Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information for: Enhanced Water Permeability across a Physiological Droplet Interface Bilayer Dopped with Fullerenes

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Comparison of Lipid Bilayer Dopped with Fullerenes or Hydrophobic Gold Nanoparticles

We produce a freestanding bilayer, in a microfluidic chip, following the method describes by Guo et al. [2]. An example of such freestanding bilayer is presented in Fig.S1.A. If some hydrophobic nanoparticles have been dispersed in the oil, they become embedded into the core of the lipid bilayer during the bilayer formation (i.e zipping process). Using this method, the formed lipid bilayer could be doped with fullerenes or hydrophobic gold nanoparticles, respectively. Taking advantage of the microfluidic platform, the lipid bilayer could be investigate via electrophysiological inspection. Without nanoparticles, a pure lipid bilayer is equivalent to a plane dieletric from the electrostatic point of view. A pure lipid bilayer has a measured capacitance around 100pF, while with fullerenes ($c \approx 10 - 40 \mu g/ml$) the measured capacitance of the bilayer drops to 60pF and 30pF respectively (Fig.S1.B). This measure indicates an increase of the thickness of the bilayer d as $d \propto C^{-1}$. As the fullerenes are

conducting, it is natural to ask if this capacitance reduction is an artefact due to conducting properties of the fullerenes. But re-doing the capactince measurement with an applied voltage of $0mV$, we obtain the same results which allow us to neglect this hypothesis. The same effect appears with gold nanoparticles, when disperse into the oily phase at $c \approx 50\mu g/ml$, we measured a reduction of the bilayer capacitance to 60pF (Fig.S1.B). Which confirms an increase of the thickness of the bilayer due to the presence of hydrophobic gold nanoparticles. Now we stop the experiments with the microfluidic freestanding bilayer, and we come back to the DiB system. Measuring the water permeability coefficient P_f of a PC/PS/PE (70:10:20) bilayer with gold nanoparticles ($c \approx 50\mu g/ml$),via the DiB technique, we measure a decrease of permeability $P_f \approx 0.1cm/s$ (at 298.15K). This is exactly the opposite effect that we measure with fullerenes, that enhanced an increase of the water permeability coefficient. As fullerenes and hydrophobic gold nanoparticles are comparable in term of hydrophobicity and in term of size. The only difference between these two nano-objects are that gold nanoparticles are not electrically conducting , while fullerenes are electrically conducting. Therefore, we can propose that the conducting properties of the fullerenes play a key role in the enhanced water permeability of our lipid bilayer.

One could ask why not do patch-clamp measurements directly on DiB droplet. It is technically duable but due to the presence of the presence of the glass pipettes, the bilayer may be stretched and thus the measured permeability coefficients may be strongly affected. This is why, we prefer to not perform electrophysiological measurements directly on DiB.

Lipid molecules All phospholipid molecules were purchased from Avanti Polar Lipids. The other chemical were purchased from Sigma-Aldrich. The electrolyte for electrophysiological measurements consisted of 100 mM NaCl (Sigma-Aldrich) in Milli-Q water.

Patch Clamping. Ag/AgCl electrodes were prepared by inserting a platinium electrode (HEKA, germany) in a borosilicate glass pipette (outer diameter 1.5 mm, inner diameter 0.86 mm, Vendor) containing an electrolyte agarose solution. Lipid membrane conductance was measured using the standard function provided by the patch clamp amplifier EPC 10

