A Microfluidic Based Bubble Formation Enables Analysis of Physical Property Change in Phospholipid Surfactant Layers by Interfacial Ozone Reaction

Supplementary Information

Supplementary Method

Ozonolysis product analysis The lipid solution is prepared by mixing 80μM NBD-PG, 40μM POPG, and 40μM DPPC in water. The final concentration of the mixed solution is adjusted to 10μM NBD-PG, 10μM POPG, and 20μM DPPC with water. The solution of NBD-PG is also adjusted to the concentration of 10μM with water for characterizing NBD-PG itself. Lipid vesicle formation is performed using freeze-thaw method, and the solution is kept in 4 °C before ozonolysis reaction. Ozone of 20 ppm is continuously bubbled into the solution during 5 min. The final concentration for electrospray ionization mass spectrometry (ESI-MS) is the same with the lipid solution. Product analysis of ozonolysis reaction is performed using Thermo Scientific LTQ Velos dual ion trap mass spectrometer in negative ion mode.

Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Figures

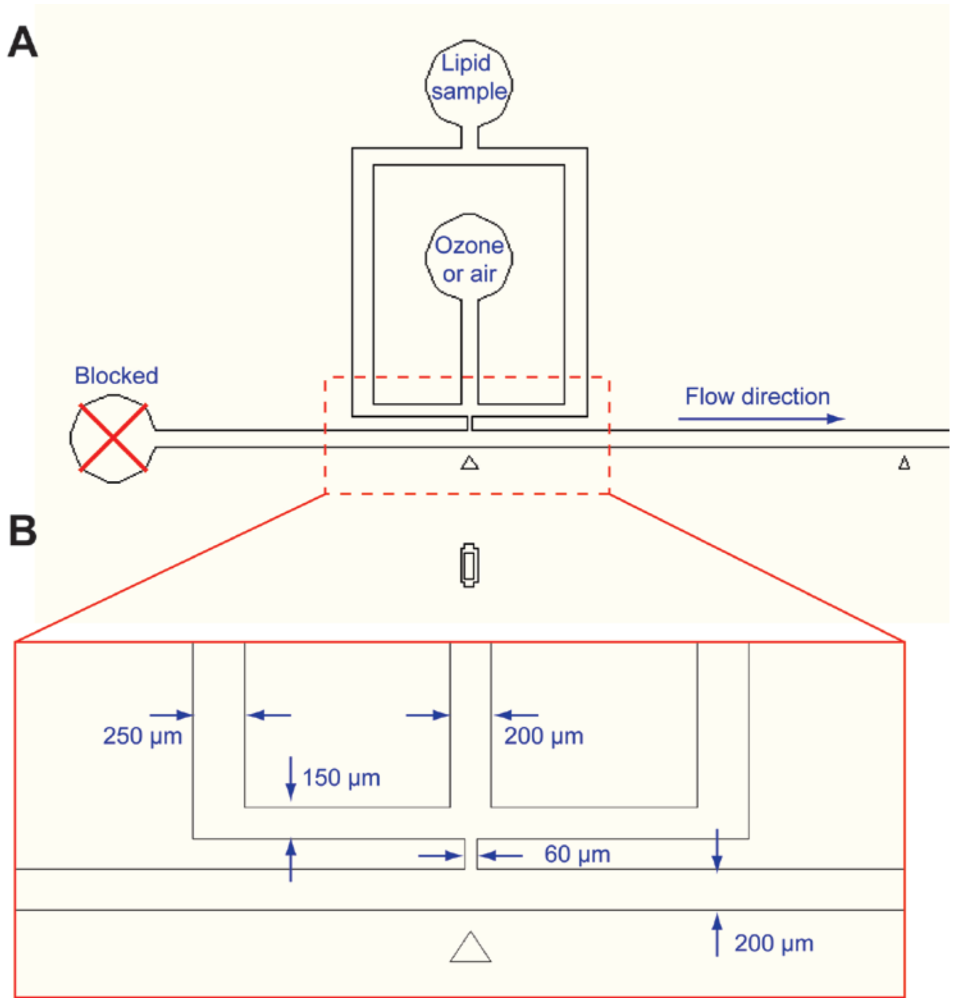

Fig. S1. Design of the microfluidic device for the bubble generation. (A) AutoCAD drawing for the bubble generation component. Either air or ozone is introduced from the center channel and the lipid sample flows in from the outer two channels. Lipid sample flow pinches off the gas to form bubbles. The hole in the left is used to introduce water right after plasma-based bonding for the hydrophilicity of the channel and remains blocked with a pin-plug for the rest of the process. (B) Zoomed in image of the main components of the bubble formation with dimensions.

