

Electronic Supplementary Information

***In vitro* selection of random peptides against artificial lipid bilayers: a potential tool to immobilize molecules on membranes**

Shota Kobayashi,^{a†} Takuya Terai,^{a†} Yuki Yoshikawa,^a Ryoya Ohkawa,^a Mika Ebihara,^a Masahito Hayashi,^b Kingo Takiguchi,^b and Naoto Nemoto^{*a}

^a Graduate School of Science and Engineering, Saitama University, 255 Shimo-Okubo, Sakura-ku, Saitama 338-8570, Japan.

^b Division of Biological Science, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan.

† These authors contributed equally.

Table S1. Distribution of the expected amino acids in the random region (coded by XYZ in Figure S2).

Amino acid	Expected appearance (%)
Gly (G)	7.0
Ala (A)	1.0
Val (V)	1.0
Leu (L)	4.0
Ile (I)	3.0
Met (M)	2.0
Pro (P)	4.0
Phe (F)	0
Trp (W)	0
Ser (S)	7.0
Thr (T)	5.0
Asn (N)	1.0
Gln (Q)	3.2
Tyr (Y)	0
Cys (C)	0
Lys (K)	4.0
Arg (R)	56.0
His (H)	0.8
Asp (D)	0.2
Glu (E)	0.8
stop	0
Total	100

Table S2. Sequences of the selected peptides.

Name	Amino acid sequence ^[a]	Number
A1 (LB-1)	RHSKSLPSRVIPRADPRTKTRRRRRRKRTL	9
A2	RHSKSLSSRVIPRADPRTKTRRRRRRKRTL	1
A3	RHSKSLPSRVIPRADPRTKTRRRRGRKRTL	1
B1	RISKITRPPTTRRRQRQRTLTPRPRRRRQI	3
B2	RISKITRPPTRTQRQRTLTPRPRRRRQI	1
C1	GSKKQPRPSGIRTRRINRRLRRLRPLKKHL	2
-	Other unique sequences	67

[a] Only the randomized moiety is shown for clarity. For full sequences, see Figure S2.

Table S3. Primers used in this study.

Name	Sequence (5' to 3')
T7-Ω	GATCCCGCGAAATTAATACGACTCACTATAGGGGAAGTATTTTACAA CAATTACCAACAACAACAACAACAACAACATTACATTTTACAT TCTACAACACTACAAGCCACCATG
Forward primer 1 (New Left)	GATCCCGCGAAATTAATACGACTCACTATAGGG
Ω-RTL-new	GGGGAAGTATTTTACAACAATTACCAACAAC
T7-Ω-new	GATCCCGCGAAATTAATACGACTCACTATAGGGGAAGTATTTTACAA CAATTACCAACA
Reverse primer 1 (New Y tag)	TTTCCCGCCGCCCGTCCT

