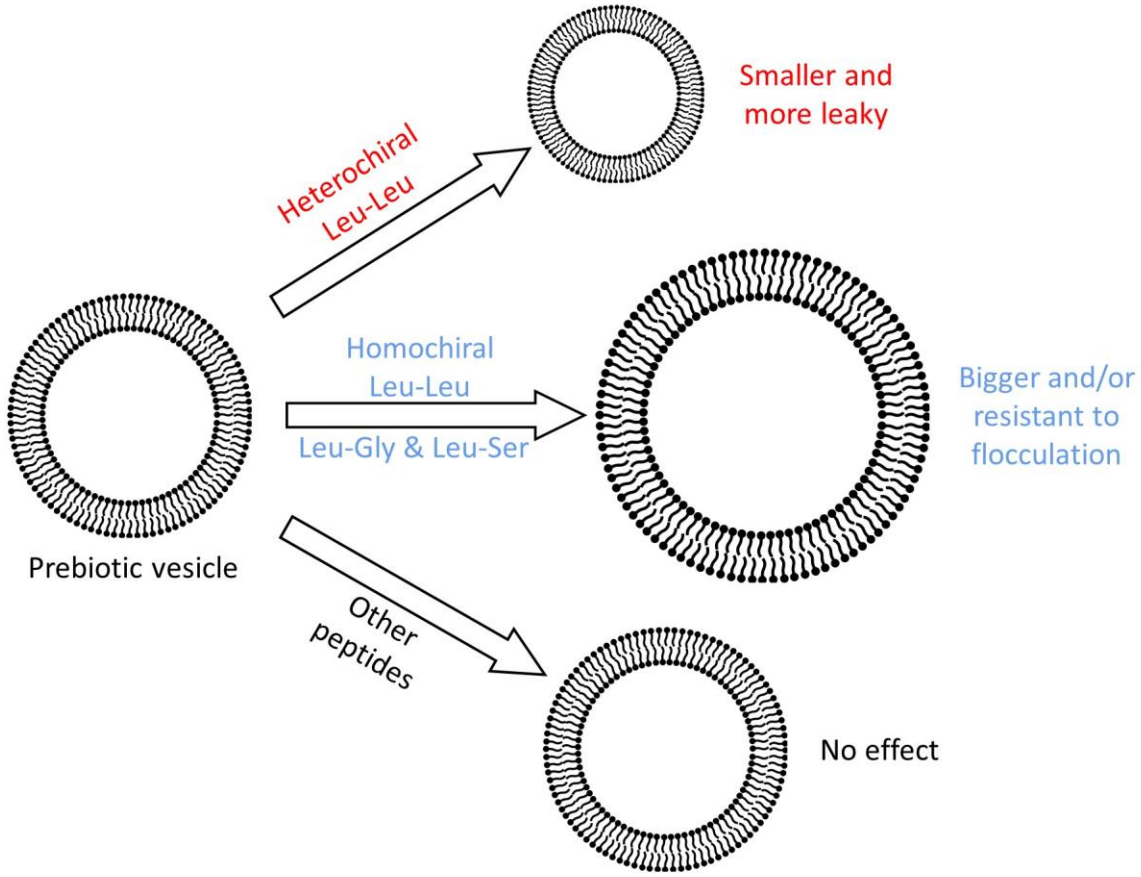
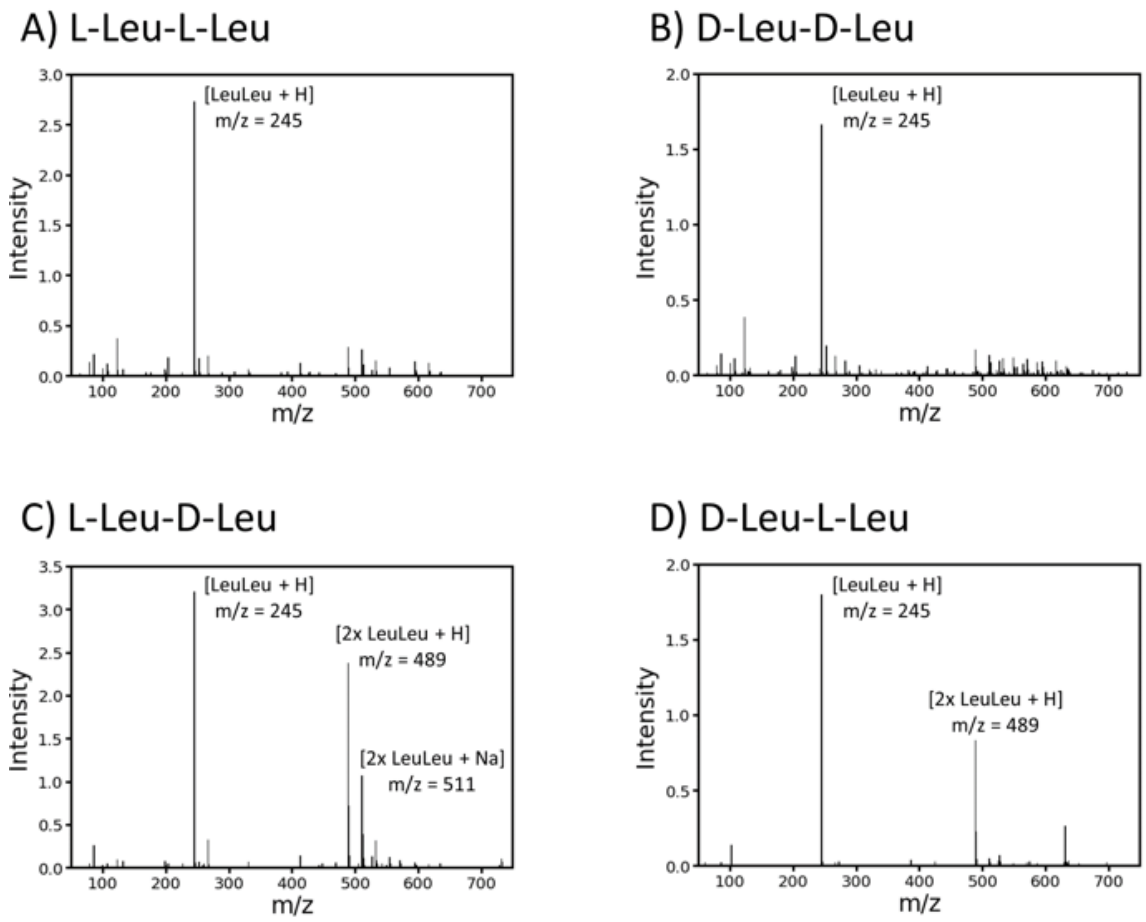
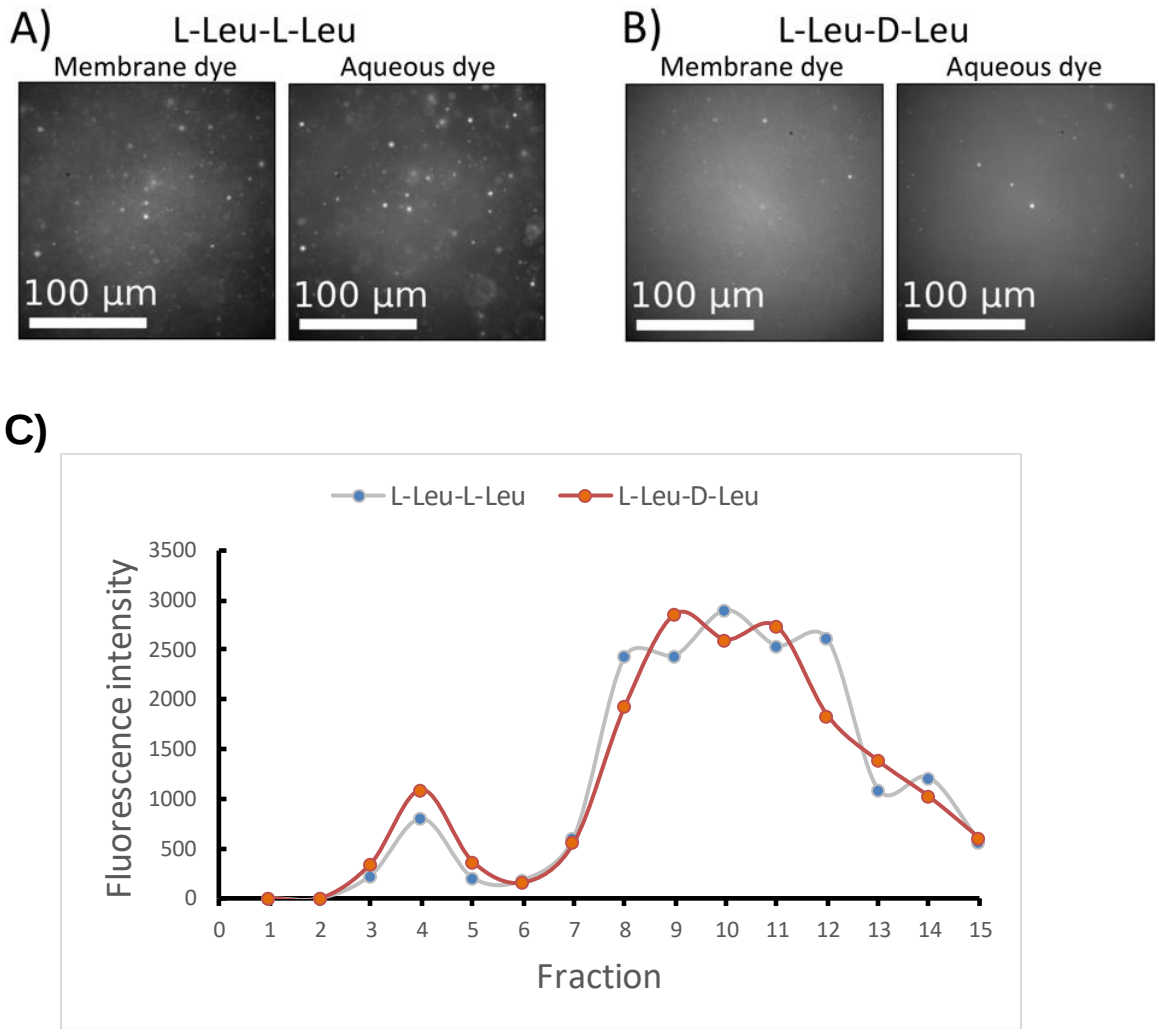
EXTENDED DATA



