Supporting Information

Formation of Giant Lipid Vesicle Containing Dual Functions Facilitates Outer Membrane Phospholipase

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Method

Orientation assay of OmpLA into liposomes with Elastase

The liposomes (10 mM; DOPC/DOPG (1:3 molar ration) or DOPC/DOTAP (19:1 molar ratio)), and OmpLA (final concentration, 0.5 mg mL^{-1}) were incubated for 1 h at 27 ℃. After the reconstitution of OmpLA into the liposomes, the residual OmpLA into the buffer solution was removed by the ultrafiltration (100 kDa cutoff). This solution containing the OmpLA-vesicles was split into half the volume. Elastase (final concentration, 800 μg mL^{-1}) was added to the OmpLA-vesicle solution. After incubation for 2 h at 37 ℃, the vesicle solution was heated for 10 min at 95 ℃ for terminating the elastase reaction. We analyzed the orientation of OmpLA by SDS-PAGE and western blotting with anti-His-tag.

Negative control of transportation assay (Figure S13)

GUVs (1 mM DOPC/DOPG (1:3 molar ratio) hydrated with HEPES buffer containing sucrose (10 mM HEPES, 140 mM NaCl, 500 mM sucrose; pH 7.4) were prepared using the gentle hydration method. 12 mM LDAO was filtered three times using HEPES buffer (20 mM HEPES, 2 mM EDTA, pH 8.3). After that, LDAO solution was filtered three times using Tris buffer (20 mM Tris, 2 mM EDTA, pH 8.3). HEPES buffer (48.4 μL) containing glucose (10 mM HEPES, 140 mM NaCl, 500 mM glucose; pH 7.4), calcein at a final concentration of 100 μM, and 1 μL of 1 mM GUVs (DOPC/DOPG (1:3 molar ratio) was gently placed on a glass coverslip with a silicon rubber spacer. The solution was incubated for 10 min at 23 °C, and 0.6 μL of LDAO solution was added. The influx of calcein into GUVs was observed for 30 min using CLSM with an oil-immersion lens (60X) at 473–520 nm (for Alexa 488) using the diode laser at 473 nm.

Negative control of dimerization assay (Figure S16)

10 mM 12-SB was filtered three times using HEPES buffer (20 mM HEPES, 2 mM EDTA, pH 8.3). After that, 12-SB solution was filtered three times using Tris buffer (20 mM Tris, 2 mM EDTA, pH 8.3).47.4 μL aliquot of the buffer (10 mM HEPES, 140 mM NaCl, 500 mM glucose; pH 7.4) was gently placed on a glass coverslip with a silicon rubber spacer. Thereafter, 1 μL of 1 mM GUVs containing rhodamine and 0.6 μL of 12-SB solution was added and incubated for 30 min at 23 ℃. After incubation, 1 μL of 5 mM calcium (final concentration, 100 μM) was added, and GUVs were observed using an OLYMPUS confocal laser scanning microscope (FV1200) with an oil-immersion lens (60X) at 559–572 nm using the diode laser at 559 nm.

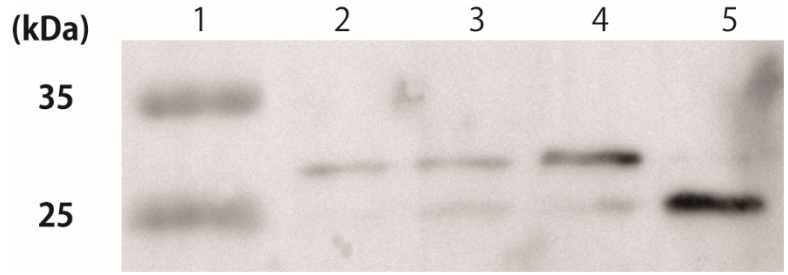

Figure S1. Western blotting of purified OmpLA. Lane1: Protein ladder. Lane2: heat OmpLA at 95 ℃ for 5 min. Lane3: 1 hour after heating. Lane4: cold in ice for 5 min after heating. Lane 5: purified OmpLA

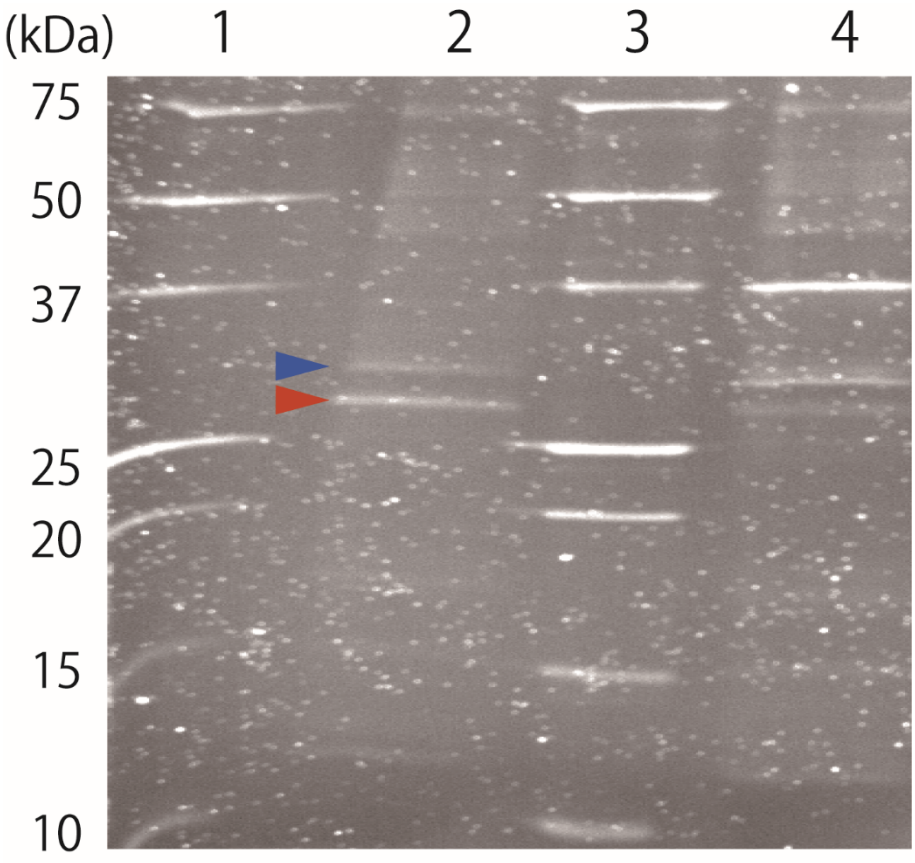

Figure S2. SDS-PAGE of purified OmpLA. Lane1: Protein ladder. Rad arrow is folded OmpLA and blue arrow is unfolded OmpLA. Lane2: purified OmpLA. Lane3: Protein ladder. Lane4: heat OmpLA at 95 ℃ for 5 min.