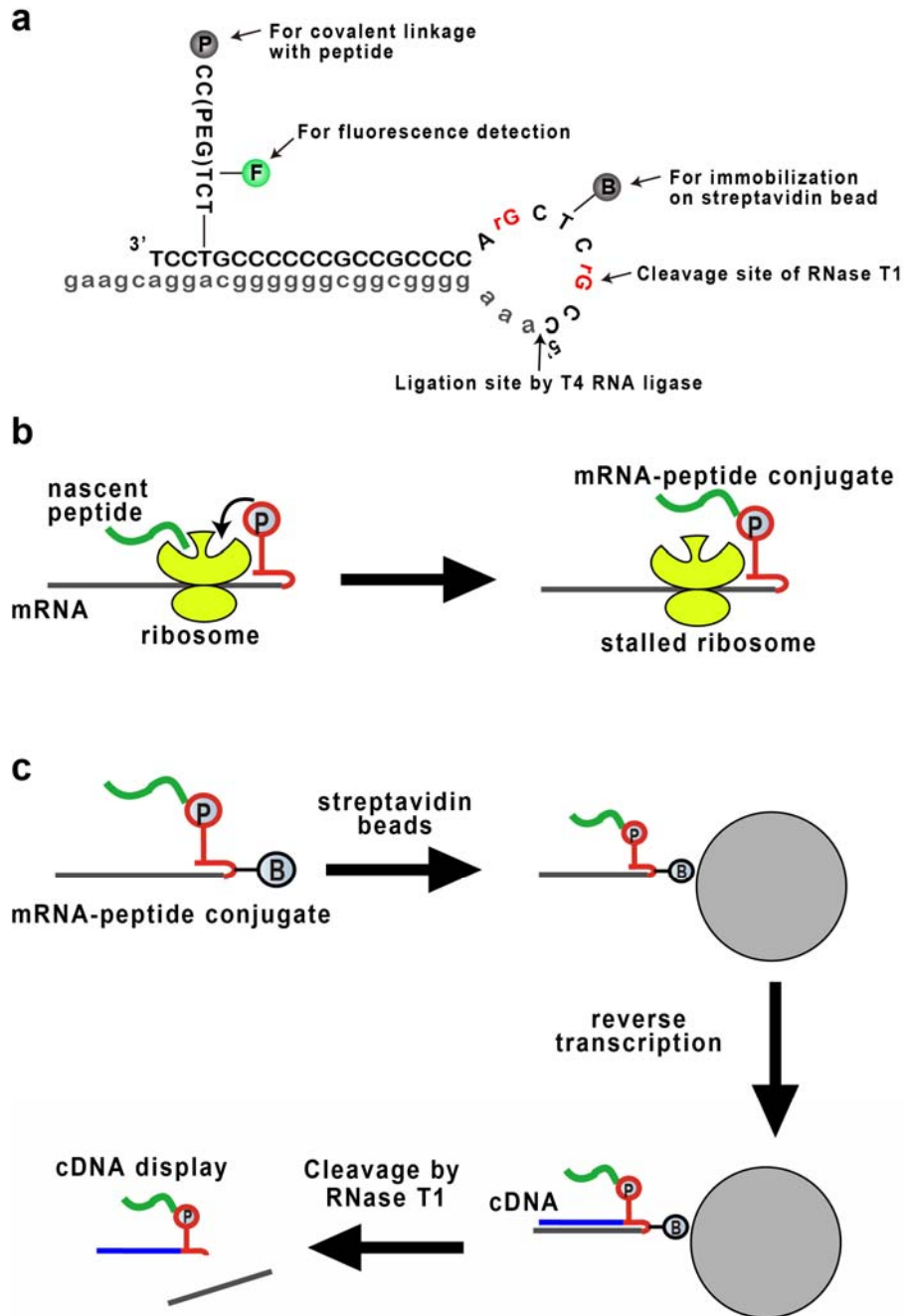


Figure S1. (a) Structure of the DNA linker (SBP linker)^{S1} used in this study. rG: ribonucleotide G, P: puromycin, F: fluorescein, B: biotin. mRNA is shown in gray lowercase letters for clarity. (b, c) Two roles of the linker in cDNA display technology. (b) The puromycin moiety is incorporated in the A site of the ribosome and forms an amide bond with the terminal amino acid of the nascent protein. (c) The biotin moiety is used to immobilize the mRNA-protein conjugate on streptavidin beads. After reverse transcription and buffer exchange, cleavage by RNase T1 is performed to yield cDNA display molecules.

5'-GATCCCGCGA AATTAATACG ACTCACTATA GGGGAAGTAT TTTTACAACA
 ATTACCAACA ACAACAACAA ACAACAACAA CATTACATTT TACATTCTAC
 AACTACAAGC CACCATGGC GGTXYZXYZX YZXYZXYZXY ZXYZXYZXYZ
 XYZXYZXYZX YZXYZXYZXY ZXYZXYZXYZ XYZXYZXYZX YZXYZXYZXY
 ZXYZXYZXYZ XYZGGGGGAG GCAGCCATAA TCATAATCAT AATCATAATC
 ATAATCACAA CGGCGGAAGC GGC GGAAGCA GGACGGGGGG CGGCGGGGAA
 A -3'

Position	Role
14 ~ 30	T7 promoter
34 ~ 36	5'cap
37 ~ 107	Ω
110 ~ 114	Kozak
115~117	Start codon
124 ~ 213	Random region
214 ~ 225	Spacer 1 (GGGS)
226 ~ 261	HN tag
262 ~ 279	Spacer 2 (GGSGGS)
280 ~ 301	Linker hybridization region

Base mixing ratio of random region

	A	T	G	C
X	0.5	0	0.1	0.4
Y	0.1	0.1	0.7	0.1
Z	0.4	0.1	0.4	0.1

Figure S2. DNA sequence of the full-length library. Explanation of each colored part is shown in the table (left bottom), together with the base mixing ratio within the random region (X, Y and Z).

Comment:

We used this XYZ codon for two reasons. First, we wanted to increase the expected appearance ratio of arginine (coded by CGN and AGA/G) to about 50%. To do this, the first letter of the codon (X) should be mainly A or C, the second letter (Y) should be mainly G, and the third letter (Z) should be mainly A or G. Second, we did not want the random region to contain stop codons (TAA, TAG, and TGA) or Cys (TGT/C) because they will reduce the library size or may form complicated disulfide bonds, respectively. To achieve this aim, we had to remove T from the first letter. All other minor cells of the table were set to 10%, yielding the above mixing ratio. With this codon, although several amino acids were also unavoidably removed from the library (see Table S1), we thought it was acceptable.

full length LB-1: RHSKSLPSRVIPRADPRTKTRRRRRRKRTL-Cys(F)-NH₂

N-20: RHSKSLPSRVIPRADPRTKT-Cys(F)-NH₂

C-10: RRRRRRKRTL-Cys(F)-NH₂

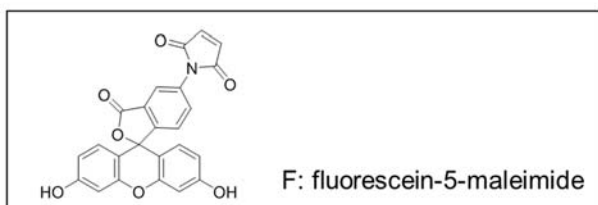


Figure S3. Structures of the chemically synthesized peptides in this work. Amino acids are written in standard one letter code. Cys(F) stands for a cysteine labeled with fluorescein-5-maleimide (inset) at the side chain.

