Supplementary Information

for

Layer-by-layer Cell Membrane Assembly

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Supplementary Methods

All chemicals were purchased from Sigma Aldrich (St. Louis, MO) unless otherwise stated. Cascade Blue-labeled 10-kDa dextran (Invitrogen, Carlsbad, CA) was used as provided. Phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.4) was prepared. Tris-acetate-EDTA (TAE) buffer was purchased (Applichem, Darmstadt, Germany). The lipids 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE), and 1-oleoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-*sn*-glycero-3-phosphocholine (NBD-PC) were obtained either as powders or chloroform stock solutions and used without further purification (Avanti Polar Lipids, Alabaster, AL). Lipids were stored in chloroform at -20 °C.

Preparation and characterization of oil-lipid solutions. Cholesterol (10 mol%) was added to chloroform-lipid stocks. Chloroform solutions of phospholipids (10 mg/ml) were dried under gentle nitrogen flow (45 min, 37 °C) using a Reacti-Vap evaporator (Thermo Scientific, Waltham, MA). The resulting lipid film was dried under vacuum (2 h, 25 °C) to remove residual solvent. Dried lipids were dissolved in squalene in glass vials with shaking overnight (1 mM, 37 °C, 100 rpm). The critical micelle concentration (CMC) was determined using the method of Kanamoto et al. [1]. Various concentrations of DOPC in squalene were prepared with 7,7,8,8-tetracyanoquinodemethane (TCNQ, 0.1 mg/mL), incubated 5 h, excess solids removed via centrifugation and absorbance measured (480 nm). The CMC was reported as the intersection of lines along low and high lipid concentration from a semi-log plot.

Microfluidic device fabrication and operation. All microfluidic device fabrication was carried out using soft lithography in poly(dimethylsiloxane) (PDMS)[2]. The PDMS mold was bonded to a glass slide by pre-treating both surfaces with a water:hydrogen peroxide:hydrochloric acid solution (5:1:1 30 min) prior to oven baking (80 °C, 1 h)[3]. The glass slides were either standard 1-mm-thick borosilicate microscope slides for routine quantitative

imaging, or #0 cover glass (24 x 60 mm, Electron Microscopy Sciences, Hatfield, PA) for high-resolution oil immersion imaging. Channels were 120 μm wide and 50 μm deep. Inlet ports were connected to syringes using micro-Tygon tubing (0.01" i.d. × 0.03" o.d., Saint Gobain) via 30 gauge stainless steel needles with Luer-Lok connectors (Small Parts Inc., Miramar, FL). Fluids were driven through the circuit with 2 syringe pumps (PHD Ultra, Harvard Apparatus, Holliston, MA) using 100- or 500-μL glass syringes (Hamilton, Reno, NV).

Alpha hemolysin gene synthesis and compartmentalized expression. The alpha hemolysin gene sequence of *S. aureus* was synthesized (GenScript, Piscataway, NJ) without the N-terminal secretory signal[4]:

ATGGCAGATTCTGATATTAATATTAAAACCGGTACTACAGATATTGGAAGCAATACTACAGTAA
AAACAGGTGATTTAGTCACTTATGATAAAGAAAATGGCATGCACAAAAAAGTATTTTATAGTTT
TATCGATGATAAAAATCACAATAAAAAACTGCTAGTTATTAGAACGAAAGGTACCATTGCTGGT
CAATATAGAGTTTATAGCGAAGAAGGTGCTAACAAAAGTGGTTTAGCCTGGCCTTCAGCCTTTA
AGGTACAGTTGCAACTACCTGATAATGAAGTAGCTCAAATATCTGATTACTATCCAAGAAATTC
GATTGATACAAAAGAGTATATGAGTACTTTAACTTATGGATTCAACGGTAATGTTACTGGTGAT
GATACAGGAAAAATTGGTGGCCTTATTGGTGCAAATGTTTCGATTGGTCATACACTCAAATATG
TTCAACCGGATTTCAAAACAATTTTAGAGAGCCCAACTGATAAAAAAGTAGGCTGGAAAGTGA
TATTTAACAATATGGTGAATCAAAATTGGGGACCATATGATAGAGATTCTTGGAACCCGGTATA
TGGCAATCAACTTTTCATGAAAACTAGAAATGGCTCTATGAAAGCAGCAGATAACTTCCTTGAT
CCTAACAAAGCAAGTTCTCTATTATCTTCAGGGTTTTCACCAGACTTCGCTACAGTTATTACTAT
GGATAGAAAAGCATCCAAACAACAAACAAATATAGATGTAATATACGAACGAGTTCGTGATGA
CTACCAATTGCATTGGACTTCAACAAATTGGAAAGGTACCAATACTAAAGATAAATGGACAGAT
CGTTCTTCAGAAAGATATAAAATCGATTGGGAAAAAGAAGAAATGACAAATTAA.