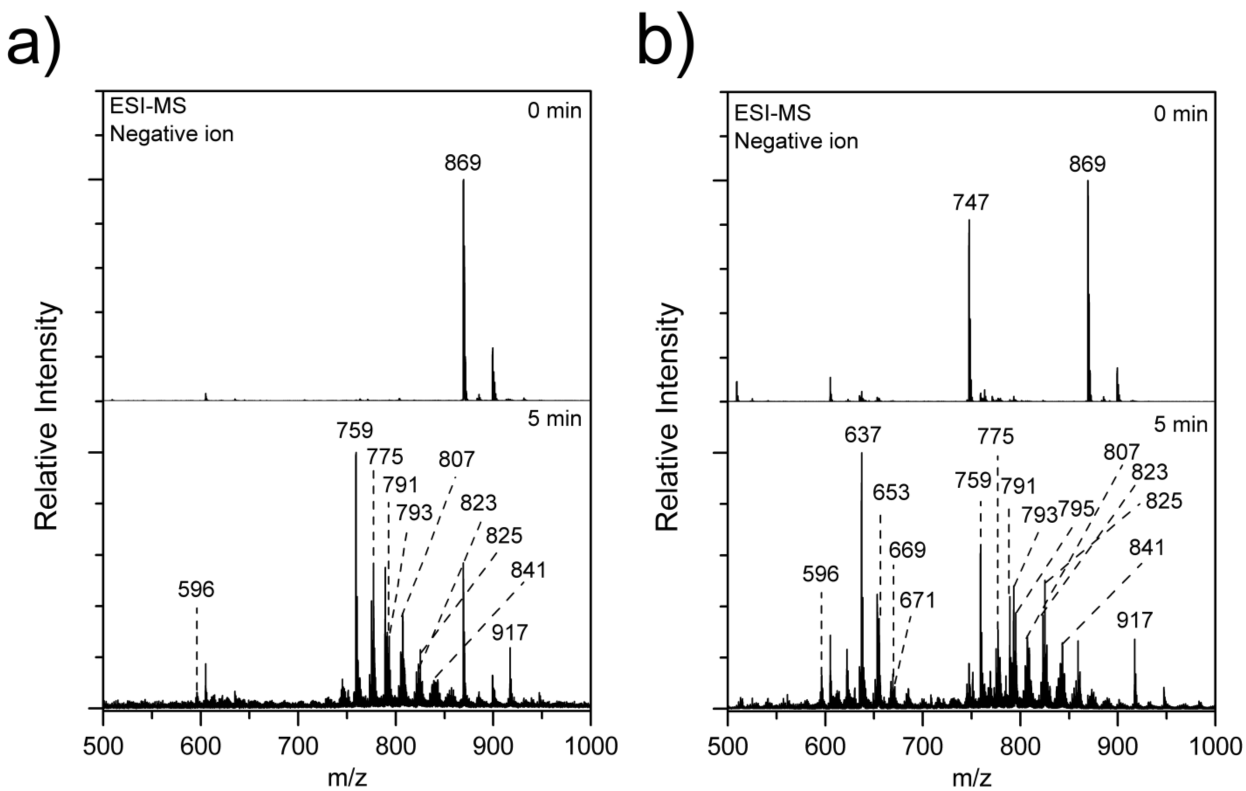

Fig. S2.(A) ESI-MS spectra of NBD-PG in negative ion mode. (B) ESI-MS spectra of the mixture composed of NBD-PG, POPG and DPPC in negative ion mode. A continuous flow of ozone (~20 ppm) is bubbled into the lipid solution for 5 min to cause ozonolysis reaction. Unidentified peaks are produced by the ozonolysis of impurity. The structures of the observed ozonolysis products are shown in Fig. S3 and S4.