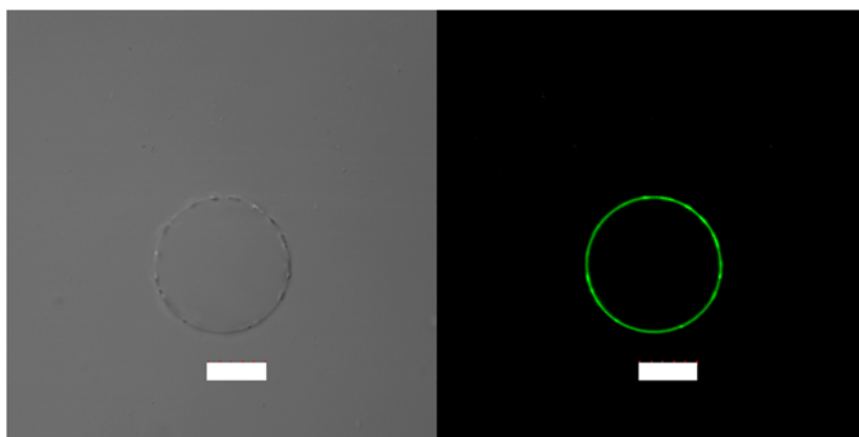


Figure S4. Confocal microscopic images (left: DIC, right: FITC filter set) of a DOPC liposome, with LB-1 (6 μM) in the inner solution. Scale bar: 10 μm.

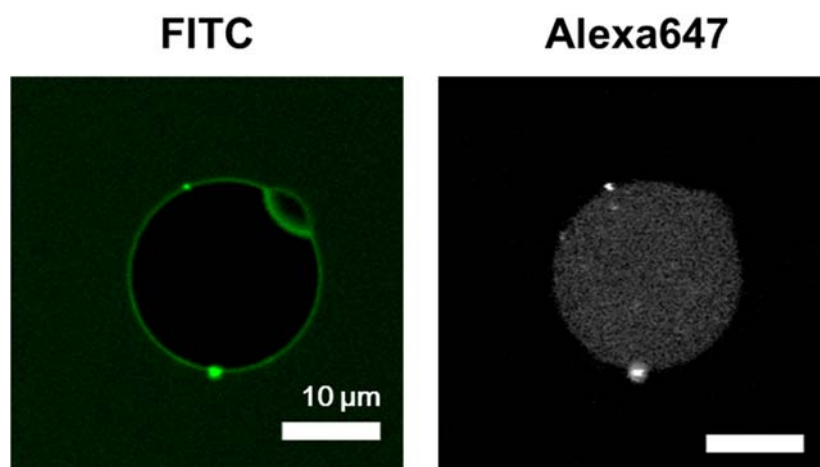


Figure S5. Confocal microscopic images of a DOPC liposome including the anti-fluorescein antibody ($0.6 \mu\text{M}$, labeled with DyLight649) with the addition of LB-1 (labeled with fluorescein) to the outer solution. Images taken with FITC (left) and AlexaFluor647 (right) filter sets are shown. Scale bar: $10 \mu\text{m}$.

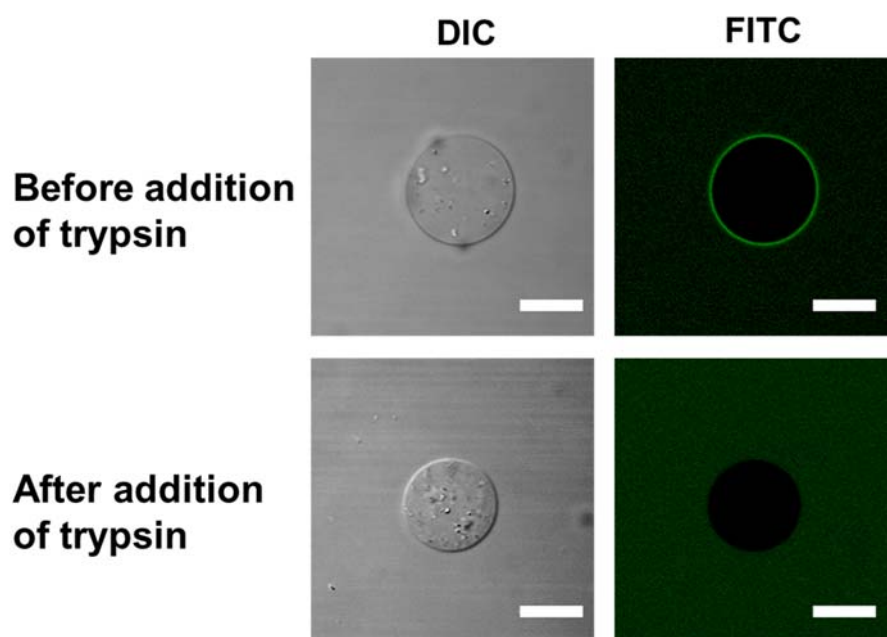


Figure S6. Trypsinization of LB-1 on a liposome. LB-1 labeled with fluorescein at the C-terminus was incubated with DOPC liposomes (as in Fig. 2a) and trypsin (0.25% , final concentration) was added to the outer solution. (Left) DIC images. (Right) Fluorescence images with the FITC filter set. Note: two different liposomes were imaged before and after trypsin digestion. Scale bar: $10 \mu\text{m}$.