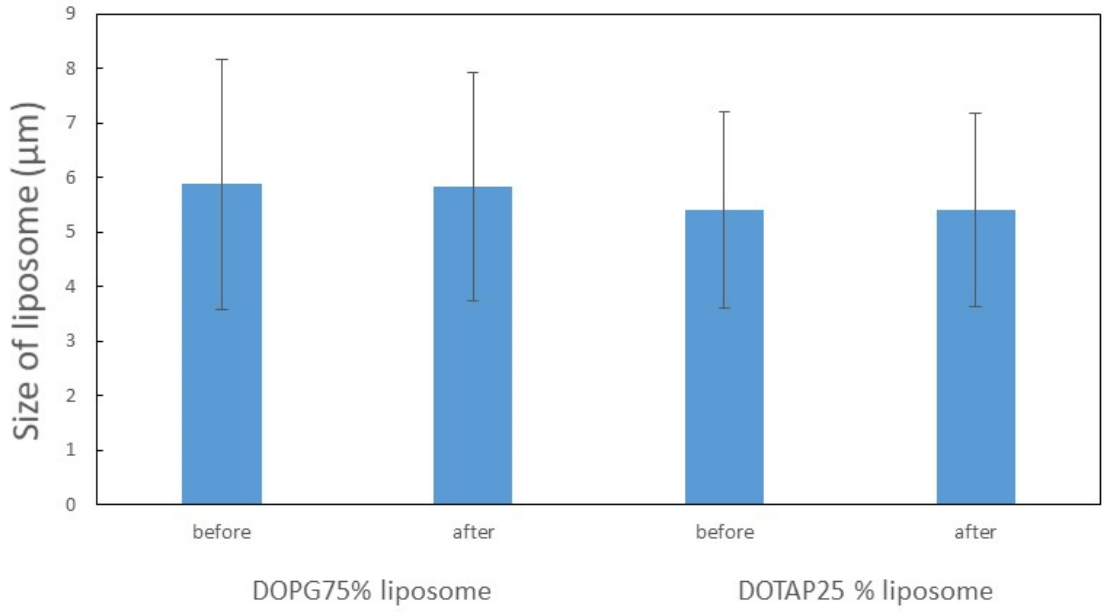

Figure S3. Size distribution of vesicles. the vesicles before and after reconstitution of OmpLA (n=30 in DOPC/DOPG, n=18 in DOPC/DOTAP). Error bars indicate the standard deviation (SD). P value<0.471 (DOPG), P value<0.474 (DOTAP).

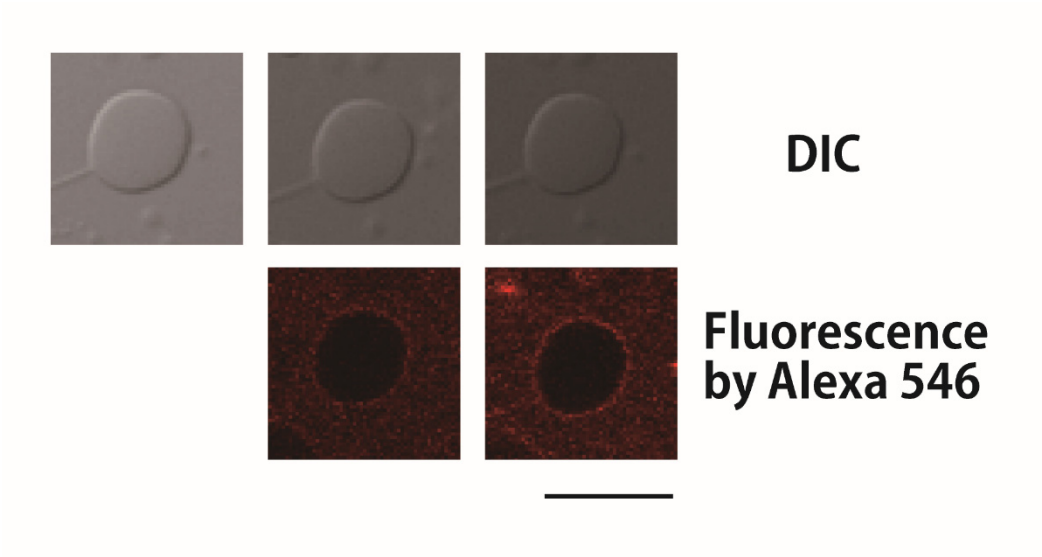

Figure S4. Morphology of vesicles before (left) and after (center and right) reconstitution of OmpLA. The images of center and right are 30 min and 60 min after reconstitution of OmpLA, respectively. Scale bar is 10 μm.

Figure S5. The current signal at 30 min of ion current measurement buffer (20 mM Tris, pH 8.3, 1 M KCl, 230 μM LDAO) without OmpLA.

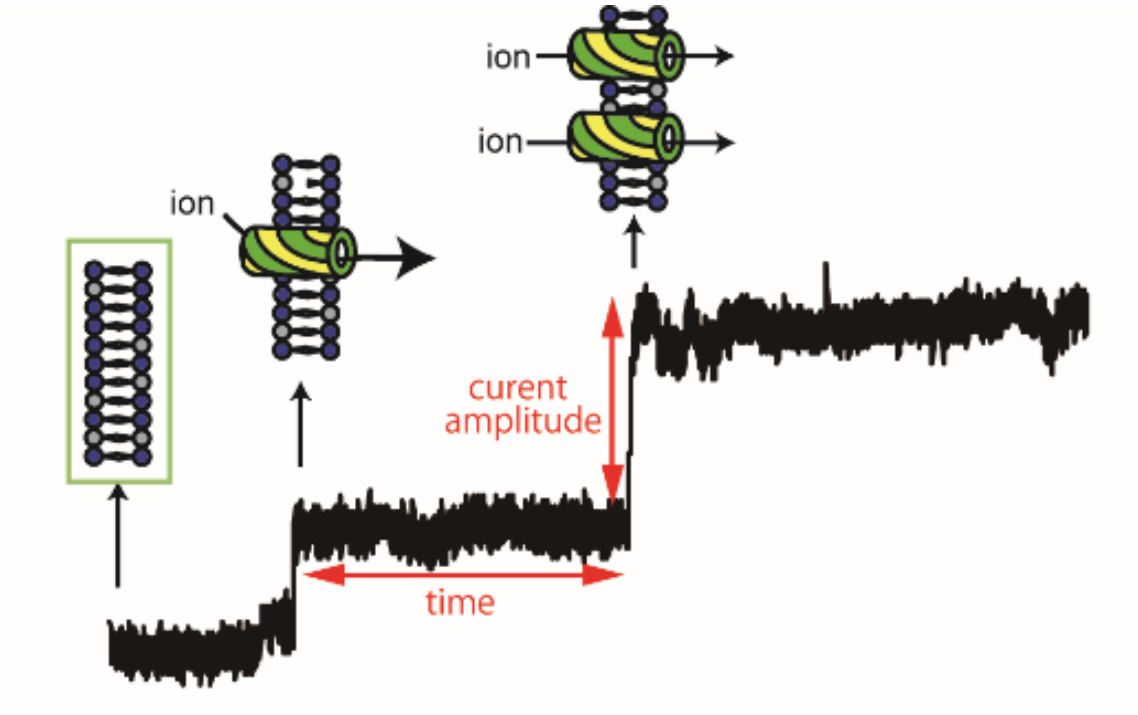

Figure S6. The typical signal of the folded OmpLA. An upward step is appeared when one folded OmpLA is reconstituted into the planer lipid bilayer. Horizontal axis represents time (second) and vertical axis represents current amplitude (pA).