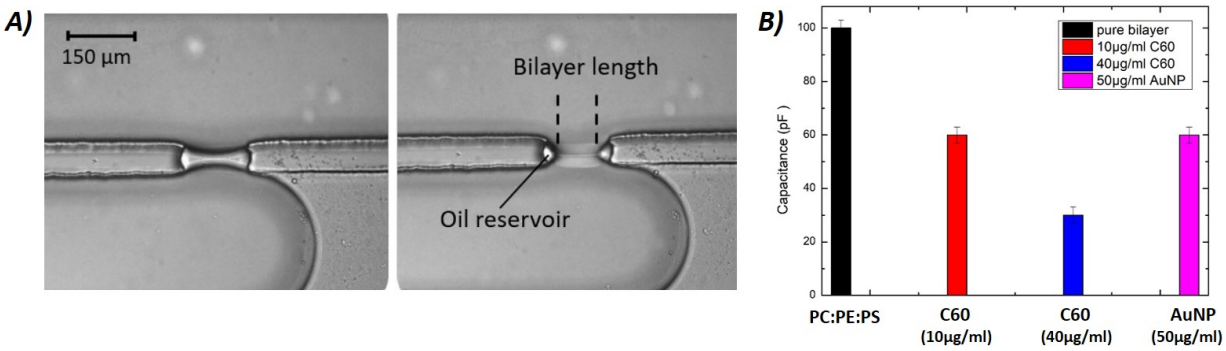

Figure 1: A) Example of lipid bilayer formation in a microfluific cip. B) Capacitance measurements of lipid bilayer dopped differently.

USB (Heka-Electronics). A 10 mV sinusoidal wave with a frequency of 20 kHz was used as an excitation signal. The electrodes are carefully introduced into the aqueous compartment of the Sylgard 184 device using micromanipulator. The total capacitance C is define by $C = \frac{\epsilon_L \epsilon_0 A}{d}$, where $\epsilon_L = 2.2$ is the dielectric constant of lipid, ϵ_O the vacuum permittivity, A is the bilayer area and d is the bilayer thickness.

Microfluidics Microchannels with two parallel rectangular sections were fabricated using typical soft lithography protocols. Channel dimensions were 300 μm in width and 140 μm in height. The device was molded in SYLGARD 184 (Dow Corning) from an SU-8 photoresist structure on a silicon wafer. The surface of the SYLGARD 184 device was exposed to oxygen plasma (Diener electronic GmbH) and sealed with a plasma-treated glass cover slide. The sealed device was rendered hydrophilic by heating it to 135°C overnight. The liquids were dispensed from syringes (Hamilton Bonaduz AG), which were connected to the microfluidic device by tubing. Custom-made, computer-controlled syringe pumps were used to control the injection of the water and the oil phase. For the fluorescence microscopy experiments, a commercial micro–particle image velocimetry setup from LaVision was used, with a sensitive CCD (charge-coupled device) camera (Imager pro X).

Vertical Freestanding Lipid Bilayer Formation In the following, all the lipid-oil mixtures are prepared with a concentration of 5 mg/mL in Squalene oil. The lipids are mixed in the squalene via magnetic steering, and let at 45°C for 3-4 hours until completely

dissolved. The freestanding bilayer formation method is based on a variant of the droplet interface bilayer method. Two buffer fingers are dispersed in an oil-lipid droplet. As the lipid molecules are amphiphilic, they are similar to a surfactant and they are covering each water-oil interface. Our chip is designed to position two buffer fingers close to each other and only separated by an oil-lipid sandwich. Then, as the PDMS chip is porous it drains the oil phase separating the two buffer phases, and eventually bring the two lipid monolayers in contact to form a lipid bilayer. Experimentally, we perform this by filling the chip with an oil-lipid solution, then the two microchannels are filled with a buffer solution using volume syringe pumps. The complete adsorption of squalene by PDMS walls changes between $\approx 10 - 20$ minutess depending on the volume of lipid-oil mixture introduced to the system.

Hydrophobic Gold Nanoparticles AuNPs were synthesized from the one-step process described by Zheng et al. [1]. These NPs were composed of a gold core (1nm) and coated with a dense monolayer of 1-dodecanethiol with a length of 1.6 nm, as described in detail by guo and al. [2] As describe in detail in [2], once inserted into the lipid bilayer they present a lipid interdigitation with the dodecanethiol layer of ≈ 1 nm.

[1] N. Zheng, J. Fan, G. D. Stucky, One-step one-phase synthesis of monodisperse noble-metallic nanoparticles and their colloidal crystals. **J. Am. Chem. Soc.** 128, 6550–6551 (2006).

[2] Y. Guo, E. Terazzi, R. Seemann, J. B. Fleury and V. A. Baulin, Direct proof of spontaneous translocation of lipid-covered hydrophobic nanoparticles through a phospholipid bilayer. **SciAdv** , 2, e1600261 (2016).