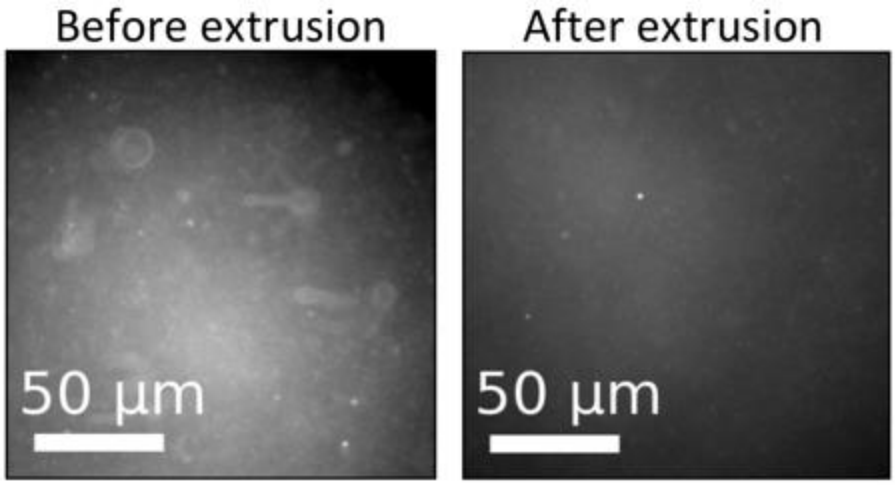
Extended Data Figure 1. Schematic summary of findings.