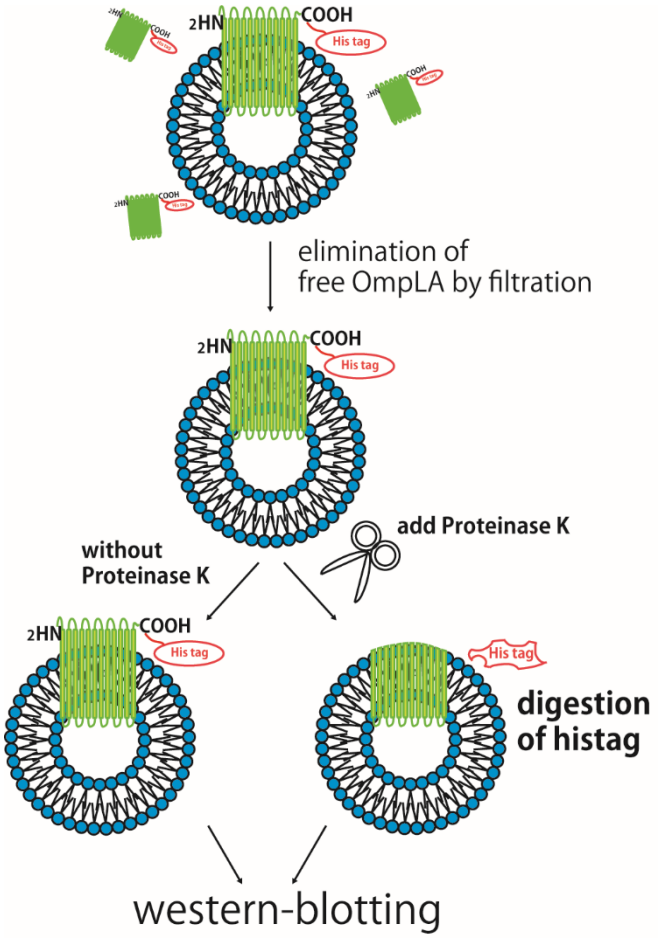

Figure S7. Schematic procedures of orientation determination assay using proteinase K.

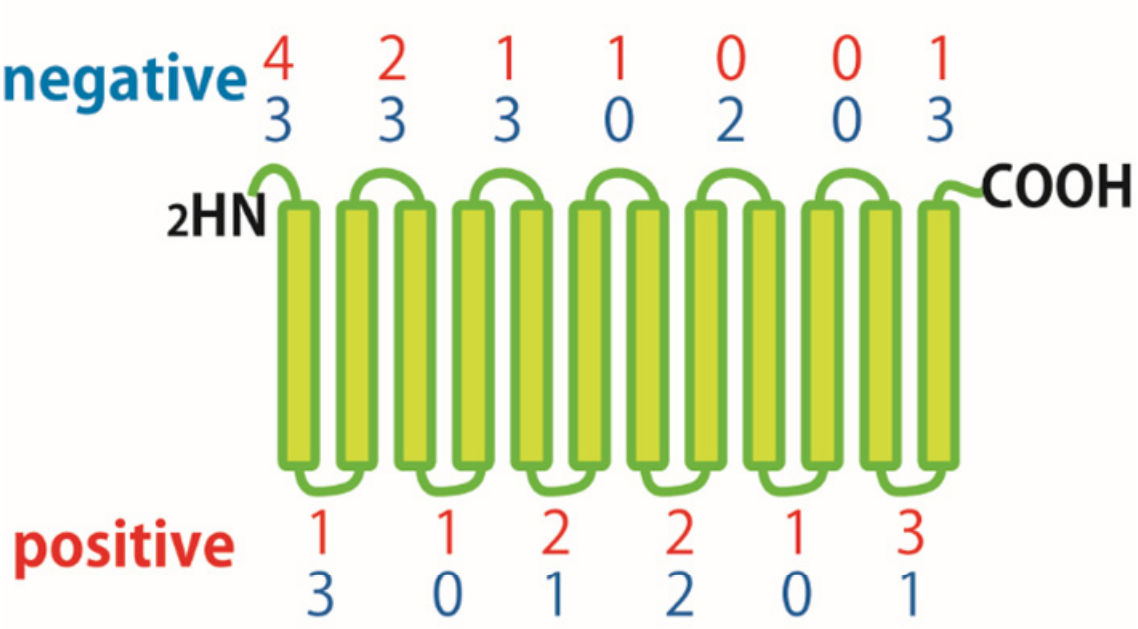

Figure S8. The structure of OmpLA. Red numbers and blue numbers represent the number of positively amino acid and negatively amino acid at hydrophilic loop, respectively. The side of N and C terminal is negatively charged while positively charged in the opposite side.

