Supplementary Methods

Fluorescence recovery after photobleaching (FRAP) microscopy
FRAP was performed on a Leica SP5 laser scanning confocal microscope with a 63X (NA 1.4) oil immersion objective where 0.1 wt% of the FUS LC was labelled with Cy3. The circular-shaped spot was bleached with a 514 nm laser (ten iterative pulses with full laser power) in the middle of a cluster and intensity changes over time were collected with 514 nm laser (with 5% laser intensity) for Cy3-labeled FUS LC in samples. The recovery curve and half-time of recovery were calculated using the EasyFRAP tool.(49)

Total Internal Reflection Fluorescence (TIRF) microscopy
TIRF was performed on a VisiScope on a Nikon spinning disk confocal microscope. TIRF excitation and imaging was performed with a 100X, 1.49 NA oil immersion objective (Nikon). Excitation was done at the laser wavelengths stated in the figures and a 2X optovar was used for extra magnification.

Coherent anti-Stokes Raman scattering (CARS) spectroscopy and microscopy
Pristine DOPS small unilamellar vesicles (SUVs) and DOPG SUVs (control samples) and those in presence of FUS LC (10 µM) were measured on a home built CARS setup described in detail previously. For CARS measurements, samples were placed in two channels created by double-sided tape strips between glass coverslip and glass slide. PBS was placed in the third channel and used as a reference measurement. Briefly, a dual-output laser source (Leukos-CARS, Leukos) generates sub-nanosecond pulse train divided internally into pump (1064 nm) and Stokes (1100-1600 nm range via photonic crystal fiber and long-pass filter) beams. Two beams are combined to overlap temporally (utilizing optical delay line in a pump beam path) and spatially (via dichroic mirror) before entering the inverted microscope (Eclipse Ti-U, Nikon), where they are tightly focused with 100X, 0.85NA air objective (LCPlan N, Olympus) on the sample sandwiched between two coverslips. Samples were raster scanned via XYZ piezo stage (Nano-PDQ 375 HS, Mad City Labs). At each scanning position, a CARS signal was collected and collimated with a 50x, 0.5NA (LMPN100, Olympus) objective, filtered through notch (NP03-532/1064-25; Semrock) and short-pass filters (FES1000; Thorlabs), and focused on a slit of Czerny-Turner type spectrometer (303i; Andor). The resulting broadband CARS spectrum 700-3900 cm⁻¹ was detected on a deep cooled CCD (NewtonDU920P-BR-DD, Andor).

Acquired hyperspectral data were analyzed via a custom-written code in Igor (WaveMetrics) and Matlab (MathWorks). Phase retrieval from CARS was performed via TDKK method and error phase subtraction via Savitzky-Golay filtering. Spectra acquired for 3 to 12 samples were averaged and depicted with the error bar of one standard deviation.

**GUV preparation**

We followed a standard electroformation method to make GUVs for our studies. Briefly, lipids in chloroform (for fluorescence experiments, 0.1 wt% of 18:1 Liss Rhod PE was doped in the lipid solution) were spread on cleaned Indium-Tin-Oxide (ITO) coated glass slides (Nanocs) to make uniform lipid film and dried under vaccume for at least 2 hours to remove the volatile chloroform. Electroformation was carried out at 55°C in glucose solution (255 mM) to match the osmolarity with the PBS buffer. The voltage was employed 10Vpp during electroformation while keeping the frequency at 10 Hz. 2 mol% of 18:1-12:0 Biotin PE lipid was doped in the pristine GUVs and then tethered to the passivated neutravidin surface as done previously.