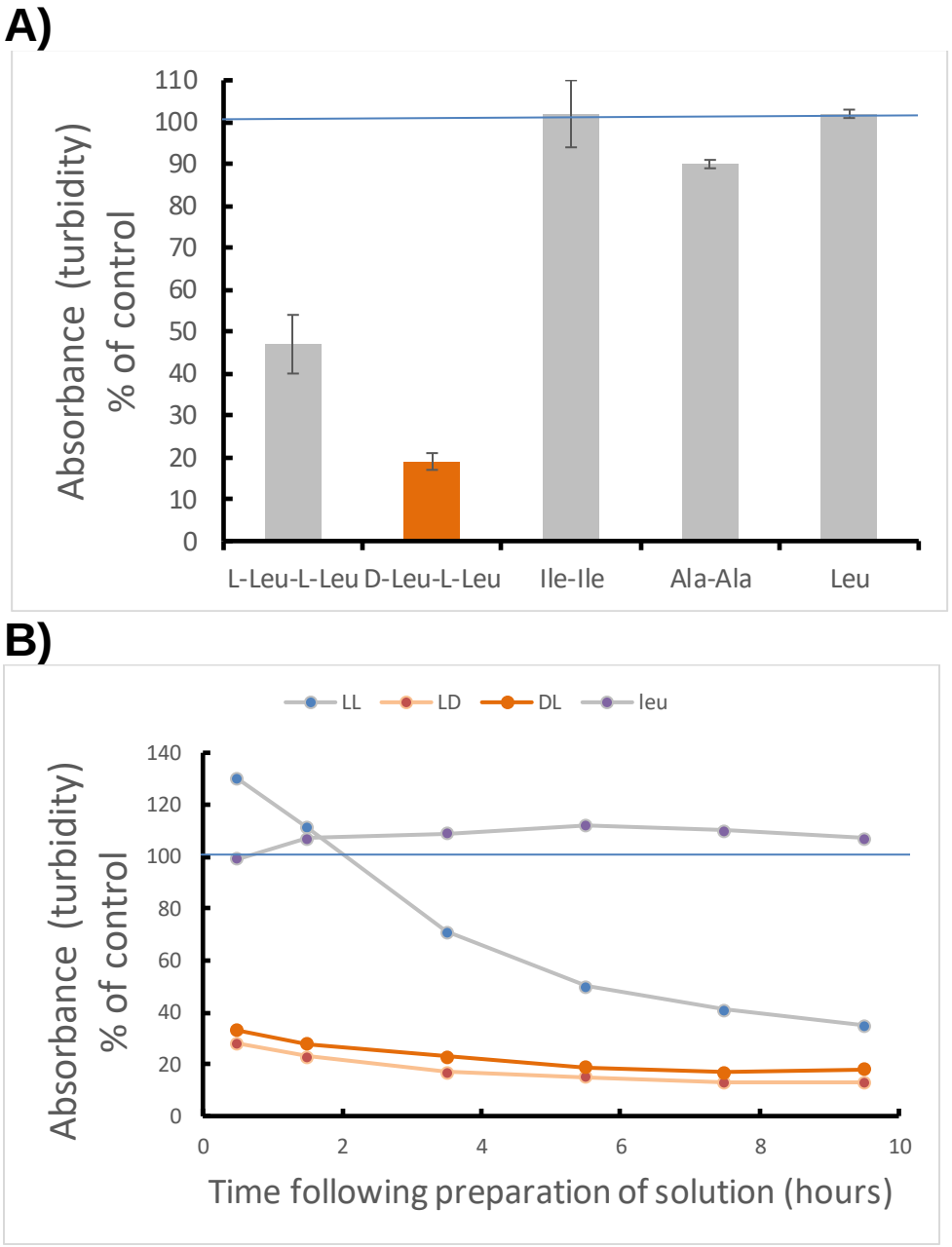
Extended Data Figure 2. Analysis of Leu-Leu dipeptides by mass spectrometry. The Leu-Leu dipeptides were analyzed on a Bruker Esquire ion trap mass spectrometer. All four dipeptides show the expected mass, and show no significant other masses. In both homochiral samples, there is major peak at m/z = 245 which corresponds to the protonated Leu-Leu molecular ion. There is a minor peak at m/z = 123 which is also present in the blank spectra. In the L-Leu-D-Leu spectrum (with material from TCI), in addition to the major peak at m/z = 244.9 there are major peaks at m/z = 489 and m/z = 511, which correspond to noncovalent clusters of two Leu-Leu molecules. The m/z 489 peak corresponds to two Leu-Leu molecules plus a proton, and the m/z 511 peak corresponds to two Leu-Leu molecules plus sodium. Fragmentation of these peaks recovers the protonated and sodiated Leu-Leu ions, respectively. The spectrum for D-Leu-L-Leu shows the major peaks at m/z 245 and m/z 489. The spectrum for the L-Leu-D-Leu from Bachem, not shown, is virtually identical to the spectrum for D-Leu-L-Leu.