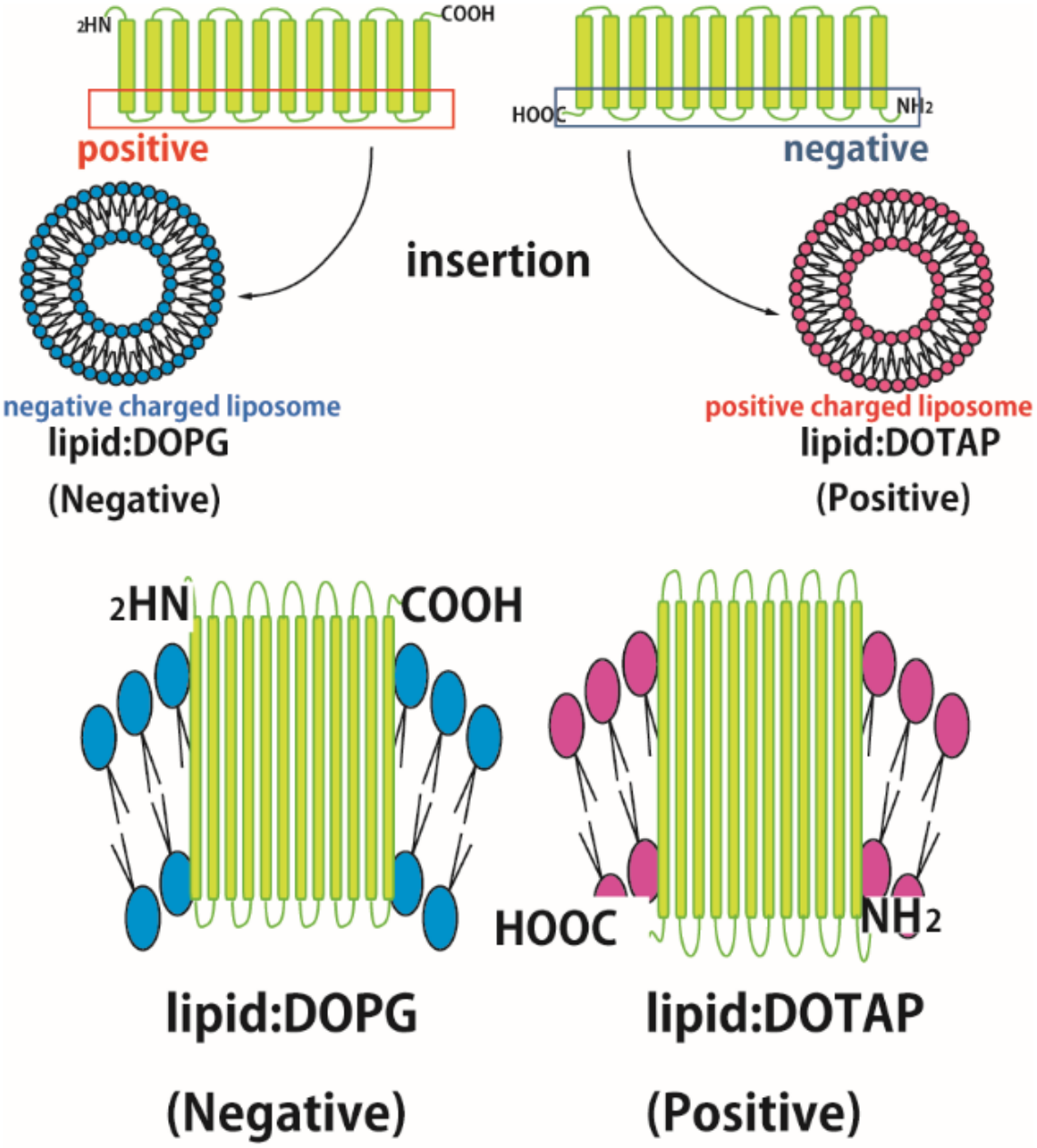

Figure S9. Schematic images of orientation of OmpLA. OmpLA is reconstituted into DOPC/DOPG giant vesicles from positive side while into DOPC/DOTAP giant vesicles from negative side (up). We estimate that OmpLA is reconstituted such as down picture.

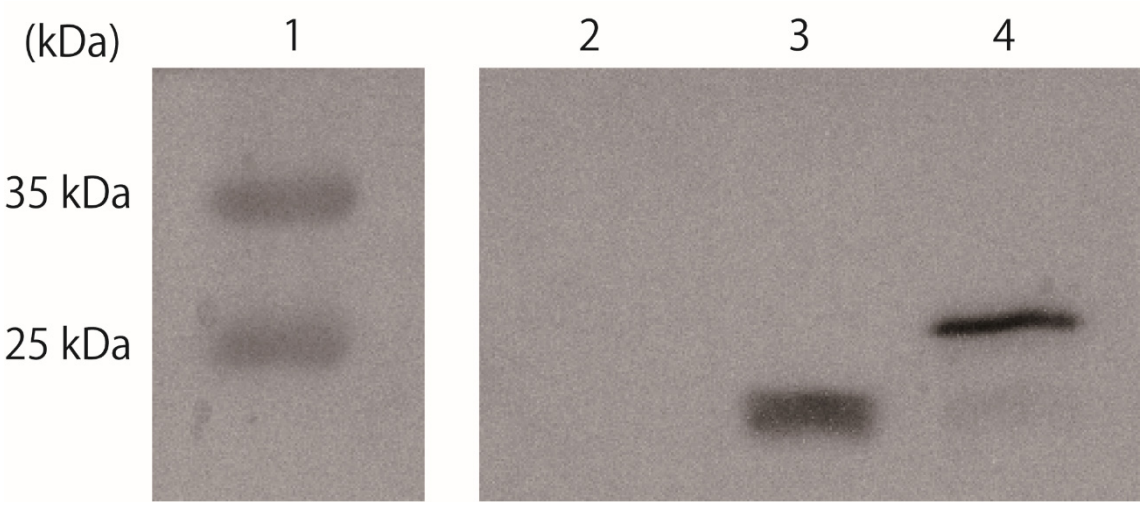

Figure S10. OmpLA digestion were investigated by proteinase K. Molecular mass marker proteins were showed in *lane 1*. Folded OmpLA (final concentration 0.728 mg/mL) were incubated with proteinase K (final concentration 9.92 mg/mL) for 16 h at 37 ℃ (*lane 2*). The folded OmpLA (0.968 mg/mL) (*lane 3*) and the folded OmpLA heated for 5 min at 95 ℃ (*lane 4)*.