**Vibrational Sum Frequency Generation (SFG) spectroscopy**

SFG is a second-order nonlinear optical spectroscopy which reports on vibrational modes of molecules with non-centrosymmetric organization. For the experiments presented here, the laser beams, one in the infrared (IR) and another in the visible (Vis) range, were overlapped spatially and temporally at the lipid
sample position, and the signal generated at sum of their frequencies was detected. Because SFG is an even-order nonlinear spectroscopy process, it is forbidden in the bulk and provides exquisite surface-specificity. Thus, the SFG signal is typically generated at an interface if the molecular species at the interface have, on average, a net orientation. Additionally, the SFG technique is chemically-specific: The fact that vibrational frequencies of different molecular moieties as well as those of different molecular vibrations are unique allows to spectrally resolve them. SFG is a widely applied tool to study bio-interphases, as the technique provides information on interfacial peptide/protein ordering and secondary structure formation.\(^{54}\)

Our SFG system consists of the following major parts: a seed laser, a pump laser, a regenerative amplifier, and an optical parametric amplifier (OPA). The seed is a Ti:Sapphire laser (Mai Tai, Spectra Physics) with an output pulses duration of ~40 fs. After stretching, the selected pulses are directed into the regenerative amplifier (Spitfire Ace, Spectra Physics). The Ti:Sapphire crystal in the amplifier is pumped with a Nd:YLF laser (Empower 45, Spectra Physics). After amplification the selected pulses are directed into the compressor which shortens their duration close to 40 fs. The output laser pulses (1 kHz repetition rate, ~800 nm center wavelength) is then divided into two paths. The first path goes to the etalon (SLS Optics) to produce a spectrally narrow Vis beam (FWHM~15 cm\(^{-1}\)). The second path goes to pump the OPA (TOPAS-C, Light Conversion) producing, with the use of the difference frequency generation (DFG) process. This produces a broadband IR beam (3-6 mW) with a tunable center frequency. Afterwards, the IR and Vis beams are focused and overlapped spatially and temporally at the sample. The generated SFG signal is then collimated and guided into the spectrometer (Acton SpectraPro 300i, Princeton Instruments). Finally, the SFG signal is detected by the electron-multiplied charge-coupled device camera (Newton EMCCD 971P-BV, Andor Technology). The polarization state of the SFG, Vis, and IR beam is controlled by polarizers and half-wave plates. In our experiments, an ssp polarization combination was used where p (s) denotes the polarization parallel (perpendicular) to the plane of incidence which is defined by the incident beam and the perpendicular to the interface.

For SFG experiments, lipid stock solutions with a concentration of 0.143 mM were prepared in chloroform and this stock was used to prepare a lipid monolayer. For the processing of each SFG spectrum, the background corrected sample spectrum was divided by the background corrected reference spectrum to correct for the spectral shape of the IR beam. The background was recorded by blocking the IR beam. As a reference, a z-cut quartz crystal was used. Each sample spectrum was acquired for 20 minutes, while the reference spectrum for 10 seconds.

SFG spectra were recorded in the amide I and carbonyl stretching region (1600-1800 cm\(^{-1}\)) and in the -CH/-OH-stretching region (2800-3600 cm\(^{-1}\)). The amide I (1600-1700 cm\(^{-1}\)) vibrational response arises from the peptide/protein backbone and carries information on peptide/protein interfacial secondary structure as
different structural motifs contribute at different vibrational frequencies.\textsuperscript{(38, 54)} The SFG response in the water OH-stretching region (3000-3600 cm\textsuperscript{-1}) provides information on the interfacial water organization and hydrogen bonding.

**Surface Pressure (SP) Measurements**

SP measurements were performed coincidentally with SFG spectroscopy to study adsorption of FUS LC to model membranes. In an SP experiment, the surface pressure change (with respect to that of the as-prepared membrane interface) was monitored as a function of time after FUS LC addition. SP experiments were performed using a DeltaPi tensiometer (KBN 315 Sensor Head, Kibron Inc.) and a FilmWareX 3.62 software.