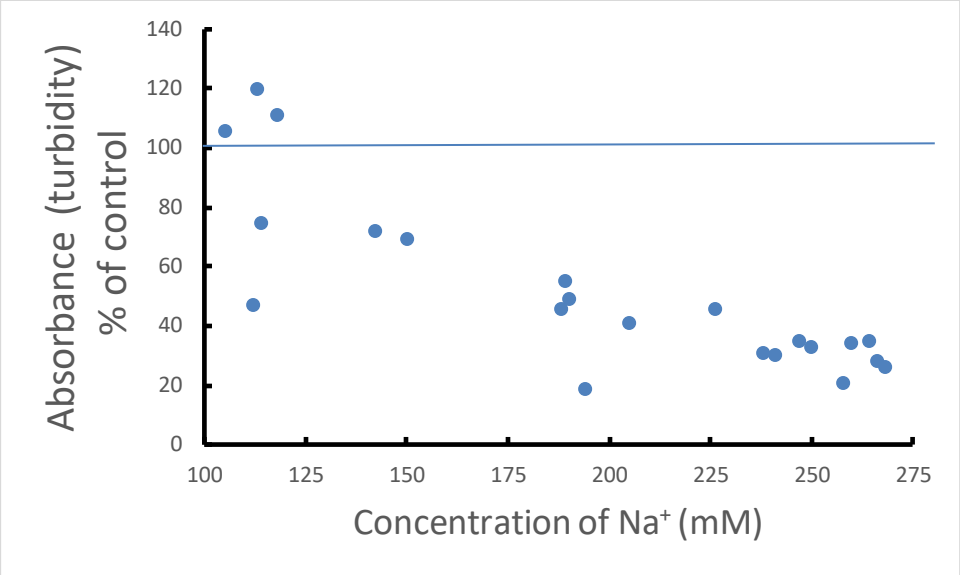
Extended Data Figure 3. L-Leu-D-Leu reduces the number of vesicles detectable by fluorescence microscopy but does not reduce fluorescence intensity of SEC-purified vesicles. A-B. Solutions of L-Leu-L-Leu and L-Leu-D-Leu were prepared as for the turbidity experiments except that 0.5 mM calcein was included during preparation of the decanoic acid solution. Vesicles were separated from free dye by SEC, and rhodamine 6G was added immediately prior to imaging. C. Fluorescence intensity of the SEC fractions was measured with a Fluoroskan plate reader with excitation at 485 nm and emission at 520 nm. The images shown in (A) and (B) are from fraction 3 for both L-Leu-L-Leu and L-Leu-D-Leu. In a separate SEC run, with L-Leu-L-Leu and D-Leu-L-Leu, the fluorescence intensity of fractions 1-5 was also similar, 2002 and 2502 respectively.



