on the viscosity of the liquid phase η and the density of the surfactant ρ.

Having in mind the tremendous advection accelerated coverage of a pendant drop with lipids, we explore the membrane formation between two aqueous fingers in a microfluidic cross geometry, Fig. 3. When the two aqueous fingers containing mixture B (top) and mixture C (bottom) are brought into contact with one another directly after formation, they coalesce immediately upon contact. But if the fingers approach slowly within about 5 min, their water/oil interfaces are sufficiently decorated with lipids prior to contact and a solvent free bilayer will form at their contact area. Compared to the static formation of a droplet interface bilayer in an oil phase, the equilibration time of about 5 min is already short. The reason for that is the advective transport in and around the liquid finger that is moved forward in a microfluidic channel. The importance of the advective transport for the short equilibration times in microfluidics becomes clear if an additional cross flow of the continuous phase containing lipid A is applied through the horizontal channel, *cf.* Fig. 3. With applied cross flow, a stable bilayer is formed immediately when the two fingers are brought into contact, where 'immediately' corresponds to the time needed to bring the liquid fingers into contact, *i.e.* less than 1 s.

This result also indicates that the membrane formation between two oil/water interfaces which are fully decorated with lipids is very fast[4] and the role of the equilibration time is just to achieve sufficient surface coverage. The time needed for membrane formation between two fully decorated oil/water interfaces is estimated by

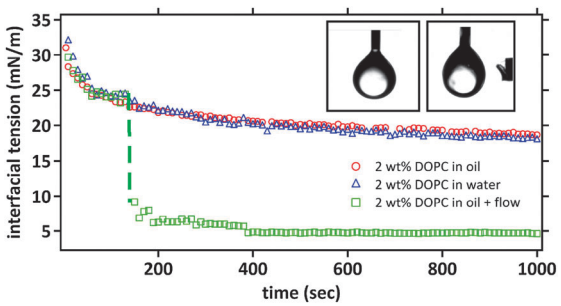

Fig. 2 Interfacial tension of water droplets in hexadecane as a function of time. The dots and triangles denote the time evolution when 2 wt% DOPC is added to the oily and the aq. phase, respectively. The squares were measured under the same conditions as the data shown as triangles, whereas at $t \approx 130$ s, a continuous flow of the oily phase with 2 wt% (DOPC) was applied to the droplet for 10 s at a flow rate of 20 μL s^{-1}. Insets: pendant drop with and without a needle to apply the additional flow. In the experiment the distance between the needle (outer diameter 500 μm) and the droplet was about 2 cm, *i.e.* about 50 times larger than shown in the inset.

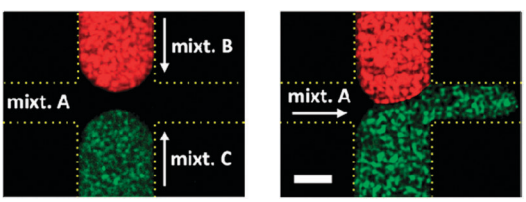

Fig. 3 Membrane formation in a microfluidic cross geometry. The images show an overlay of two different fluorescent micrographs of the fluorescein and rhodamine dyes in mixtures B and C, respectively. The surrounding phase consists of squalane containing lipid A. The channel height is 100 μm, the scale bar represents 125 μm. (a) Image is taken before the two aqueous fingers are brought into contact. (b) An asymmetric lipid bilayer has been formed between the two aqueous fingers and due to the applied flow of the continuous phase A, some of the mixture C enters the right channel, while still having a bilayer with the other finger.

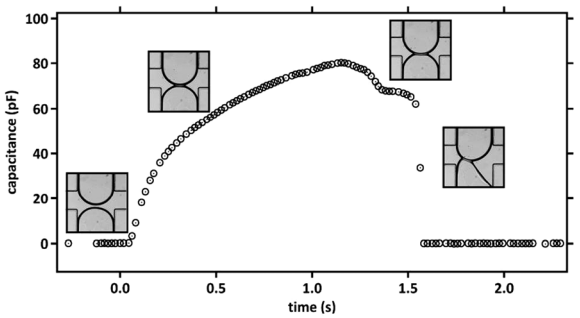

Fig. 4 Capacitance measurements of an asymmetric bilayer membrane consisting of lipids A, B and C. The dots show a single trace upon approach and withdrawal of two aqueous fingers.