**Experimental Protocol of SFG and SP experiments**

Each experiment was performed at 22.5±1.0 °C with 20 mL of PBS buffer (Dulbecco’s Phosphate Buffered Saline 1X, Gibco, in H\textsubscript{2}O) as the subphase in a cleaned trough. Surface pressure (SP) measurements were calibrated at the air/water interface, and rotation of the trough was started (12 rotations per minute) to minimize laser damage to the sample. Rotation of the trough was also used to average over different spots on the sample as well.\textsuperscript{(55)} The lipid solution was spread at the air/water interface dropwise with a Hamilton click-syringe (0.5 μL droplet volume) until the SP change reached a value of 20 mN/m.\textsuperscript{7} After the equilibration of the monolayer, an SFG spectrum (for the monolayer without protein) was acquired. Afterwards, FUS LC (200 μL of 75 μM FUS LC in CAPS buffer, pH 11) was added with a glass syringe into the bulk solution in the trough. The final FUS LC concentration in the sample was 3 μM. The rotating trough also served to assist in sample homogenization. Following protein addition, SFG spectra for the monolayer in presence of FUS LC were taken at increasing incubation times until the SP curve and SFG spectra appeared to no longer change. The SP was monitored during the whole experiment. The sample box was flushed with nitrogen both to prevent unsaturated lipids from oxidation, and to remove water vapor from the sample box which would have absorbed the IR radiation (undesired effect especially in the amide I and carbonyl stretching spectral region).

**Preparation of a supported lipid bilayer (SLB) from SUVs**

Before preparation of SLB samples, all glass slides and coverslips were pre-cleaned with a Piranha solution. Afterwards, coverslips were hydrated with PBS followed by addition of SUVs dispersed in PBS, and the mixture was incubated for 30 mins at room temperature to allow formation of an SLB. Excess SUVs were
removed by washing with PBS (3 times). The lipid mixture for SLBs (both for DOPS and DOPG) contained 0.1 wt% of 18:1 Liss Rhod PE as a label. FUS LC in CAPS buffer at pH 11 was added to the SLB, and the final concentration and pH of the mixture was adjusted to 10 µM (with 0.1 wt% of Cy5-labelled FUS LC) and pH 7.4, respectively. The mixture was incubated for 1 h at 22 °C before adding Thioflavin T(ThT) to a final concentration of 10 µM. Unbound ThT was gently washed after 15 minutes, and samples were imaged in TIRF. All mixing was performed at a room temperature. The TIRF imaging was done on the Spinning Disc Confocal from Visitron with an ablation laser on a Nikon stand w using 100X TIRF objective. Image analysis was carried out with FIJI.
Supplementary Figures

Figure S1. DLS measurements lipid SUVs and a bead standard. DLS measurements of (a) 50 nm polystyrene beads, (b) DOPC SUVs, (c) DOPG SUVs, and (d) DOPS SUVs in PBS buffer at pH 7.4. Experimental temperature was 22 °C.
To calculate the concentration of SUVs, we use the equation (1):

\[
c_{\text{SUVs}} = c_{\text{beads}} I_{\text{SUVs}} \left( \frac{d_{\text{beads}}}{d_{\text{SUVs}}} \right)^6 \left[ \left( \frac{n_{\text{beads}}/n_{\text{solution}}}{n_{\text{SUVs}}/n_{\text{solution}}} \right)^2 - 1 \right] \times \left[ \left( \frac{n_{\text{SUVs}}/n_{\text{solution}}}{n_{\text{beads}}/n_{\text{solution}}} \right)^2 + 2 \right]^2,
\]

where \( c_{\text{SUVs}} \) is the concentration of SUVs; \( d_{\text{SUVs}} \) and \( d_{\text{beads}} \) are diameter of the SUVs and the polystyrene beads (50 nm beads, the standard sample); \( I_{\text{SUVs}} \) and \( I_{\text{beads}} \) are the scattered light intensities stemming from SUVs dispersion in PBS and the polystyrene beads, respectively; \( n_{\text{solution}} \), \( n_{\text{beads}} \) and \( n_{\text{SUVs}} \) are the refractive indices of the solution, the beads, and the SUVs, respectively. Calculation results are presented in Table S1.