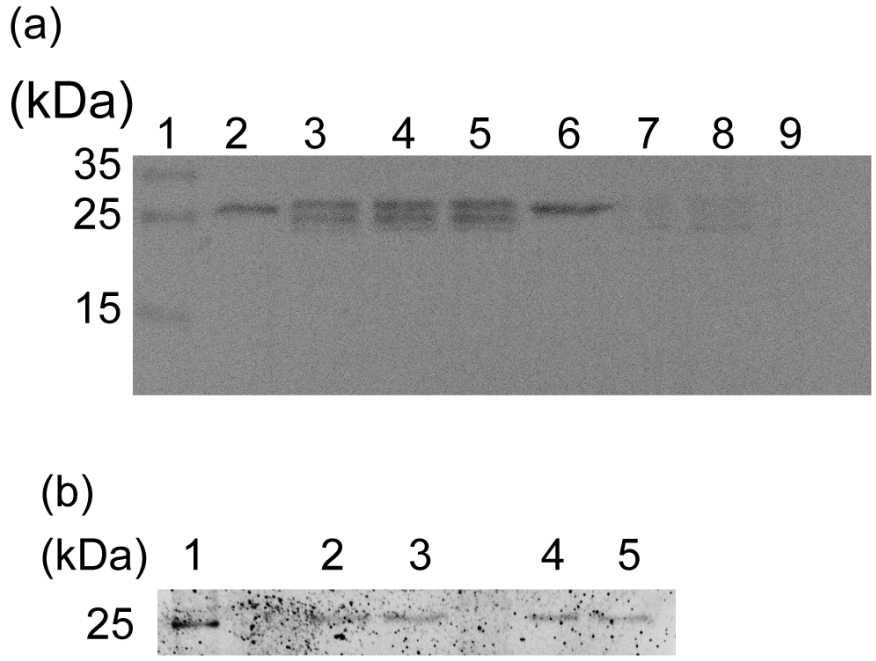

Figure S11. OmpLA digestion into the OmpLA-liposome solution were investigated by elastase. (a) Western blot analysis of OmpLA-reconstituted vesicles in the presence of elastase. Lane 1: protein molecular weight ladder, lane 2: without elastase (DOTAP 5 % vesicles containing OmpLA), lane 3: with elastase #1 (DOTAP 5 % vesicles containing OmpLA), lane 4: with elastase #2 (DOTAP 5 % vesicles containing OmpLA), lane 5: with elastase #3 (DOTAP 5 % vesicles containing OmpLA), lane 6: without elastase (DOPG 75 % vesicles containing OmpLA), lane 7: with elastase #1 (DOPG 75 % vesicles containing OmpLA), lane 8: with elastase #2 (DOPG 75 % vesicles containing OmpLA), lane 9: with elastase #3 (DOPG 75 % vesicles containing OmpLA). (b) SDS-PAGE analysis of OmpLA-reconstituted vesicles in the presence of elastase. Lane 1: protein molecular weight ladder, lane 2: without elastase (DOPG 75 % vesicles containing OmpLA), lane 3: with elastase #1 (DOPG 75 % vesicles containing OmpLA), lane 4: without elastase (DOTAP 5 % vesicles containing OmpLA), lane 5: with elastase #1 (DOTAP 5 % vesicles containing OmpLA).

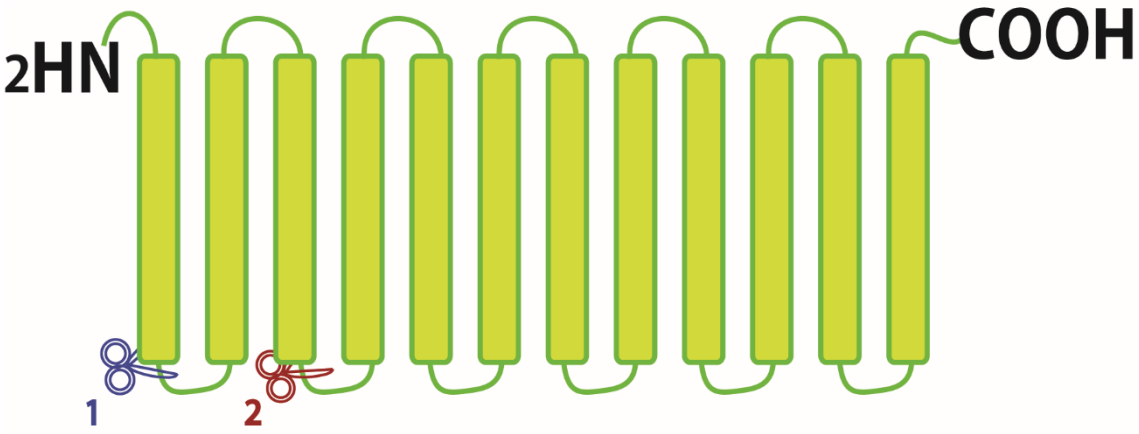

Figure S12. Area of digestion of OmpLA by proteinase K. When OmpLA is digested at 1, the molecular weight between N-terminal and 1 scissor is approximately 4.7 kDa. In the other hand, the molecular weight between N-terminal and 2 scissor is approximately 11.0 kDa.

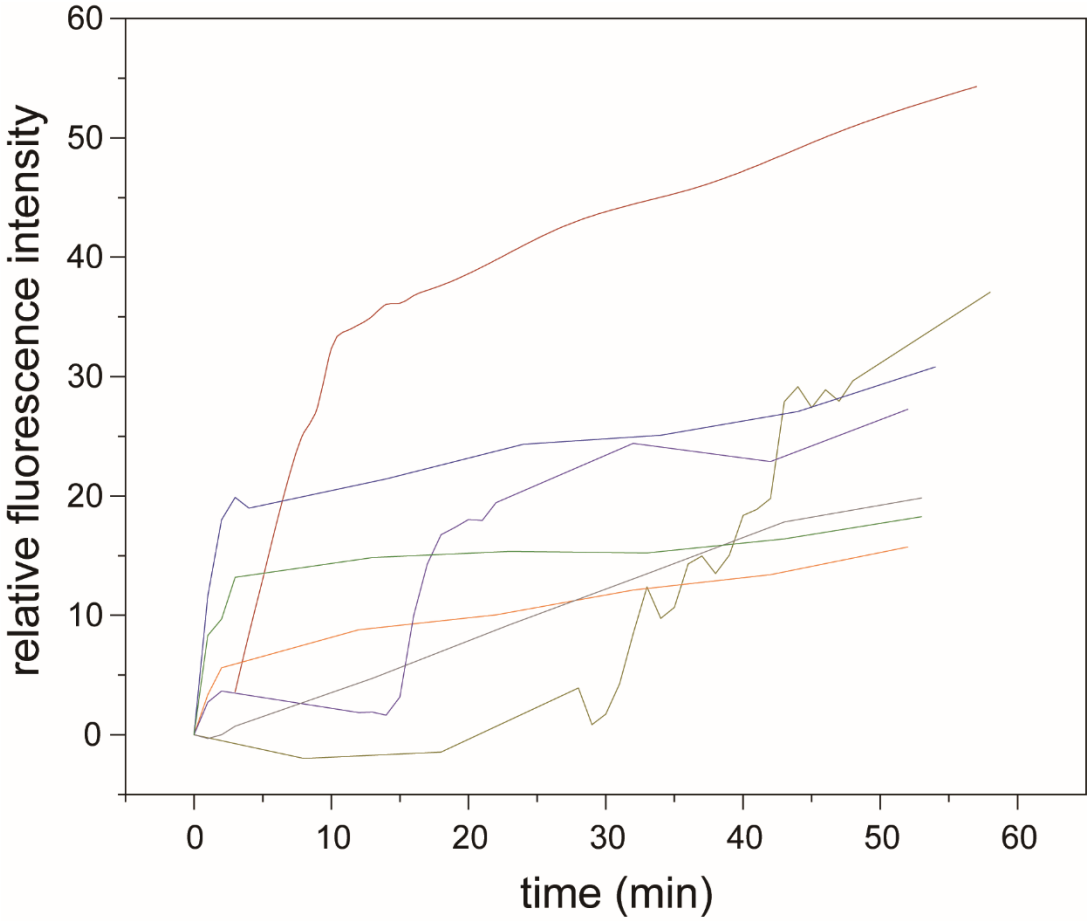

Figure S13. Time-lapse graph of calcein fluorescence in the giant vesicles. Every line showed increased fluorescence around 10 min.