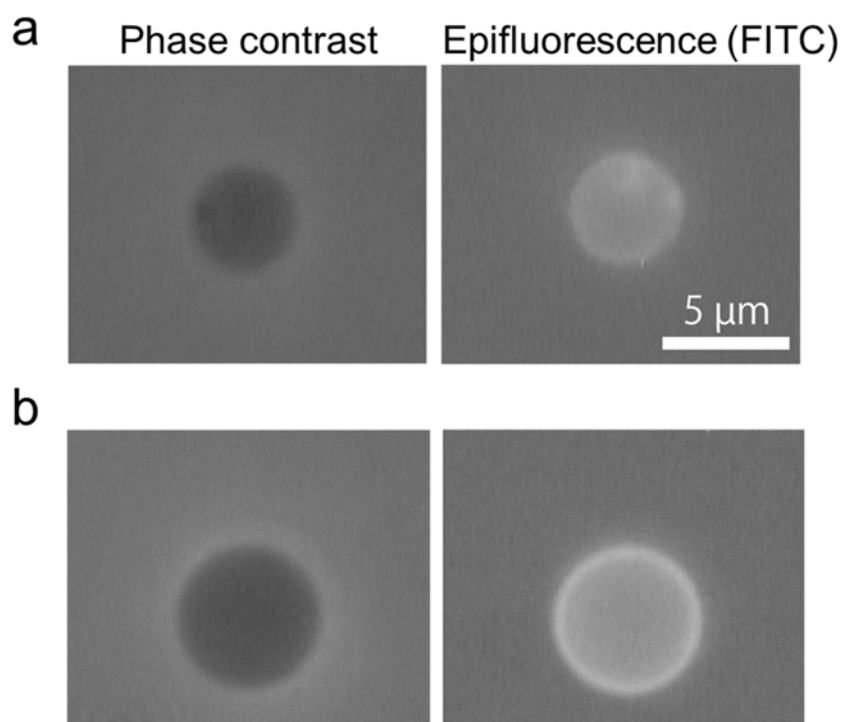


Figure S7. Observation of liposomes using an epifluorescence microscope. (a) DOPC liposomes and (b) DOPC/DOPS = 1/1 liposomes were made and incubated with fluorescently labeled LB-1. Both phase contrast images (left) and fluorescence images (right) are shown.

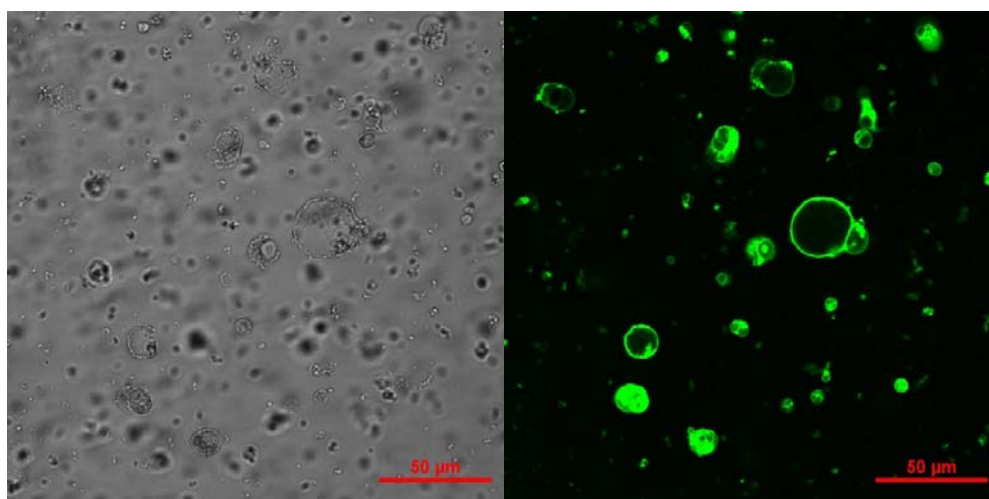


Figure S8. DIC (left) and confocal fluorescence (right) images of DOPC/DOPG = 1/1 liposomes incubated with fluorescently-labelled LB-1 (10 μM), with low magnification. In addition to correctly formed single giant liposomes, other objects are also seen, which obstructed quantitative or statistical analysis.

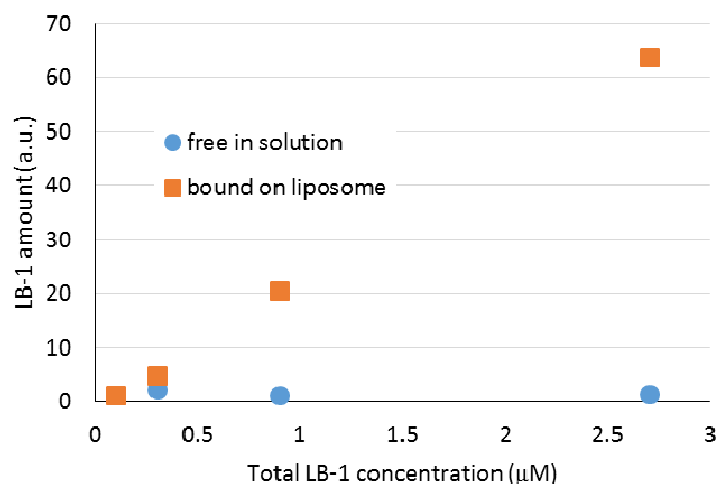


Figure S9. Fluorescence titration of LB-1 and DOPC/DOPG liposome. Fixed amount of liposomes were incubated with fluorescently-labelled LB-1 (final 0.1, 0.3, 0.9, 2.7 μM) for 1 h, and liposomes were removed by centrifugation. Then, by measuring the fluorescence of supernatant, the amount of LB-1 bound to liposomes was calculated. Data are mean of three samples. It appears that the binding is linear and no saturation takes place at this concentration range. Moreover, concentration of free LB-1 remains low regardless of total concentration. So, we could not determine the dissociation constant.