Table S1. Hydrodynamic diameter and concentration of SUVs with various composition.

| Sample | \( d_h \) (nm) | \( c_{\text{SUVs}} \) (particles/mL) |
|--------|----------------|-------------------------------------|
| DOPC   | 45.2±1.1       | \( 0.98 \times 10^{15} \)          |
| DOPS   | 19.5±3.0       | \( 1.85 \times 10^{15} \)          |
| DOPG   | 22.0±1.8       | \( 1.88 \times 10^{15} \)          |
Figure S2. Bright field images of SUVs and FUS LC mixtures at fixed SUVs concentration and various FUS LC concentrations. Images of FUS LC-lipid clusters for (a) DOPS and (b) DOPG SUVs in the presence of FUS LC at the indicated concentrations in PBS at pH 7.4. DOPS and DOPG SUV concentration was $1.85 \times 10^{15}$ and $1.88 \times 10^{15}$ vesicles/mL, respectively. Scale bar is 10 µm.
Figure S3. Bright field images of SUVs and FUS LC mixtures at fixed FUS LC concentration and various SUV dilutions. Images of FUS LC-lipid clusters with FUS LC (10 µM) in the presence of different concentrations of SUVs in PBS at pH 7.4 for (a) DOPG SUVs and (b) DOPS SUVs. 1X dilution for DOPG and DOPS SUVs corresponds to SUV concentrations of $1.88 \times 10^{15}$ and $1.85 \times 10^{15}$ vesicles/mL, respectively. Scale bar 10 µm.
Figure S4. BCARS spectra of FUS LC protein condensates (200 µM) in phosphate buffer, pH 7.4 containing 150 mM of NaCl.

Figure S5. Negatively charged GUVs drive peripheral FUS LC accumulation. Confocal microscopy images of GUVs (doped with 0.1 mol% of 18:1 Liss Rhod PE) incubated with 5 µM of FUS LC (doped with 0.1 mol% of Cy5 labeled FUS LC) in PBS buffer, pH 7.4. From left to right: lipid channel, protein channel, and merged fluorescence images. Top panel is for FUS LC+DOPS GUVs and bottom panel is for FUS LC+DOPG GUVs. Scale bar is 10 µm.
SFG spectral fitting of the amide I and C=O stretching region for FUS LC at the DOPS monolayer.

Fitting of SFG spectra was performed according to the equation (2):\(^{(57)}\)

\[
\chi^{(2)}(\omega) = A_{NR}e^{i\phi_{NR}} + \sum_{j} A_j \frac{1}{\omega - \omega_{IR,j} + i\Gamma_j}
\]

where \(A_{NR}\) and \(\phi_{NR}\) are the amplitude and the phase of the non-resonant contribution and \(A_j, \omega_{IR,j},\) and \(\Gamma_j\) are amplitude, center frequency, and half-width at half maximum (HWHM) of the \(j\)-th resonant contribution, respectively.

Spectral fitting was performed to extract the resonant contribution of \(\chi^{(2)}\) for the amide I. For the spectra presented in Fig. 3b of the main text, the amide I and C=O stretching region, the following resonant contributions were considered: 1) the amide I signal was assumed to have two contributions and fitted with two peaks centered at \(\sim1650\ \text{cm}^{-1}\) and \(\sim1675\ \text{cm}^{-1}\). These initial guess values were chosen to account for possible random-coil as well as \(\beta\)-sheet and/or \(\beta\)-turn interfacial FUS LC structures, respectively. 2) the C=O contribution was fitted with two peaks according to the data obtained from heterodyne-detected SFG spectroscopy measurements reported for lipid monolayers at air/solution interface.\(^{(58},59)\) Global fitting was performed for all spectra. During the global fitting procedure, the central frequency \(\omega\) and FWHM (i.e. \(2\Gamma\)) parameters of each resonant contribution were linked throughout the time-dependent set of measurements for all SFG spectra. Amplitude \(A\) values for each resonant contribution were not linked and were fitted independently. The non-resonant contribution parameters (amplitude \(A\) and phase \(\varphi\)) were not linked and were fit independently for each SFG spectrum. Results of the fitting are presented in Table S2 and plotted in Figure S6a. Figure S6b presents the extracted amide I contribution calculated plotted based on fitting results.