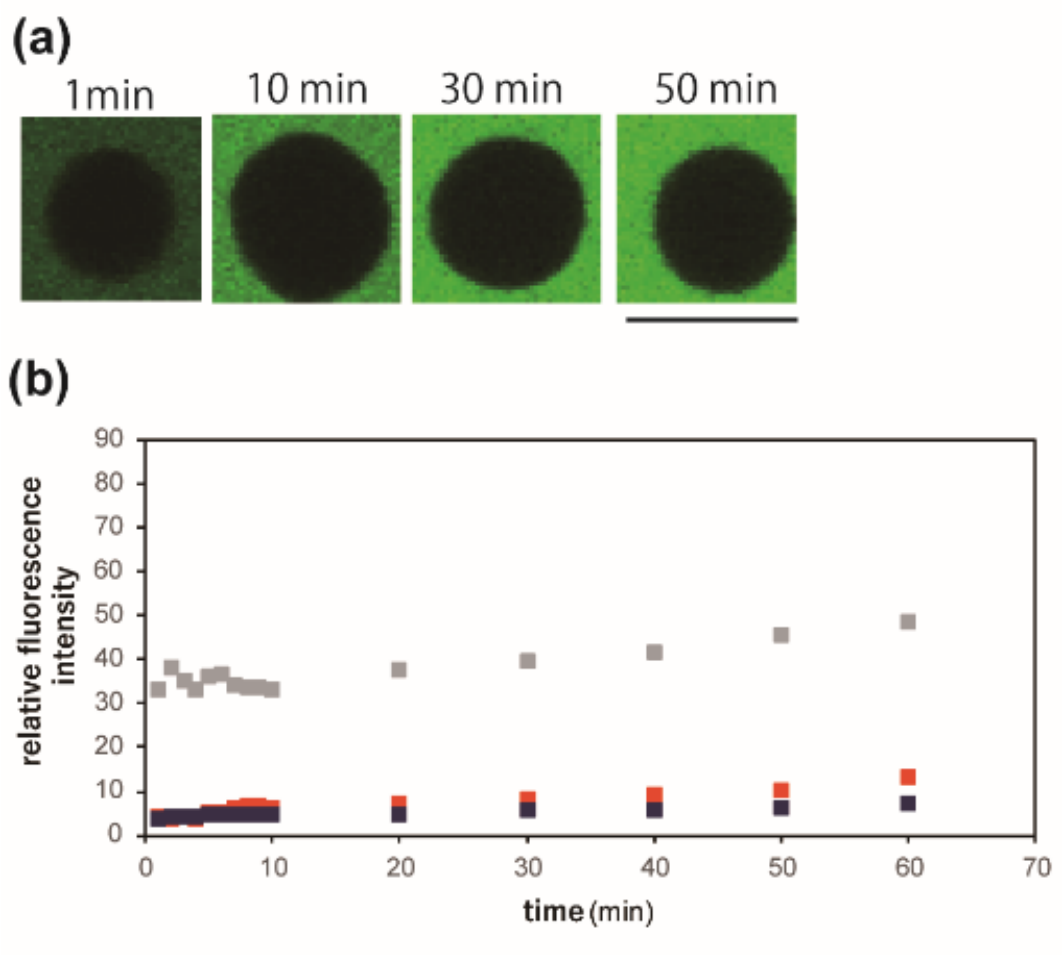

Figure S14. CLSM time-lapse images of the giant vesicles (DOPC/DOPG (1:3 molar ratio)) in the presence of calcein (final concentration 12.2 μg/mL) at outer solution of the giant vesicles. (a) The scale bar represents 10 μm. (b) Time-lapse graph of calcein fluorescence in the giant vesicles. The vesicles observed green fluorescence in vesicles were also not changed fluorescence intensity (gray).

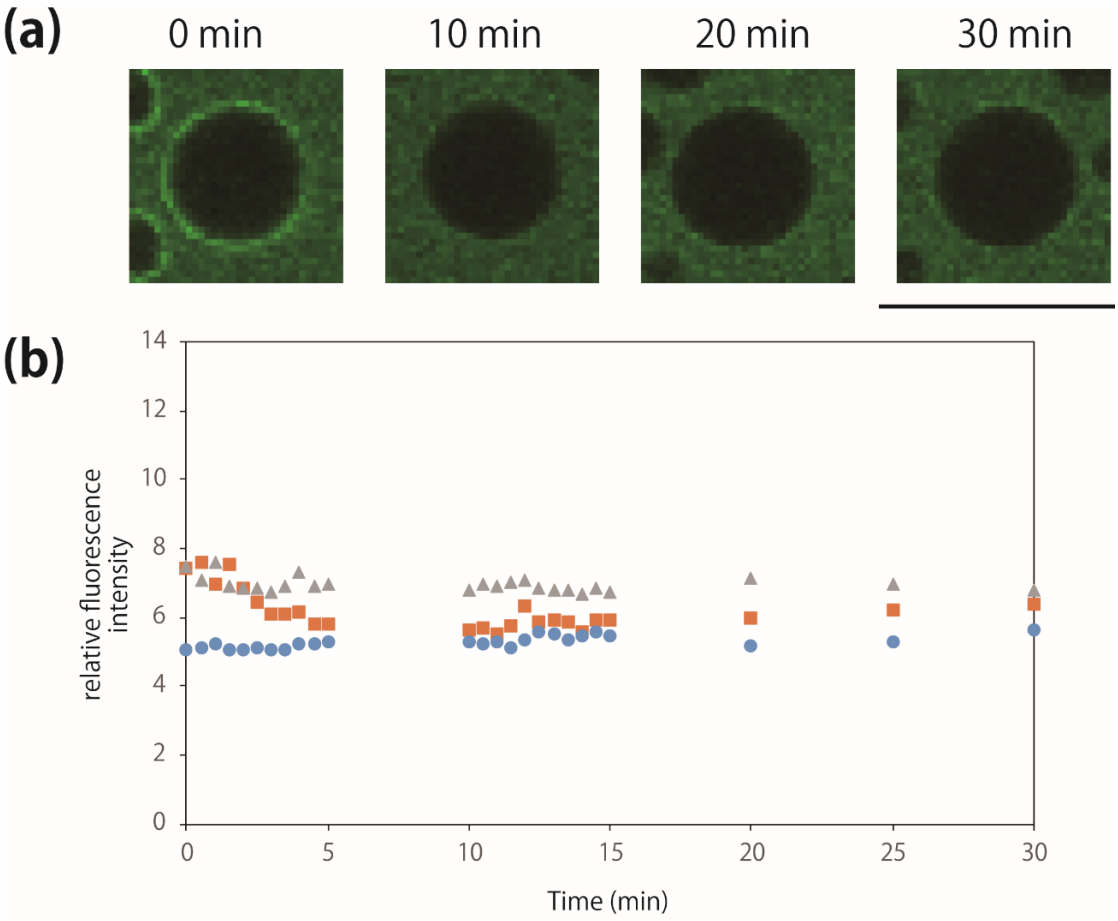

Figure S15. CLSM time-lapse images of the giant vesicles (DOPC/DOPG (1:3 molar ratio)) in the presence of calcein (final concentration 12.2 μg/mL) plus detergent (LDAO) at outer solution of the giant vesicles. (a) The scale bar represents 10 μm. (b) Time-lapse graph of calcein fluorescence in the giant vesicles.