Restriction endonuclease recognition sequences for *Nco*I and *Blp*I (New England Biolabs, Beverly, MA) were introduced using PCR into the 5′ and 3′ termini, respectively. The modified gene was then directionally inserted into pBEN-SBPb (Agilent, Santa Clara, CA) downstream of the T7 expression cassette. The template for cell-free expression (PureSystem, Cosmo Bio USA,

Carlsbad, CA), including fusion protein, open reading frame and upstream signal sequences was generated by PCR using primers 5′-CTAGTTATTGCTCAGCGGTGG-3′ and 5′-TCTCGATCCCGCGAAATTAATACG-3′ and purified on a spin column (Qiagen, Valencia, CA). Purified DNA (25 ng) was added to a 50-μL reaction and encapsulated in droplets, which were subsequently transformed into vesicles as described with the exception that AQ_{ex} was tris-buffered saline (TBS, 150 mM NaCl). Selective bilayer permeabilization assays were conducted as described with a 75-min incubation period to allow for gene expression.

Linear regression analysis. Quantitative layer-by-layer deposition fluorescence data were fit using a linear model with two parameters (mx + b, x = # of leaflets, m = slope, b = intercept) yielding m = 0.247, Δm = .007, b = 0.02, Δb = 0.02, R^2 = 0.998. Selective external leaflet fluorescence quenching data were fit to an asymptotic curve with two parameters (a - c/x, a = asymptote, c = rise) yielding a = 0.95, Δa = .03, c = 0.92, Δc = .04, R^2 = 0.995.

Supplementary Figures & Legends

Figure S1| Raw image data for vesicle encapsulation yield quantitation. A trapped droplet containing fluorescein dye (*left*) becomes a vesicle (*right*) following layer-by-layer deposition of a phospholipid monolayer. The encapsulation yield is the integrated intravesicular cargo fluorescence intensity divided by the droplet fluorescence immediately before layer-by-layer deposition. A white dashed circle indicates the region of interest (ROI). scale = 60 μm

Figure S2 | Replicate selective permeabilization data. Individual traces are plotted in light green (FAM) or cyan (DEX). Average values over all replicates are plotted in bold green (FAM) and bold blue (DEX). Hemolysin treatment (▼) correlates with FAM permeabilization. Traces are from 5 arrays (n = 30 total vesicles).

Figure S3 | Raw image data for selective permeabilization assay. A vesicle containing 10-kDa dextran (*blue*) and fluorescein (*green*) size exclusion markers exhibits selective membrane permeabilization when treated with soluble α-hemolysin. Integrated fluorescence intensity data were collected based on the ROI (white dashed circle). scale = 60 μm

Figure S4 | Time-resolved raw image data for dithionite quenching of asymmetric unilamellar vesicle intermediates. **a,** unilamellar vesicles prepared with fluorescent head-labeled reporter phospholipid **2** in the outer (L_2) leaflet exhibit concerted quenching of the membrane fluorescence when treated with dithionite; **b,** When reporter lipid resides in the inner (L_1) leaflet, the structures resist quenching. Integrated membrane fluorescence intensity data were collected based on the ROI (white dashed circle). Mean integrated membrane fluorescence was 6,100 ± 100 counts (t = 1 min) and 6,200 ± 200 counts for L_1-labeled vesicles (t = 23 min); and 17,400 ± 400 counts (t = 1 min) and 120 ± 60 counts (t = 23 min) for L_2-labeled vesicles. scale = 60 μm