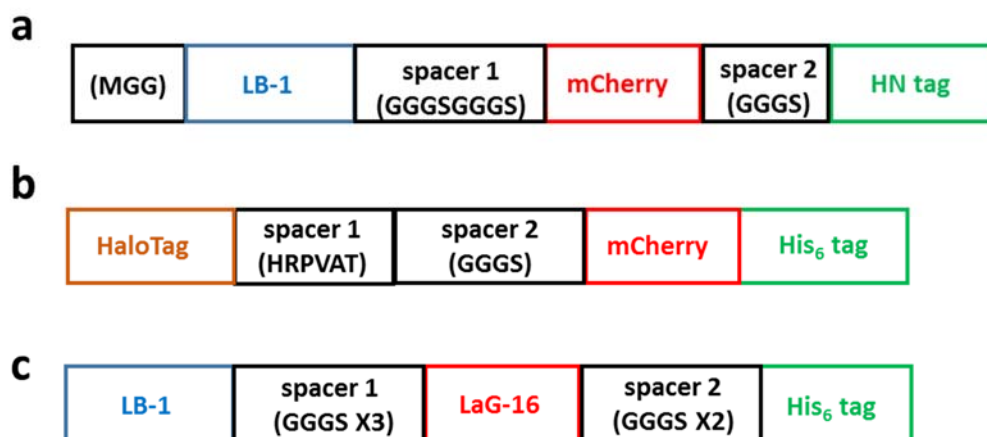


Figure S10. Construction of recombinant proteins. (a) Schematic representation of the LB-1-mCherry fusion protein. (b) Schematic representation of the control mCherry protein (HaloTag-mCherry). (c) Schematic representation of the LB-1-LaG-16 fusion protein. Letters in parentheses indicate amino acids in one letter codes. See experimental procedures for details.

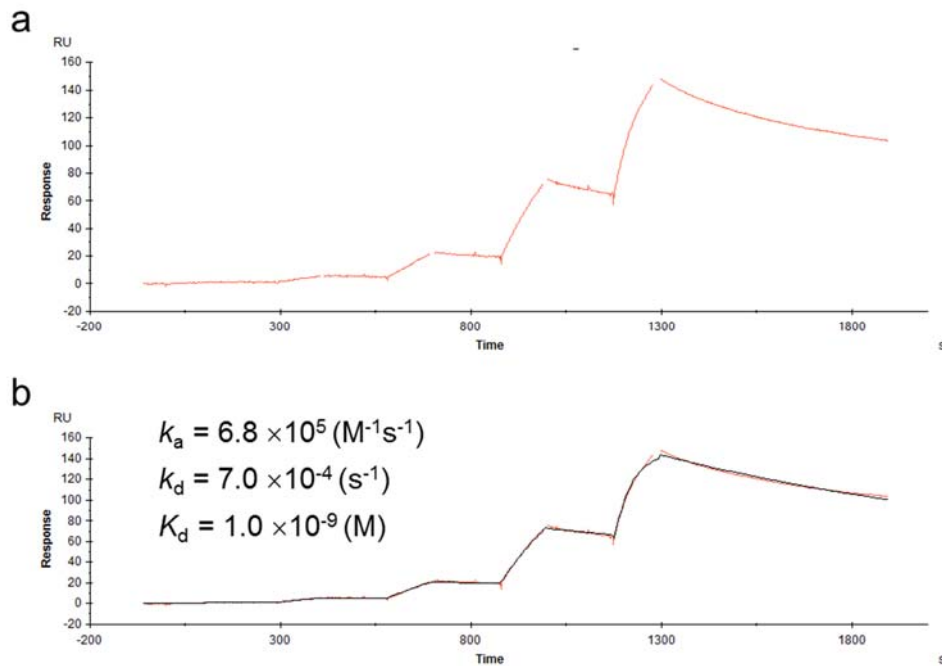


Figure S11. SPR analysis of the binding of GFP to LB-1-LaG-16. (a) Blank-subtracted sensorgram of a single-cycle experiment. LB-1-LaG-16 was immobilized onto a CM5 sensorchip using amine coupling and GFP was injected at increasing concentrations. Some data points were omitted from the analysis to eliminate potential artifacts arising from injection shock. (b) Overlay of the observed (orange) and fitted (black) sensorgrams. Fitted parameters are shown in the inset.

a)

CATATGGGTGGCCGCCATAGCAAAAAGTCTGCCCTCACGCGTTATTCTCGTGCCGATCCG
 CGTACCAAAACTCGCCGTCGTCGTCGCCGTAAACGCACATTAGGCGGAGGCAGCGGTGG
 TGCCAGCATGGTGTCAAAGGCGAAGAGGATAATATGGCCATTATCAAGGAATTTATGCG
 GTTTAAAGTGCACATGGAGGGTAGTGTGAATGGCCATGAGTTGAGATCGAAGGCGAAG
 GCGAAGGACGCCCATACGAGGGTACGCAAACCGCGAAGCTCAAAGTGACGAAAGGTGG
 CCCGTTACCGTTTGCATGGGACATTCTGTCCCCTCAGTTCATGTATGGGTCGAAAGCCTAT
 GTTAAGCATCCAGCGGATATTCCCATTACCTTAAACTGAGCTTCCGGAAGGTTTCAAAT
 GGGAACGGGTAATGAACTTTGAGGATGGCGGAGTTGTCACCGTAACCCAGGATTCTCT
 CTGCAAGACGGCGAATTCATCTACAAGGTGAAACTGCGTGGCACTAACTTTCCGTCTGAT
 GTCCAGTCATGCAGAAGAAAACGATGGGGTGGGAAGCAAGCTCGGAACGCATGTATCC
 GGAAGATGGGGCGTTGAAAGGGGAAATCAAACAGCGCCTGAAACTGAAAGATGGTGGT
 CATTATGACGCAGAAGTGAAAACGACCTATAAAGCGAAGAAACCGGTTCAATTGCCGGG
 TGCTTACAACGTCAACATCAAACCTCGACATTACCTCGACAATGAGGACTACACCATTGT