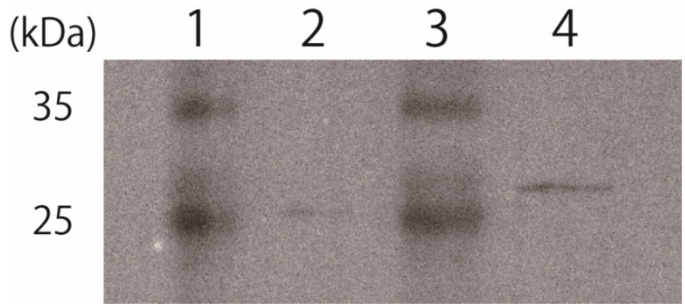

Figure S16. Western blotting of purified OmpLA including 12-SB. Lane1: Protein ladder. Upper is 35 kDa and down is 25 kDa. Lane2: purified OmpLA. Lane3: Protein ladder. Lane4: heat OmpLA at 95 ℃ for 5 min.

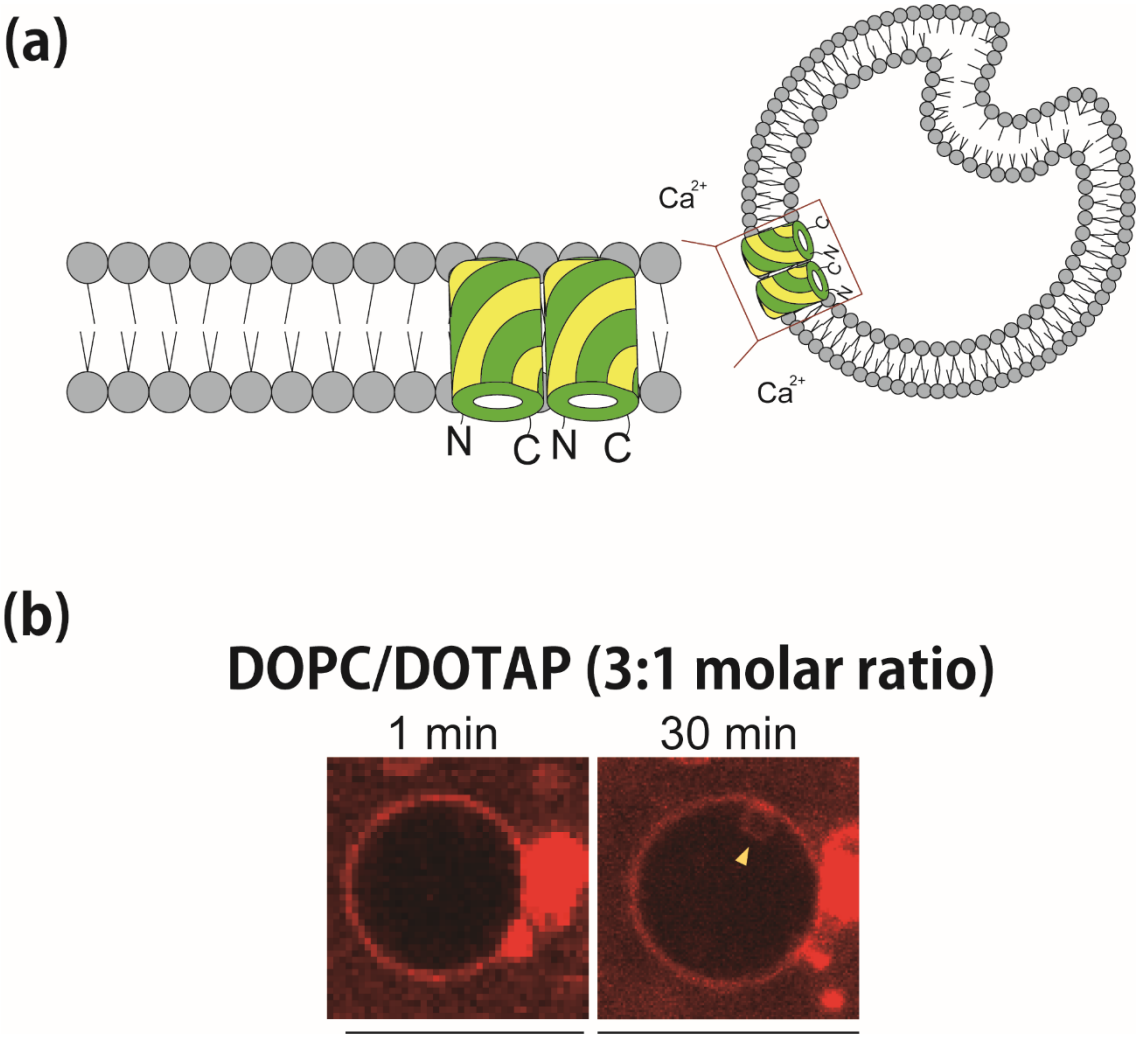

Figure S17. Phospholipid hydrolysis by OmpLA in the presence of calcium ions. (a) Dimer OmpLA activates degradation of phospholipids on the outer leaflet to lysophospholipids and fatty acids on the giant vesicle membrane. (b) We observed the budding vesicle into the giant vesicle containing DOPC/DOTAP (3:1 molar ratio). The scale bar represents 10 μm.