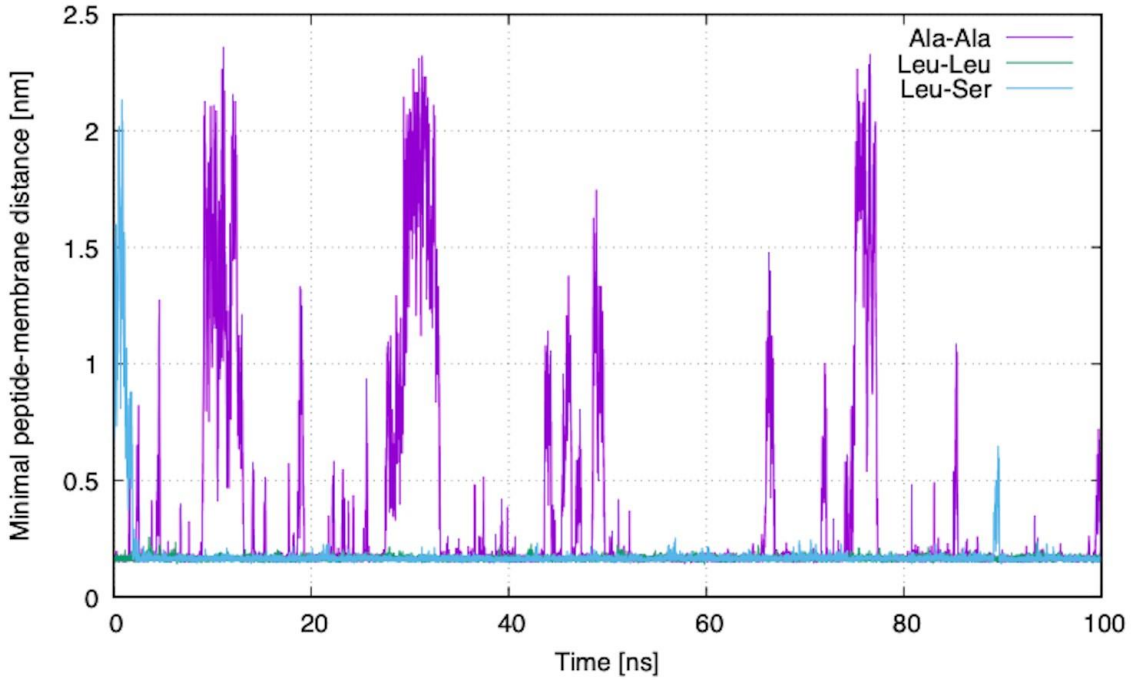
Extended Data Figure 4. Extrusion to 100 nm largely eliminates vesicles detectable by fluorescence microscopy. A solution of 80 mM decanoic acid/0.1 M HEPES/pH 7.65 was imaged before and after extrusion through a 100 nm filter. Rhodamine 6G was added immediately prior to imaging.



Extended Data Figure 5. L-Leu-L-Leu reduces turbidity of a decanoic acid vesicle solution during an extended incubation. Turbidity was determined as described in the Methods except that the absorbance of the solutions was measured (A) 24 hours or (B) at various times after preparation of the solutions, instead of within 30 minutes. The dipeptides were 30 mM and leucine was 60 mM. The values shown in (A) are the averages from two independent experiments. Error bars represent the average error. The line at 100% indicates the normalized value of the control with no compound added in both (A) and (B).