AGAACAGTATGAACGCGCTGAAGGACGCCATTCCACAGGCGGGATGGACGAACTGTATA
AAGGCGGTGGCAGTCATAATCACAACCATAACCACAACCACAATCACAATTAAGCGGCC
GC

b)

CATATGCGCCACTCAAAAAGCCTGCCGTCTCGTGTTATCCCGCGTGCCGATCCGCGTACC
AAAACCCGTCGCCGCCGTCTCGTCAAACGTACCCTGGGCGGTGGCAGCGGTGGCGGTA
GCGGCGGTGGCAGCATGGCCAGGTGCAGCTGGTTGAAAGCGGTGGCCGTCTGGTGCA
GGCGGGTGATAGCCTGCGTCTGAGCTGTGCCGCAAGCGGTCGCACCTTTAGCACCAGCG
CCATGGCATGGTTTCGTCAGGCCCGGGCCGTGAACGCGAATTTGTGGCGGCCATTACCT
GGACCGTTGGTAACACCATCCTGGGCGATAGCGTGAAAGGTCGTTTTACCATTAGCCGTG
ATCGCGCCAAAAACACCGTGGATCTGCAGATGGATAATCTGGAACCGGAAGATACCGCG
GTTTATTATTGTAGCGCCCGTAGCCGCGGTTATGTGCTGAGCGTTCTGCGCAGCGTTGATA
GCTATGATTATTGGGGTCAGGGCACCCAGGTTACGGTCAGCGGCGGGCGGCTCGGGCGGC
GGTTCCCATCATCATCATCATCATTGAGGATCC

Figure S12. DNA sequences of the synthesized genes. a) LB-1-mCherry. b) LB-1-LaG-16.

Experimental procedures

Materials and instruments

Lipids were purchased from Avanti Polar Lipids. Oligonucleotides were synthesized by Eurofins Genomics, Gene World Co. Ltd., or GenScript Japan Inc. DNA sequencing was performed by Eurofins Genomics. Peptide synthesis was performed by Scrum Inc. Anti-fluorescein antibody (goat) labeled with DyLight649 was purchased from Funakoshi Co., Ltd. When other special chemicals, enzymes and kits were used, the names and suppliers are mentioned in the procedures below. Unless otherwise mentioned, general chemicals were purchased from Wako Pure Chemicals.

DNA construction

The full length random DNA library (shown in Figure S2) for the first round was synthesized by: (1) annealing and extension of synthetic single strand nucleotides to make a double strand 3' fragment (positions 98–301 in Figure S2); and (2) an overlap PCR with the primer, T7-Ω, to add a T7 promoter and the tobacco mosaic virus “omega” untranslated region. Full construct DNA libraries were purified and checked by polyacrylamide gel electrophoresis (PAGE) and DNA direct sequencing. The LB-1-mCherry fusion gene (Figure S12a) was chemically synthesized and inserted into pET21a (Novagen) using NdeI and NotI restriction sites. The control mCherry protein, HaloTag-mCherry, included a His×6 tag at the C-terminus. The plasmid coding this protein was a kind gift from the laboratory of Prof. Tetsuo Nagano (The University of Tokyo). The LB-1-LaG-16 fusion gene (Figure S12b) was chemically synthesized and inserted into pET3a (Novagen), using NdeI and BamHI restriction sites.

Protein expression and purification

The *Escherichia coli* (*E. coli*) BL21 (DE3) strain was transformed with plasmids coding proteins (LB-1-mCherry, HaloTag-mCherry, or LB-1-LaG-16), and expression was induced with IPTG (200 μM) in standard LB medium containing ampicillin (100 μg/mL). After incubation overnight at 18 °C (for LB-1-LaG-16) or 28 °C (for LB-1-mCherry and HaloTag-mCherry), the cells were harvested by centrifugation and lysed with BugBuster Master Mix (Millipore) according to the manufacturer's instructions.

For LB-1-LaG-16, which was expressed in inclusion bodies, the lysate was centrifuged at 16,000 *g* for 20 min at 4 °C and the pellet was washed with 0.1× BugBuster, according to the manufacturer's instructions. Then, solubilization buffer (8 M urea, 20 mM sodium phosphate (pH 7.5), 500 mM NaCl, 20 mM imidazole, 5 mM DTT) was added to the pellet and the mixture was applied to a HisTrap HP column (GE Healthcare). The column was washed with

solubilization buffer and the target protein was eluted with elution buffer (8 M urea, 20 mM sodium phosphate (pH 7.5), 500 mM NaCl, 400 mM imidazole, 5 mM DTT). The eluted protein solution was dialyzed with a dialysis device (TORU-kun, MWCO: 3 kDa, Nippon Genetics, Co. Ltd.) over PBS containing 2 M urea overnight, and then over PBS for one day at 4 °C. Precipitate formed during dialysis was removed by centrifugation and the soluble supernatant was used for imaging experiments.