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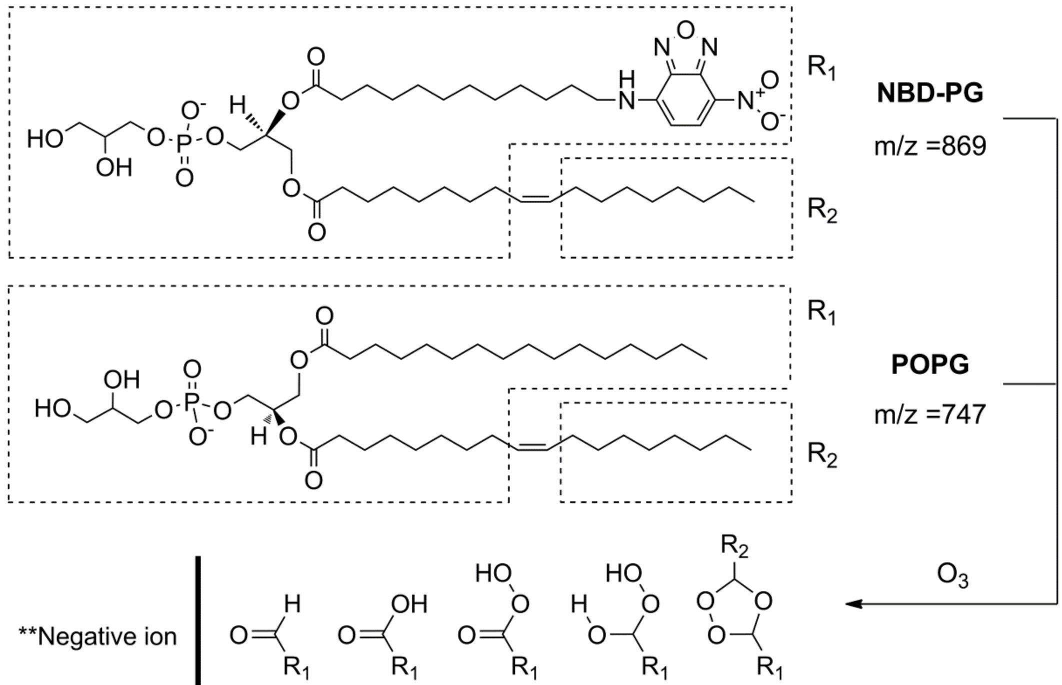

**Negative ion					
NBD-PG	759	775	791	793	917
POPG	637	653	669	671	795

Fig. S3. The product table of NBD-PG and POPG under ozone environment in negative ion mode