**Table S2.** Fitting parameters obtained from SFG spectral fitting of the DOPS monolayer before vs. after FUS LC protein addition to the subphase performed for spectra acquired in the amide I and C=O stretching region.

| DOPS | + 3 \(\mu\)M FUS LC |
|---|---|
| t=2000 s | t=3900 s | t=4800 s | t=5900 s | t=7300 s | t=10000 s | t=12700 s |
| nonresonant A | -0.01 | 0.03 | -0.08 | 0.06 | -0.08 | 0.09 | -0.10 | -0.1 |
| nonresonant \(\varphi\) | -1.6 | -2.3 | -3.6 | -0.1 | 3.2 | 0.2 | -2.84 | -2.8 |
| amide I (\(\sim1650\) 1/cm) A | none | -0.29 | 1.16 | 2.55 | 3.55 | 4.86 | 5.41 | 5.3 |
| amide I (\(\sim1650\) 1/cm) \(\omega\) | 1668.4 | 1668.4 | 1668.4 | 1668.4 | 1668.4 | 1668.4 | 1668.4 |
| amide I (\(\sim1650\) 1/cm) \(2\Gamma\) | 33 | 33 | 33 | 33 | 33 | 33 | 33 |
Figure S6. Amide I region SFG spectra of FUS LC at the DOPS lipid interface. (a) SFG spectra for FUS LC at the DOPS monolayer interface at indicated time points after the FUS LC addition to the subphase and corresponding fitting curves. (b) Amide I contribution intensity obtained from the fitting of SFG spectra presented in a.

SFG spectral fitting of the -CH and -OH stretching region for FUS LC at the DOPS and DOPG monolayer.

A similar global fitting routine was applied to extract the resonant contribution of $\chi^{(2)}$ for the CH and OH region. The peak assignments and signs of -CH and -OH contributions were chosen according to the data.
reported for negatively charged PG and PS lipids obtained from heterodyne-detected SFG spectroscopy measurements.\(^{(60)}\) Fitting results are presented in Table S3.

**Table S3.** Fitting parameters obtained from SFG spectral fitting of the DOPS and DOPG monolayer before vs. after FUS LC (3 µM) protein addition to the subphase.

|                  | DOPS       | DOPS + FUS LC | DOPG       | DOPG + FUS LC |
|------------------|------------|---------------|------------|---------------|
| nonresonant      | A          | -0.08         | 0.02       | 0.01          |
|                  | φ          | 12.7          | 2.5        | 3.4           |
| CH\(_2\) symm    | A          | 3.0           | 2856.7     | 2845          |
|                  | ω          | 2856.7        | 2845       | 2845          |
|                  | 2Γ         | 39            | 39         | 31            |
| CH\(_3\) symm    | A          | 1.3           | 2.3        | 0.4           |
|                  | ω          | 2885.9        | 2876       | 2875          |
|                  | 2Γ         | 22            | 18         | 18            |
| CH\(_2\) FR      | A          | 1.4           | 1.2        | 0.9           |
|                  | ω          | 2930.8        | 2924       | 2823          |
|                  | 2Γ         | 27            | 24         | 24            |
| CH\(_3\) FR      | A          | 4.3           | 6.5        | 2.8           |
|                  | ω          | 2949.2        | 2941.5     | 2940          |
|                  | 2Γ         | 40            | 35.7       | 29.3          |
| CH\(_3\) asymm   | A          | -8.0          | -12.7      | -6.2          |
|                  | ω          | 2963.8        | 2950       | 2850          |
|                  | 2Γ         | 59            | 60         | 60            |
| CH vinyl         | A          | 1.0           | 1.3        | 1.4           |
|                  | ω          | 3017.5        | 3005       | 3006          |
|                  | 2Γ         | 30            | 30         | 60            |
| NH               | A          | -12.2         | -23.3      | -              |
|                  | ω          | 3305.1        | 3305.1     | -              |
|                  | 2Γ         | 153           | 153        | -              |
| OH (I)           | A          | -18.8         | -19.5      | -16.3         |
|                  | ω          | 3212          | 3212       | 3267          |
|                  | 2Γ         | 170           | 170        | 283           |
| OH (II)          | A          | -75.0         | -100.8     | -37.1         |
|                  | ω          | 3472          | 3472       | 3499          |
|                  | 2Γ         | 393           | 393        | 316           |