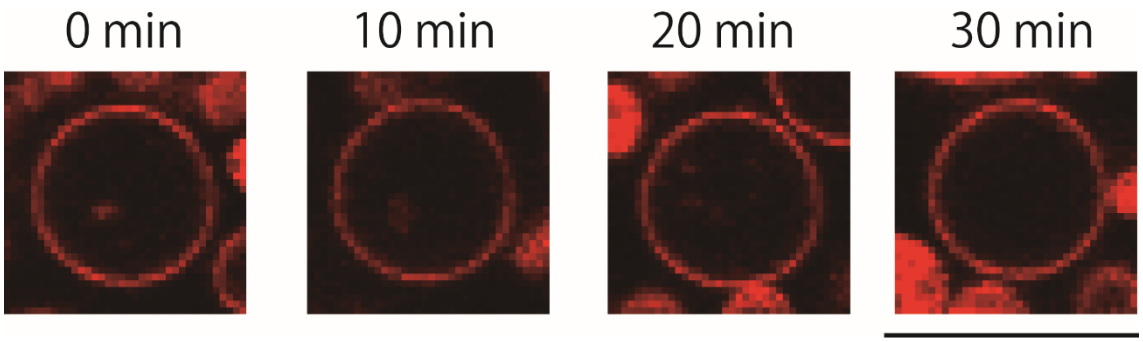

Figure S18. CLSM time-lapse images of the giant vesicles (DOPC/DOPG (1:3 molar ratio)) in the presence of detergent (12-SB) at outer solution of the giant vesicles. Budding of small vesicle is not observed. The scale bar represents 10 μm.

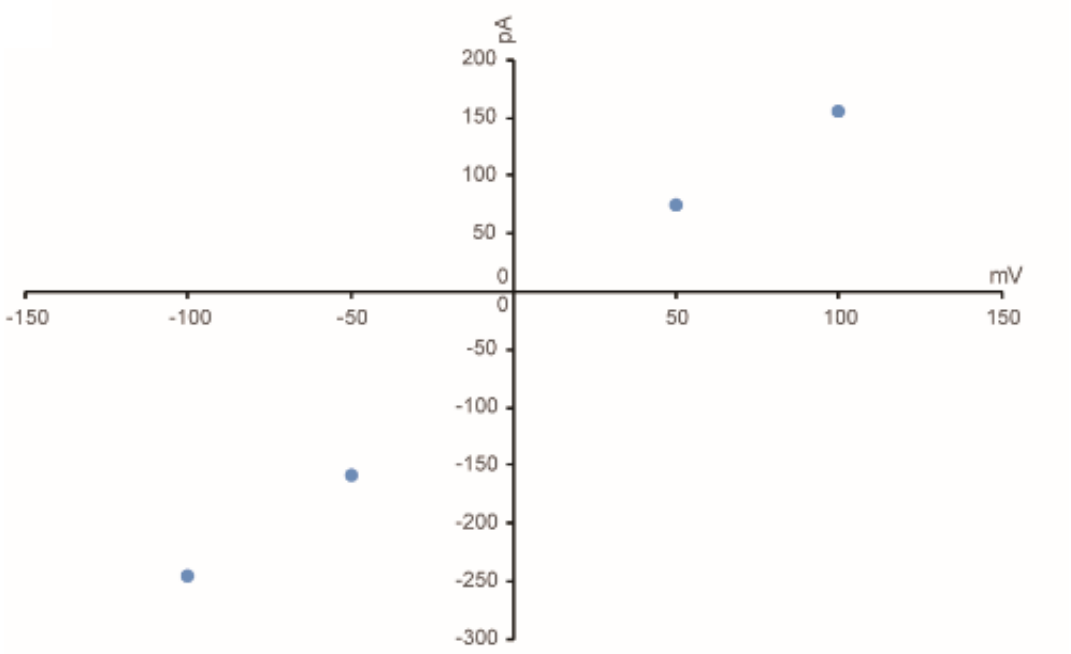

Figure S19. The I-V plot of single OmpLA.

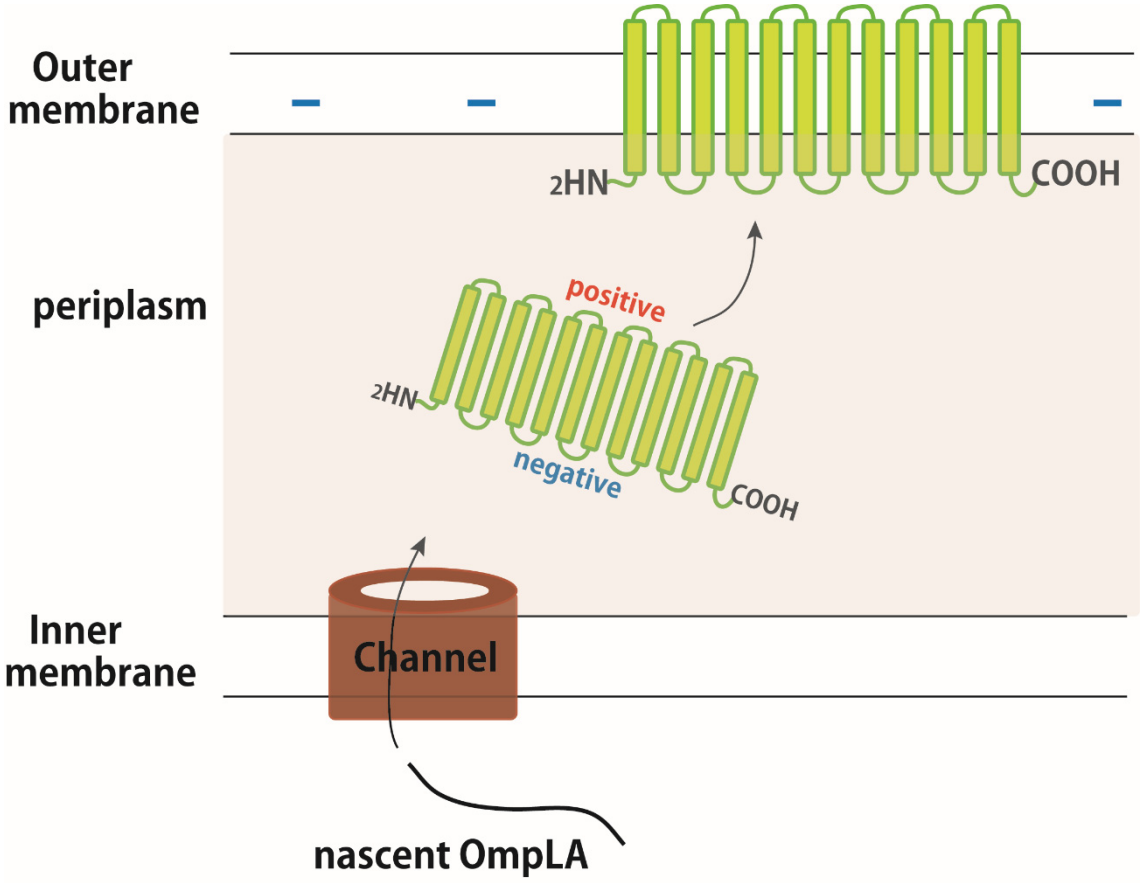

Figure S20. OmpLA insertion into outer membrane of *E.Coli.*