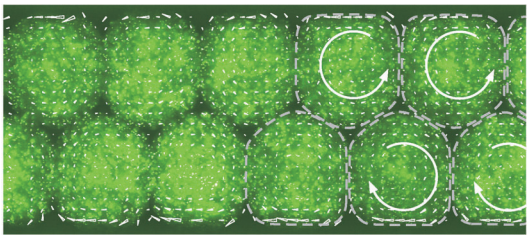

Fig. 5 Flow profiles in the rest frame of the droplet (white arrows) in an experimental situation similar to Fig. 1, with flow direction from left to right. The channel dimensions were 280 μm in width and 130 μm in height. Green fluorescent polystyrene beads were used as tracer (500 nm in diameter, Duke Scientific).

measuring the capacitance of a 'membrane' during the approach of two aqueous fingers in the same microfluidic setting as shown in Fig. 3. Two Ag/AgCl electrodes, electrochemically chlorinated using a 1 M NaCl solution, are introduced into the aqueous fingers through the soft PDMS of the microfluidic device and the capacitance between them is monitored using a patch clamp amplifier (EPC10, HEKA GmbH, Germany) by the standard lock-in technique.[9] The evolution of the capacitance between the fingers as they approach each other and during the subsequent withdrawal is shown in Fig. 4. At $t \approx 0$ the interfaces of the two liquid fingers touch each other as determined from optical measurements. At $t \approx 45$ ms an increase in the capacitance was measured. While continuously pumping liquid into the fingers they increase their contact area until $t \approx 1$ s. Assuming the contact area of a circular disc, the membrane area between the liquid fingers can be determined by microscopic inspection and the specific capacitance can be estimated to be $\sim$1.1 μF cm^{-2} which corresponds well with known literature values for lipid bilayers.[1] Within experimental accuracy the same specific capacitance is measured during the increase and decrease of the membrane area. This indicates a fast membrane formation from the lipid covered oil/water interface with a typical time scale of $\sim$50 ms in agreement with results for mono-olein membranes.[3]

Obviously the fast saturation of the involved water/oil interfaces with phospholipids is the key to the fast formation of stable membranes. This can be achieved effectively by a flow induced advective transport of lipids and micelles to the interface. Interestingly, the externally generated flow in the pendant drop measurement and the microfluidic cross, Fig. 2 and 3, is generated intrinsically in a dynamic droplet based microfluidic setting, as shown in Fig. 1. Whenever a confined droplet moves in a microfluidic channel there is a friction induced convective motion both inside the droplet[10] and in the continuous phase[11] explaining the surprisingly fast formation of stable membranes. This type of convective flow pattern is also present in a dense array of droplets as determined by particle image velocimetry (PIV)[10] in the center of the microfluidic channel in the moving frame of the droplets, Fig. 5. In the shown example the maximum flow velocity measured inside the moving droplets (61 μm s^{-1}) is almost 40% of their traveling velocity whereas the velocity gradients are very large. This promotes fast mixing inside droplets and advective transport of surfactants to the interfaces. The importance of this advection supported transport in droplet based microfluidics is demonstrated by the experimental finding that trains of stable aqueous droplets in hexadecane containing 2 wt% DOPC can be produced at a higher volume fraction when being produced at larger flow velocity. Using a coflow geometry (*cf.* Fig. 1) droplet packings produced at a total flow rate of 0.3 μL s^{-1} are unstable with a coalescence rate of $\sim$30% within the first seconds – even at low dispersed phase volume fraction of 0.4. For the same DOPC concentration and for an increased flow rate of 1.5 μL s^{-1}, droplets could be stabilized at volume fractions of up to about 0.8.

The time needed to form a stable droplet interface bilayer is limited by the decoration time of droplet interfaces with phospholipids. In static settings, which are dominated by diffusion of the lipids to the interface, the decoration time takes several tens of minutes. Due to advection supported transport of lipids to the droplet interface, the decoration time can be massively reduced and droplet interfaces can be decorated within fractions of a second. The subsequent membrane formation time after contacting two decorated droplet interfaces is in the range of about 50 ms. It was shown that the intrinsic friction induced motion in droplet based microfluidics provides a fast advective transport of lipids toward the water/oil interface promoting the formation of stable membranes three to six orders of magnitude faster compared to conventional bulk settings.

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