Figure S5 | Time-resolved raw image data for Co(II) quenching of triple monolayer water-in-oil droplet intermediates. **a,** Triple monolayer droplets prepared with fluorescent tail-labeled reporter phospholipid **3** in the outer (L_3) leaflet exhibit concerted quenching of membrane fluorescence when treated with Co(II); **b,** When reporter lipid resides in the inner (L_2) leaflet, the structures resist quenching. Integrated membrane fluorescence intensity data were collected based on the ROI (white dashed circle). Mean integrated droplet interface fluorescence was 20,400 ± 200 counts (t = 1 min) and 19,000 ± 1,000 counts for L_2-labeled droplets (t = 23 min); and 16,200 ± 600 counts (t = 1 min) and 600 ± 300 counts (t = 23 min) for L_3-labeled droplets. scale = 60 μm

Figure S6 | Time-resolved raw image data for dithionite quenching of double bilayer vesicles. **a,** double bilayer vesicles prepared with fluorescent head-labeled reporter phospholipid **2** in the outer (L_4) leaflet exhibit concerted quenching of the membrane fluorescence when treated with dithionite; **b,** When reporter lipid resides in the inner (L_3) leaflet, the structures resist quenching. Integrated membrane fluorescence intensity data were collected based on the ROI (white dashed circle). Mean integrated double bilayer membrane fluorescence was 10,200 ± 200 counts (t = 1 min) and 10,200 ± 100 counts for L_3-labeled vesicles (t = 23 min); and 17,300 ± 200 counts (t = 1 min) and 230 ± 50 counts (t = 23 min) for L_4-labeled vesicles. scale = 60 μm

Figure S7 | Determination of the CMC for DOPC in squalene. Solubilized TCNQ dye concentration (A_{480}) increases sharply in the presence of micellar amphiphile. The CMC, reported as the inflection of lines through low and high [DOPC], was 2 mM.

Movie S1 | High-resolution DIC movie of LbL membrane assembly. Movie data were collected over ~7 min. The movie frame rate is 10-times faster than real time.