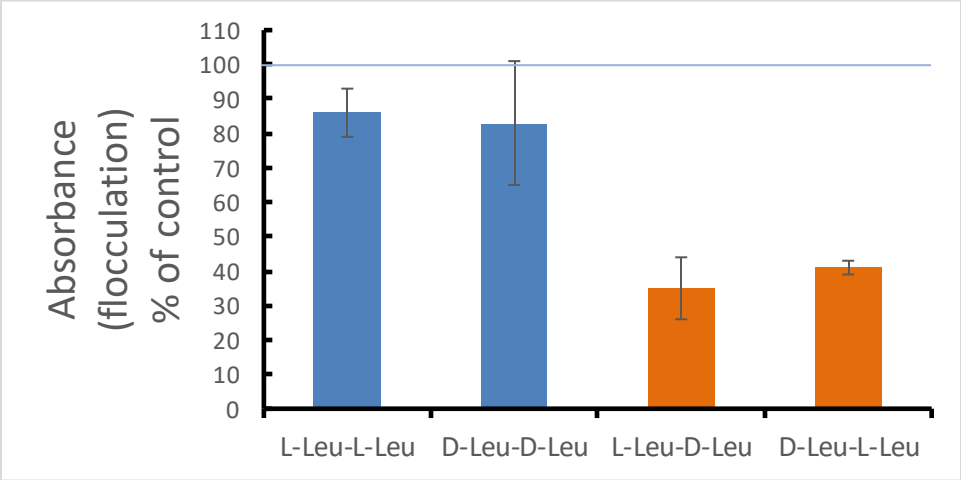


Extended Data Figure 6. The reduction in turbidity of a decanoic acid vesicle solution caused by D-Leu-L-Leu is dependent on the concentration of Na^+. Samples were prepared as described in the Methods, except that the amount of NaCl was varied and 0.1 M instead of 0.2 HEPES was used to achieve the Na^+ concentrations below 125 mM (less NaOH is required to adjust the pH to 7.6 when less HEPES is present). The concentration of D-Leu-L-Leu was 30 mM. The line at 100% indicates the normalized value of the control with no compound added.

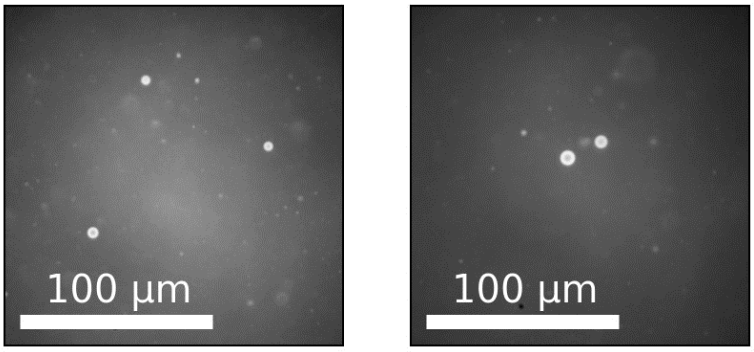


Extended Data Figure 7. Minimal distance between the indicated dipeptide and the fatty acid bilayer, measured as the shortest distance between any peptide atom and any fatty acid atom, along a 100 ns simulation trajectory. Leu-Leu is embedded in the bilayer throughout the entire trajectory. Leu-Ser starts out in solution, but quickly binds to the bilayer and remains there (with a single short-lived detachment). Ala-Ala binds to the bilayer only transiently, showing multiple adsorption and desorption events.

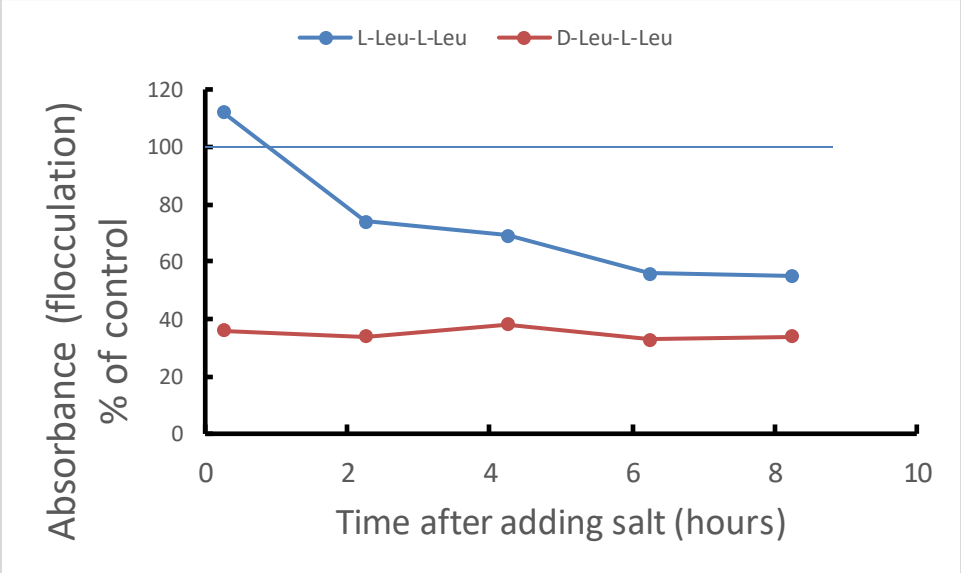
A)