\(^{(60)}\)
**Table S4.** Analysis of the CH\(_2\) symmetric and CH\(_3\) symmetric stretching contributions in SFG spectra in the CH/OH region.

|          | Area (CH\(_2\) symm) | area (CH\(_3\) symm) | ratio(CH\(_2\)/CH\(_3\)) by area | I\(_{\text{max}}\) (CH\(_2\) symm) | I\(_{\text{max}}\) (CH\(_3\) symm) | ratio(CH\(_2\)/CH\(_3\)) by max intensity |
|----------|-----------------------|------------------------|-----------------------------------|-----------------------------------|-----------------------------------|---------------------------------------------|
| DOPS     | 1.1                   | 0.4                    | 2.8                               | 0.024                             | 0.014                             | 1.7                                         |
| DOPS + FUS LC | 1.3                 | 1.2                    | 1.1                               | 0.028                             | 0.04                              | 0.7                                         |
| DOPG     | 0.35                  | 0.05                   | 6.8                               | 0.008                             | 0.002                             | 4                                           |
| DOPG + FUS LC | 0.49                | 0.12                   | 4.1                               | 0.010                             | 0.004                             | 2.5                                         |

Note, these values are calculated from analysis of the re-calculated \(\chi^{(2)}(\omega)\)\(^2\) using the fitting parameters in **Table S3** using Eq. 2.
Figure S7. CH region SFG spectra of FUS LC at the DOPS lipid interface. (a) SFG spectra for the pristine (black) DOPS monolayer and that in presence of FUS LC (green) in the -CH/-OH stretching region with corresponding fitting curves as smooth lines. Fitted peaks of the (b) OH-stretching region, (c) CH$_2$ symmetric stretching, and (d) CH$_3$ symmetric stretching contributions for the pristine (black) DOPS monolayer and that in presence of FUS LC (green).
Figure S8. TIRF images of pristine SLBs. TIRF microscopy images of pristine (a) DOPG and (b) DOPS SLBs in PBS at pH 7.4 visualized by 0.1 mol% of 18:1 Liss Rhod PE fluorescently labeled lipid. The imaging was done using 561 nm wavelength laser. Scale bar represents 1 μm.
Figure S9. FUS LC-lipid clusters on DOPS bilayers bind more ThT than clusters on DOPG bilayers.

TIRF microscopy images of SLBs (doped with 0.1 mol% of 18:1 Liss Rhod PE) incubated with 10 µM of FUS LC (doped with 0.1 mol% of Cy5 labeled FUS LC) and bound ThT in PBS buffer, pH 7.4. From left to right: lipid, protein, and ThT fluorescence channels, merged protein-lipid and protein-ThT fluorescence images. Top panel is for FUS LC with a DOPG SLB; bottom panel is for FUS LC with a DOPS SLB. The imaging was done at 405 nm, 561 nm, and 641 nm wavelength laser for ThT, lipid, and protein, respectively. Scale bar represents 1 µm.
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