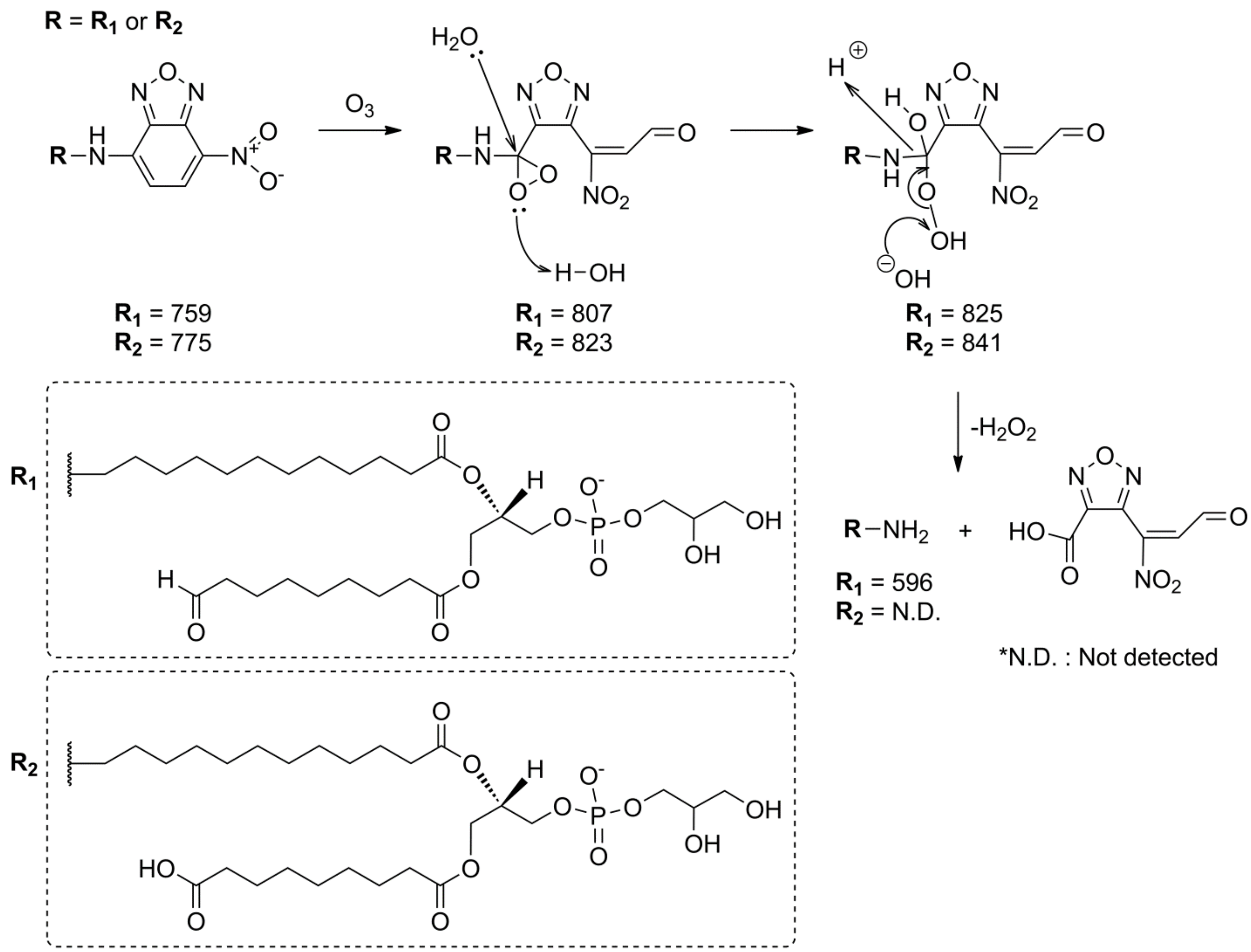

Fig. S4. The reaction mechanism of ozonolysis of the NBD fluorescent tag.

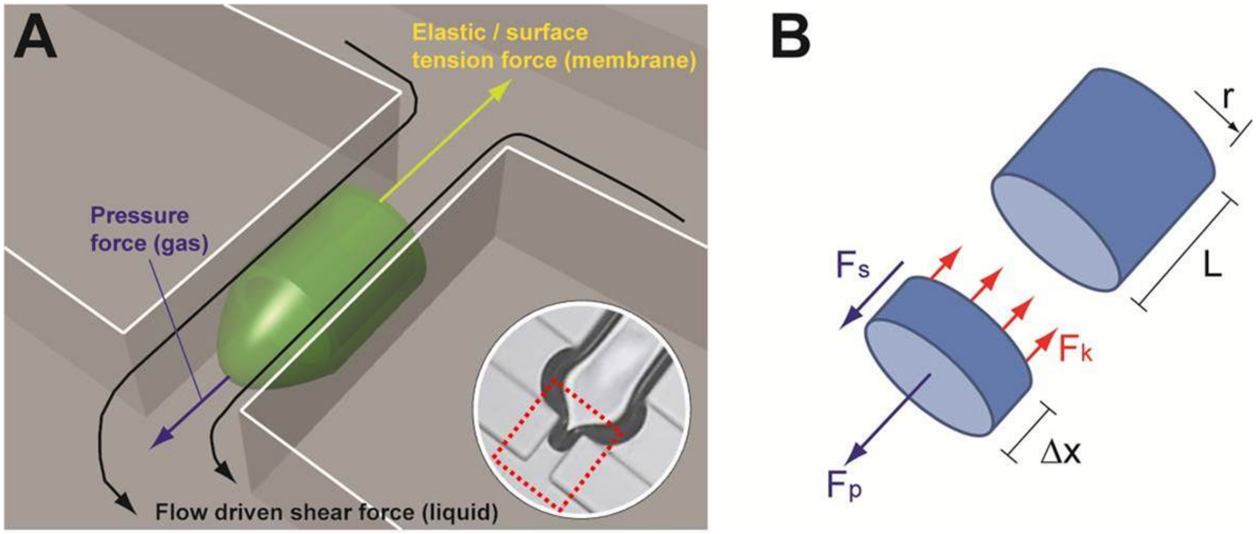

Fig. S5. (A) Major force components for the bubble generation on the lipid monolayer in the narrow channel region. Elastic force of the phospholipid monolayer at the air-liquid interface counter act to the pressure force from the gas and the shear force from the bulk lipid flow. Since the pressure for the gas and the flow rate for the bulk lipid flow are fixed, the physical characteristics of the lipid monolayer plays a major role in the bubble formation process. Inset: Actual device picture illustrating the components corresponding to the schematic. (B) Free body diagram for the force balance. The interface in the thread (the narrow channel region) is assumed as a cylinder.