Figure S1

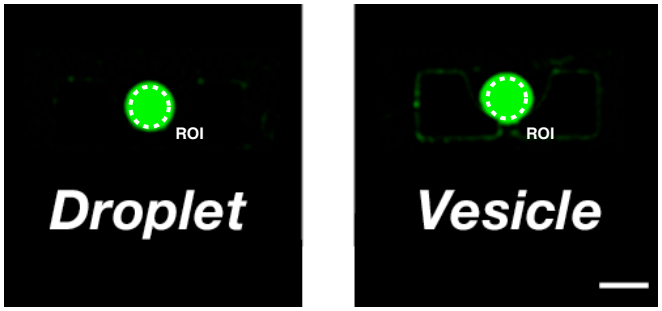

Figure S2

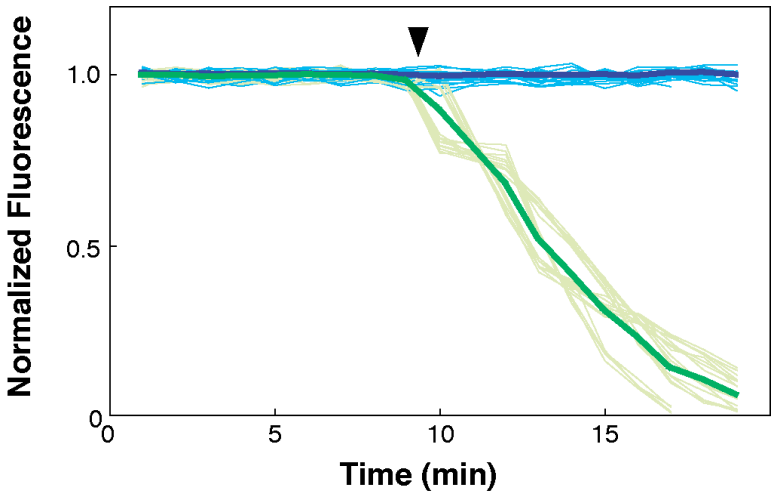

Figure S3

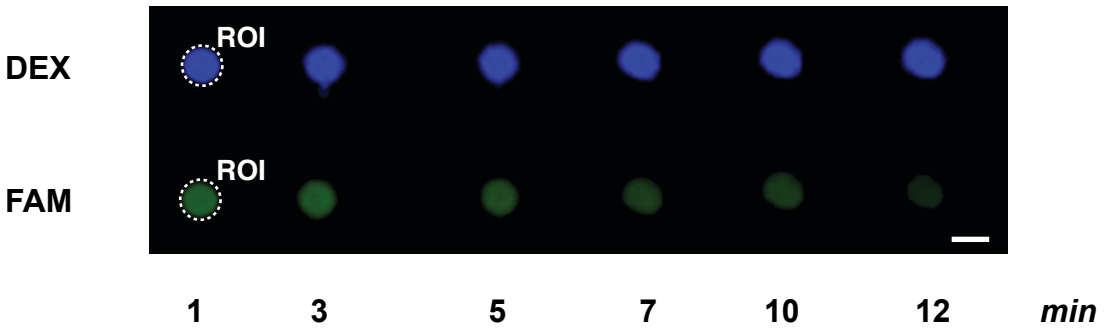

Figure S4

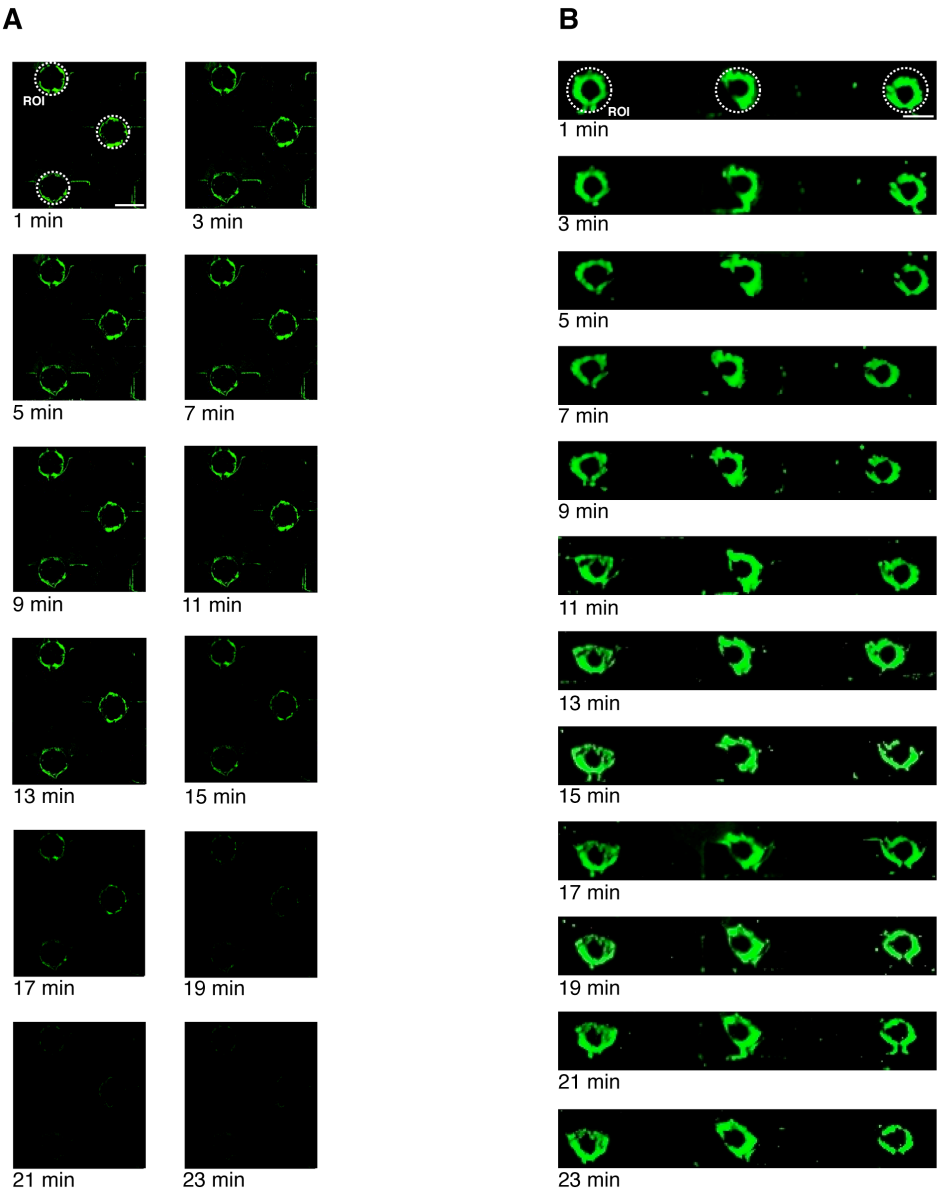
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Figure S5