For LB-1-mCherry and HaloTag-mCherry, which were expressed in the cytosol, the lysate was directly applied to Ni Sepharose 6 Fast Flow resin (GE Healthcare). The resin was washed with wash buffer (50 mM Tris-HCl (pH 7.5), 500 mM NaCl, 40 mM imidazole) and the target protein was eluted with elution buffer (50 mM Tris-HCl (pH 7.5), 500 mM NaCl, 500 mM imidazole), according to the manufacturer's instruction. The eluted protein sample was dialyzed with a dialysis device (TORU-kun, MWCO: 3 kDa, Nippon Genetics, Co. Ltd.) against PBS overnight at 4 °C.

For each expressed protein, the protein concentration was measured by absorbance at 280 nm, and the integrity and purity of the samples were confirmed by SDS-PAGE.

Transcription and ligation of the puromycin linker (1st round)

DNA libraries (10 pmol) were in vitro transcribed by the RiboMAX Large scale RNA Production System-T7 (Promega, Madison, WI, USA) for 4 h at 37 °C. Reactions were terminated by adding DNase and incubated for 15 min at 37 °C. Generated mRNA were purified by the RNeasy MinElute Cleanup Kit (Quiagen). The mRNA (total 300 pmol) was ligated with the puromycin DNA linker (SBP linker, 900 pmol), as described in a previous paper^{S1} with minor modifications. Briefly, the 3'-terminal region of the mRNA molecules was hybridized to the complementary DNA sequence in the SBP linker under annealing conditions. Then, T4 polynucleotide kinase (0.25 U/pmol mRNA, Takara Bio) and T4 RNA ligase (0.25–1.25 U/pmol mRNA, Takara Bio) were added and the mixture was incubated at 25 °C for 20 min for ligation. The annealing and ligation process was repeated three times, and ligated RNA was purified by the RNeasy MinElute Cleanup Kit (Quiagen). The obtained ligated RNA was estimated to be 255 pmol from absorption measurements.

In vitro translation (1st round)

Ligation products (total 200 pmol) were translated with rabbit reticulocyte lysate (Promega) in a 1.0 mL reaction mixture. The cell free translation mixture was incubated at 30 °C for 30 min and the mixture was incubated further at 37 °C for 40 min after addition of KCl and MgCl₂ (final concentrations of 800 and 80 mM, respectively). This process enhances the formation of mRNA-protein fusions. The total final volume was 1.6 mL.

Immobilization to streptavidin beads (1st round)

Streptavidin (SA)-coated 1.0- μ m magnetic beads (Dynabeads MyOne C1 streptavidin, Invitrogen, total 400 μ L) were washed twice with solution A (DEPC-treated water, 0.1 M NaOH, 0.05 M NaCl) and once with solution B (DEPC-treated water, 0.1 M NaCl) according to the manufacturer's instructions. The washed SA beads and 2 \times binding buffer (20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 2 M NaCl, 0.2% Triton X-100, total 1.6 mL) were added to a mixture containing mRNA-protein fusions and incubated for 20 min at room temperature. The beads were subsequently washed three times with 1 \times binding buffer.

Reverse transcription (RT, 1st round)

RT was performed at 42 °C for 30 min by adding 360 μ L of the RT reaction mixture (1 \times RT buffer (TOYOBO), 1 mM dNTP mix) and 1800 U of ReverTra Ace reverse transcriptase (TOYOBO) to the beads. The supernatant was removed and to release the mRNA/cDNA-protein fusion molecules from the beads, 180 U of RNase T1 (Ambion) in 180 μ L reaction buffer (0.01 M Tris-HCl (pH 7.5), 0.01 M MgCl₂, 0.05 M NaCl) was added. The mixture was incubated at 37 °C for 10 min and the supernatant containing mRNA/cDNA-protein fusion molecules was collected.

HN tag purification (1st round)

To TALON magnetic beads (Clontech, 30 μ L) prewashed with water and HN wash buffer (0.05 M sodium phosphate (pH 7.0), 0.3 M NaCl, 0.05% Tween 20), the supernatant prepared above and 1/5 volume of 5 \times HN wash buffer was added. The sample was rotated at 4 °C for 1 h and the beads were washed with HN wash buffer three times. Elution buffer (500 mM imidazole in HN wash buffer, 50 μ L) was added and the sample was rotated at 25 °C for 30 min before collecting the supernatant. Then, new elution buffer (30 μ L) was added to the beads and the sample was rotated at 25 °C for 30 min before collecting the supernatant. The two elutes were combined and used for selection.

Preparation of liposomes (for selection)

Giant unilamellar vesicles (GUVs) were prepared by the thin-film method. DOPC in chloroform (1 mM, 1 mL) was added to a glass petri dish and the solvent was slowly evaporated under nitrogen flow. Then, the inner solution (0.1 M sucrose containing 20 mM MOPS buffer, 20 mL) was carefully added and the mixture was incubated at 37 °C for > 3 h. Formation of GUVs were monitored using a bright field microscope.

Selection

cdNA display molecules and liposomes (2 mL) were added to a 15 mL centrifuge tube and incubated under gentle shaking at 37 °C overnight (from 1st to 4th round) or 3 h (5th and 6th round). Then, outer solution (0.1 M glucose containing 20 mM MOPS buffer, 12 mL) was added and the sample was centrifuged at 2500 g for 5 min. Fractions containing liposomes at the bottom (0.1 mL × 5) were collected by syringe and heated at 85 °C for 5 min to destroy the liposomes. This mixture should contain cdNA display molecules that bind to liposomes and this sample was used directly as a DNA template for PCR.