Absorbance (flocculation) % of control
110
100
90
80
70
60
50
40
30
20
10
0
L-Leu-L-Leu
D-Leu-D-Leu
L-Leu-D-Leu
D-Leu-L-Leu

B)

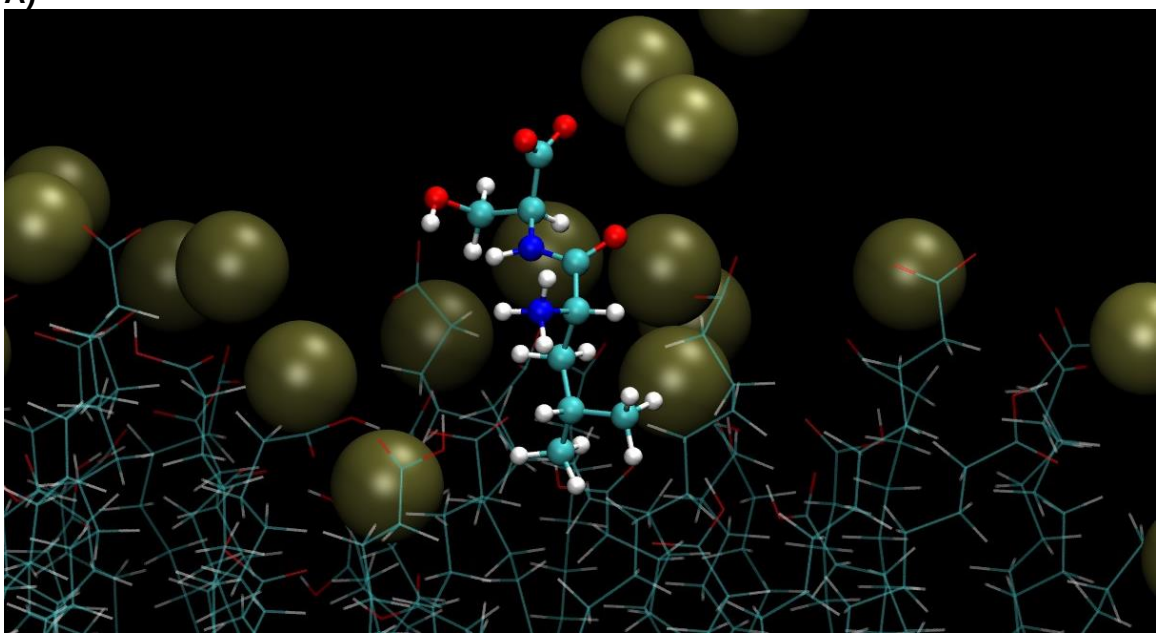

100 µm
100 µm

C)