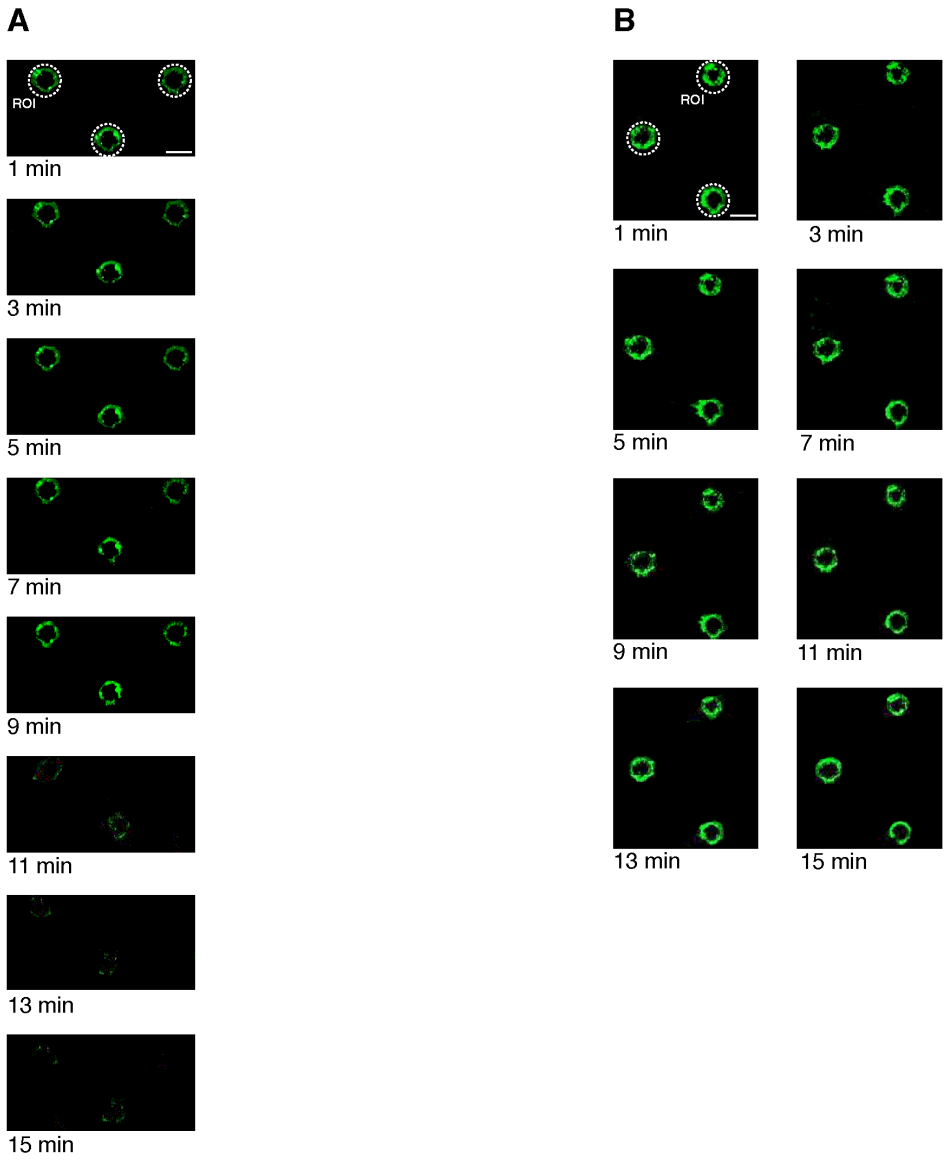
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Figure S6