Preparation of the next library

The obtained DNA template was first amplified by PCR using primers Ω-RTL-new/Reverse primer 1 (Table S2) and EX Taq DNA polymerase (Takara). Then, an overlap extension PCR of the product with a primer T7-Ω-new (Table S2) and EX Taq DNA polymerase (Takara) was performed to construct the full-length DNA library for the next round. When necessary, slight PCR amplification of the full sequences using Forward primer 1/Reverse primer 1 was performed. The library was purified by gel extraction after electrophoresis and used for transcription.

Preparation of cdNA display molecules (2nd to 6th round)

Starting from the selected DNA library, cdNA display molecules were created by essentially the same methods as described above for the 1st round (see “Transcription and ligation of puromycin linker” to “HN tag purification”). The differences were as follows. In the 2nd to 4th round, the amount of mRNA used for ligation was decreased to 60 pmol. The reaction volumes of subsequent procedures were also decreased accordingly. In the 5th and 6th round, the amount of mRNA used for ligation was further decreased to 30 pmol.

Cloning and sequencing

The selected DNAs in the final round were cloned using pGEM-T Easy Vector Systems (Promega) and Competent high DH5α *E. coli* cells (Toyobo), according to the manufacturers' instructions. The randomly selected 84 clones were sequenced and analyzed by Genetyx software (Genetyx).

Preparation of liposomes (for imaging)

Giant unilamellar vesicles (GUVs) were prepared from water-in-oil emulsions, according to a published procedure^{S2} with slight modifications. In a small glass test tube, 15 μL of the lipid solution (10 mg/mL in chloroform) was added and the solvent was evaporated. Liquid paraffin

(0.2 mL) was added to the tube and sonication was performed to dissolve the lipid. 20 μ L of inner solution (0.1 M sucrose, 0.1 M Tris-HCl (pH 7.5), 50 mM NaCl) was added to the mixture, and water-in-oil (W/O) emulsion was formed by mixing. For the experiment in Figure S4, LB-1 (6 μ M) was added to the above inner solution. Also, for the experiment in Figure S5, anti-fluorescein antibody (0.6 μ M) was added to the inner solution. The emulsion was slowly layered on top of 0.2 mL of outer solution (0.1 M glucose, 0.05 M Tris-HCl (pH 7.5), 50 mM NaCl) in a new plastic microcentrifuge tube. After incubation at 4 °C for 1 h, the W/O emulsion was centrifuged at 2,000 g for 10 min using a swing bucket centrifuge. During this process liposomes were formed and precipitated at the bottom. The solution at the bottom was carefully collected using a pipette, incubated with a LB-1 derivative at an indicated concentration for 1–2 h and used for imaging. For the experiment in Figure S7, we used slightly different solutions: inner solution (0.5 M sucrose, 20 mM MOPS-KOH (pH 7.0)) and outer solution (0.5 M glucose, 20 mM MOPS-KOH (pH 7.0))

Confocal fluorescence imaging

Imaging was performed at room temperature by a Nikon A1 confocal fluorescence microscope system equipped with 20 \times objective lens. Standard laser/filter settings for FITC (to visualize fluorescently labeled peptides and GFP) and mCherry were used, according to the manufacturer's instruction. Differential interference contrast (DIC) images were also taken. For imaging, samples (10 μ L) were loaded onto a cover glass equipped with a hand-made frame seal.

Epifluorescence imaging

Imaging for the liposomes incubated with fluorescently labeled LB-1 was performed at room temperature by a phase-contrast and fluorescence microscope (IX70, Olympus, Tokyo, Japan), with a camera (WAT-910HX, Watec, Tsuruoka, Japan). The acquired images were recorded in HDD using a PC through image capture unit (DFG/USB2aud, The Imaging Source, Bremen, Germany), and then analyzed using ImageJ software (<http://imagej.nih.gov/ij/>). For imaging, microscopic specimens (10 μ L) were sealed by valap sealant (Cold Spring Harbor Protocols, Cold Spring Harbor Laboratory Press 2015).

SPR measurement

SPR experiments were performed on a Biacore X100 (GE Healthcare). LB-1-LaG-16 was immobilized onto a CM5 sensor chip using an amine coupling kit (both provided by GE Healthcare). GFP samples (0.156, 0.625, 2.5, 10 and 40 nM) were injected using a “single-cycle” measurement procedure (contact time: 120 s, dissociation time: 600 s) in a buffered solution

containing 10 mM HEPES (pH 7.4), 150 mM NaCl, 3 mM EDTA and 0.05% Tween 20, flowing at a constant rate (30 μ L/min). The binding kinetics and the equilibrium dissociation constant (K_d) with respect to the ligand were determined using Biacore X100 evaluation software, assuming a 1:1 binding mode.

Supplementary References

- S1. Mochizuki, Y.; Biyani, M.; Tsuji-Ueno, S.; Suzuki, M.; Nishigaki, K.; Husimi, Y.; Nemoto, N. *ACS Comb. Sci.* **2011**, 13, 478-485.
- S2. Hamada, S.; Tabuchi, M.; Toyota, T.; Sakurai, T.; Hosoi, T.; Nomoto, T.; Nakatani, K.; Fujinami, M.; Kanzaki R. *Chem. Commun.* **2014**, 50, 2958-2961.