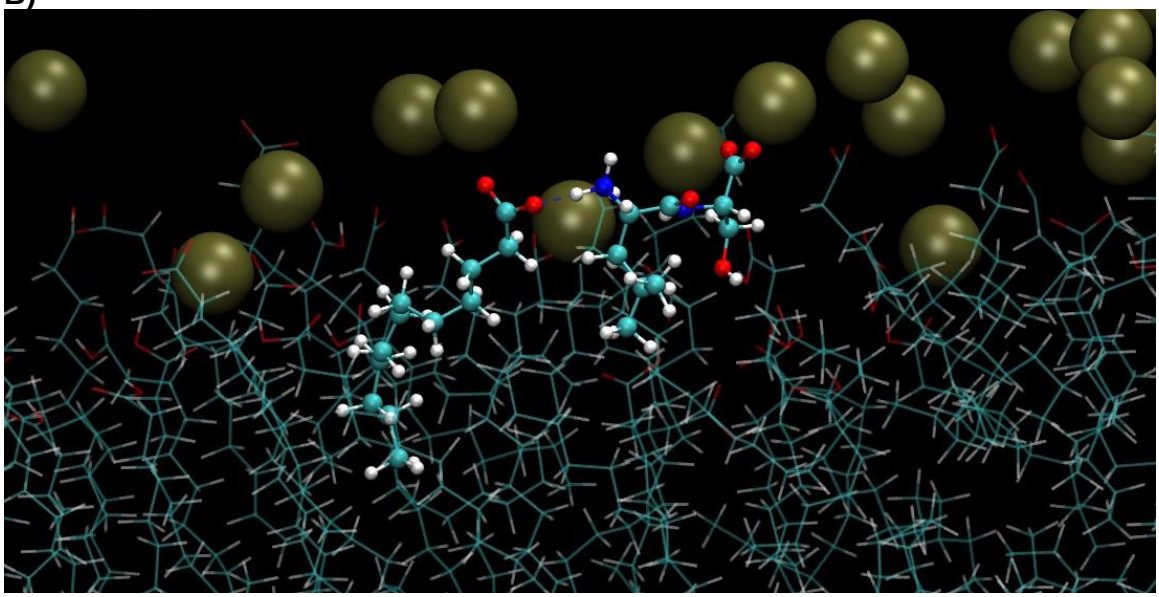

L-Leu-L-Leu
D-Leu-L-Leu
Absorbance (flocculation) % of control
120
100
80
60
40
20
0
0
2
4
6
8
10
Time after adding salt (hours)

Extended Data Figure 8. Heterochiral Leu-Leu inhibits flocculation more effectively than homochiral Leu-Leu. A. Heterochiral Leu-Leu inhibits flocculation more than homochiral Leu-Leu does. The dipeptides were 5 mM instead of the standard 15 mM to avoid maxing out the effects. Error bars represent the average error from two independent experiments (7, 3, 3, 2). The line at 100% indicates the normalized value of the control with no compound added. B. Under the conditions of the flocculation assay, with a dipeptide:decanoic acid ratio of 15:80, heterochiral Leu-Leu does not eliminate vesicles detectible by fluorescence microscopy. An aliquot of the solution from an assay of D-Leu-L-Leu, taken at about 24 hours following the addition of salt, was imaged as described in the Methods, and two representative fields are shown. C. Timecourse of reduction in flocculation. The assay for salt-induced flocculation was carried out as described in the Methods, with decanoic acid solutions containing L-Leu-L-Leu or D-Leu-L-Leu as indicated, except that samples were taken at the indicated times instead of at about 24 hours. The line at 100% indicates the normalized value of the control with no compound added. L-Leu-D-Leu also substantially reduced flocculation, by 60%, at the first time point (15 minutes).

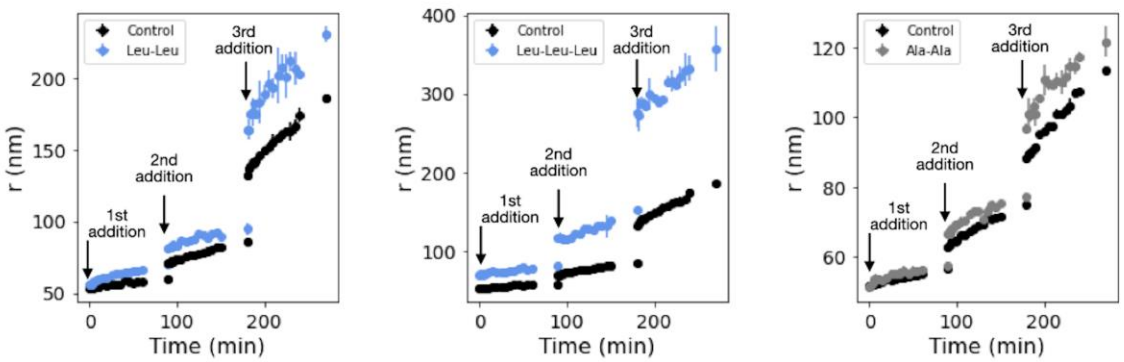
A)



B)



Extended Data Figure 9. A molecular simulation indicates how Leu-Ser could dock to a fatty acid membrane. A model fatty acid bilayer was generated with dodecanoic acid as described in the Methods. Leu-Ser was added to the medium and the simulation was run for 100 ns. Carbon atoms are cyan, hydrogen white, nitrogen dark blue, oxygen red, and potassium ions brown. Different frames of the video show different configurations: A. This image shows the hydroxyl group of the sidechain protruding into the medium. B. This image shows it more closely associated with the bilayer. One molecule of dodecanoic acid is shown with the ball-and-stick representation on the left.



Extended Data Figure 10. The effect of peptides on decanoic acid vesicle radius after multiple rounds of micelle addition. These plots are examples of the results obtained with the procedure described in the Methods. A small volume of micelles was added to the vesicles at the times indicated by the arrows, and the radii (r) were monitored by FRET analysis.