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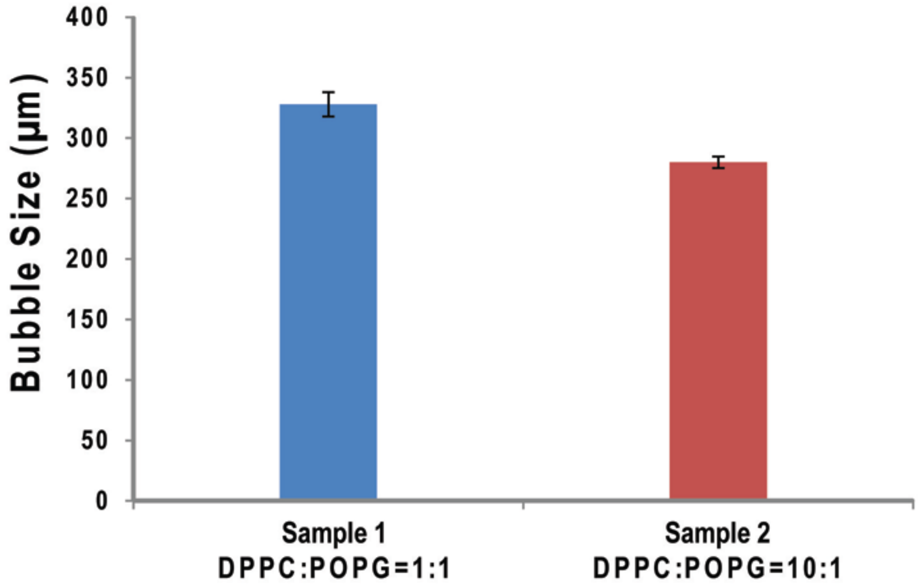

Fig. S6. Bubble size in terms of length for different phospholipid compositions. Sample 1 was prepared in 1:1 ratio of 20 µM DPPC and 20 µM POPG and Sample 2 was prepared in 10:1 ratio of 20 µM DPPC, 20 µM POPG. 6µl/min and 0.33 psi are used for the lipid sample flow rate and the pressure for the air, respectively. It can be noticed that bubble size decreases as the amount of POPG decreases, which further supports the ozone effect on the bubble size in the main text.

Supplementary Tables

Supplementary Table 1. Bubble size (in μm) and the polydispersity index

	Air	**Air/Ozone**
Average	403.01	386.05
Standard deviation	4.16	7.19
Polydispersity Index (%)	1.032	1.863

$$polydispersity\ index = \frac{standard\ deviation}{average} \times 100$$

Supplementary Table 2. Parameters for the effective elastic modulus calculation

	Air	**Air/Ozone**
$\boldsymbol{p}$	0.42 psi	
$\boldsymbol{L}$	75 μm	
$\boldsymbol{r}$	30 μm	
$\boldsymbol{\Delta x}$	4.575 μm	3.575 μm
$\boldsymbol{f}^*$	3.48 Hz	3.52 Hz
$\boldsymbol{m}$**	1.68×10^{-14} kg	1.31×10^{-14} kg
$\boldsymbol{E_{eff}}$	37973.5 Pa	48595.5 Pa

* Frequency was obtained from the stable region in oscillation: for the air condition, 1.25~3.55 sec and for the ozone condition, 13.75 ~ 17.30 sec regions were chosen to obtain the frequency (Fig. 3(B)).

** m is defined as the mass of oscillatory working gas in the thread. We assumed that low concentration of ozone (~20 ppm) does not change the density of working gas from air to air/ozone. The volume of the plug can be simplified as a cylinder in the thread. $\boldsymbol{\rho}$ is the density of air at room temperature.

$$\boldsymbol{m = \Delta x \cdot \pi r^2 \cdot \rho}$$