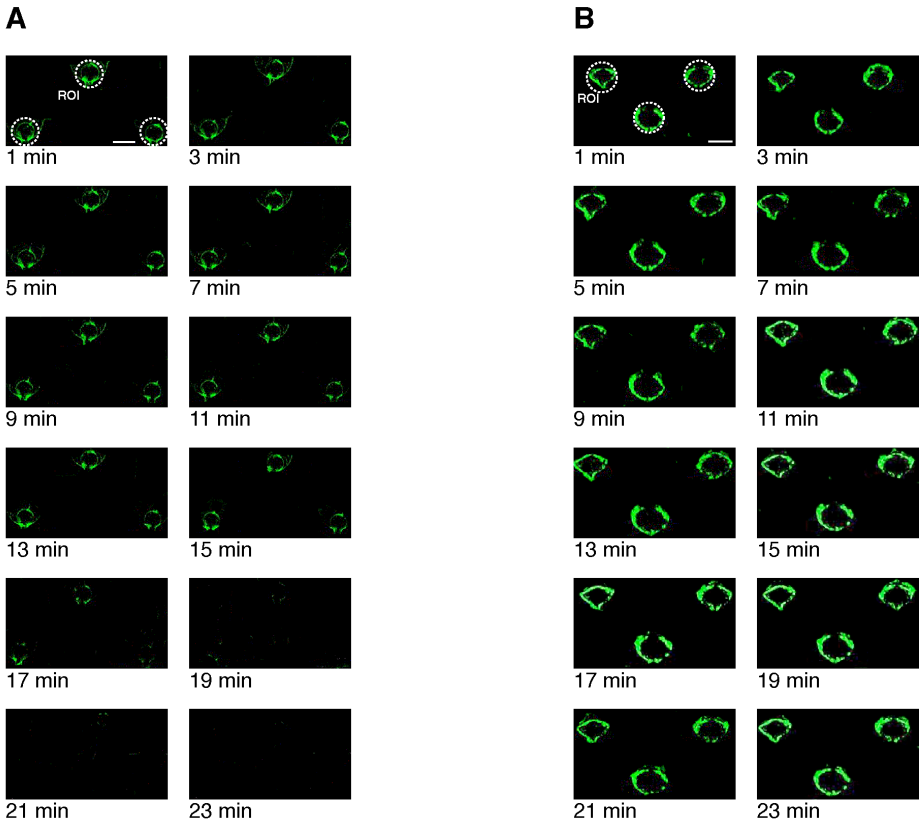
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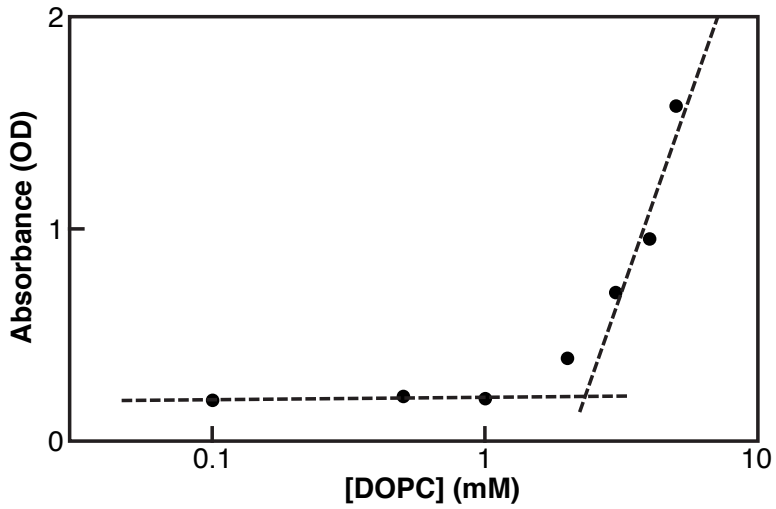
Figure S7



Supplementary References

1.Kanamoto, R., Wada, Y., Miyajima, G. & Kito, M. Phospholipid-phospholipid interaction in soybean oil. *J Am Oil Chem Soc* **58,** 1050–1053 (1981).

2.Duffy, D., McDonald, J., Schueller, O. & Whitesides, G. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal Chem* **70,** 4974–4984 (1998).

3.Sui, G. *et al.* Solution-phase surface modification in intact poly(dimethylsiloxane) microfluidic channels. *Anal Chem* **78,** 5543–5551 (2006).

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