Electronic Supplementary Material (ESI) for Nanoscale.

Bilayer Lipid Membrane Formation on Surface Assemblies with Sparsely Distributed Tethers

Martynas Gavutis[‡a], *Eric Schulze-Niemand*[‡b,c], *Hung-Hsun Lee*[§d], *Bo Liedberg*[d], *Matthias Stein*[+b] *and Ramūnas Valiokas*[+a]

[a] Department of Nanoengineering, Center for Physical Sciences and Technology, Savanorių 231, 02300 Vilnius, Lithuania;

[b] Molecular Simulations and Design Group, Max Planck Institute for Dynamics of Complex Technical System, Magdeburg, Germany;

[c] Institute of Experimental Internal Medicine, Medical Faculty, Otto von Guericke University, 39120 Magdeburg, Germany;

[d] Division of Molecular Physics, Department of Physics, Chemistry and Biology, Linköping University, 581 83 Linköping, Sweden**;**

[§] Present address: Swedish National Forensic Centre, Brigadgatan 13, 587 58 Linköping, Sweden

[‡] Dr. Martynas Gavutis and Dr. Eric Schulze-Niemand contributed equally to this article

[+] Corresponding author: Dr. Ramūnas Valiokas, valiokas@ftmc.lt

[+] Corresponding author for simulations part: Dr. Matthias Stein, matthias.stein@mpi-magdeburg.mpg.de

Supplementary Information

1. Raw QCM-D sensorgrams.

2. Summary of tBLM formation at a positive osmotic pressure (SUVs in hypertonic buffer) with preadsorbed SOPC lipids.

3. Summary of tBLM formation at a neutral osmotic pressure (isotonic solution).

1. Raw QCM-D sensorgrams

Raw QCM-D sensorgrams of tBLM formation on substrates with various surface molar densities (χ) of tethers presented in the Figure 1 and Figure 2 in the article.

The SUV deposition and removal cycles consisted of repetitive injections marked I, II and III, which correspond to HEPES buffer 1M NaCl, SUVs in HEPES buffer 1M NaCl and ethanol solution, respectively. Injection IV is deionized water. Injections V and VI are Hepes bufer 150mM NaCl and SUVs in HEPES buffer 150mM NaCl, respectively.

The first run of tBLM formation was always performed on the substrate, which contained preadsorbed SOPC (see the Methods for details). After the removal of SOPC by ethanol solution, several cycles of SUV deposition and removal injections were performed on the lipid-free surface. Vesicle solution injections in the overlays are color-coded: black, red and green are the first, second and third injections, respectively.

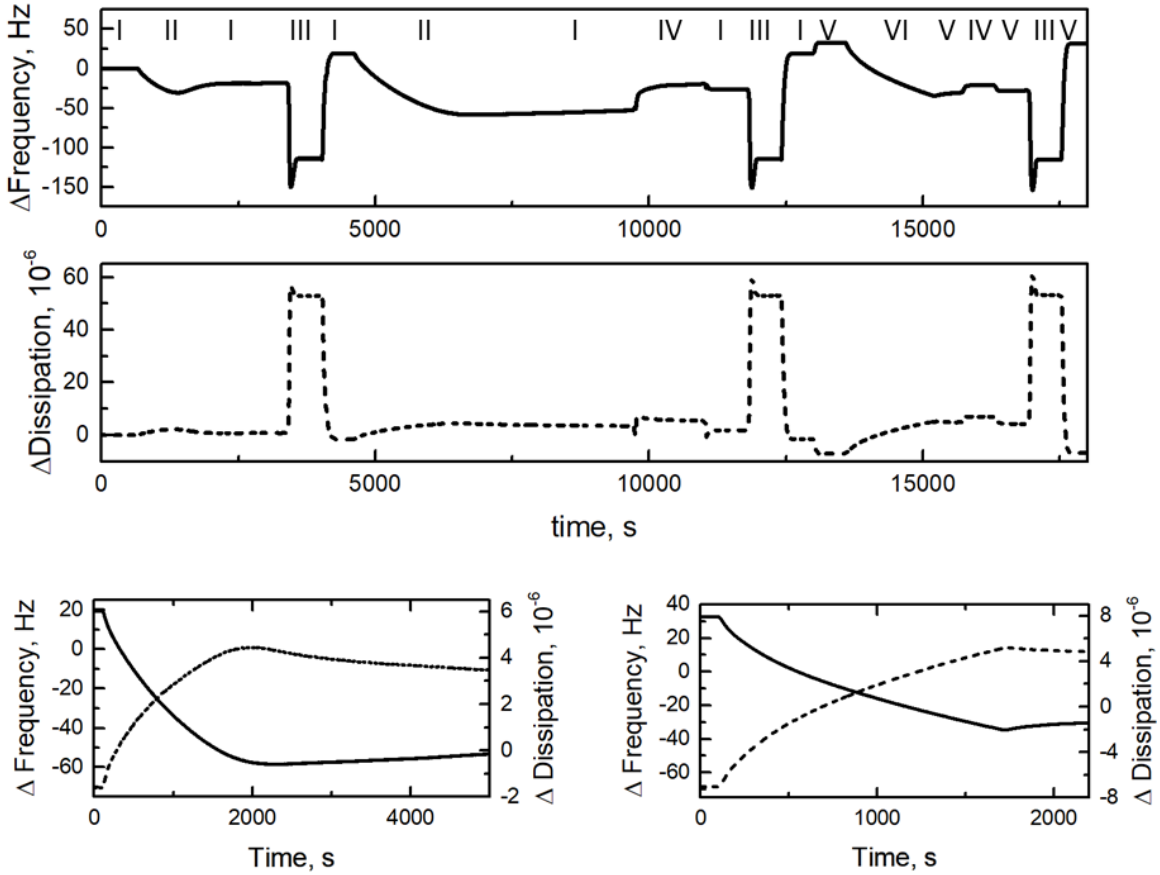

Figure S1.1. QCM-D sensorgrams of SUV solution injection on the tetherless **EG_1H** substrate, i.e.. The injection sequence is marked in roman numerals above the frequency curve. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively. Note the increase in dissipation upon injection of deionized water (injection IV).

Figures S1.2 to S1.7 show the SUV deposition and removal sensorgrams on the randomly distributed EG_6AC_8D tethers:

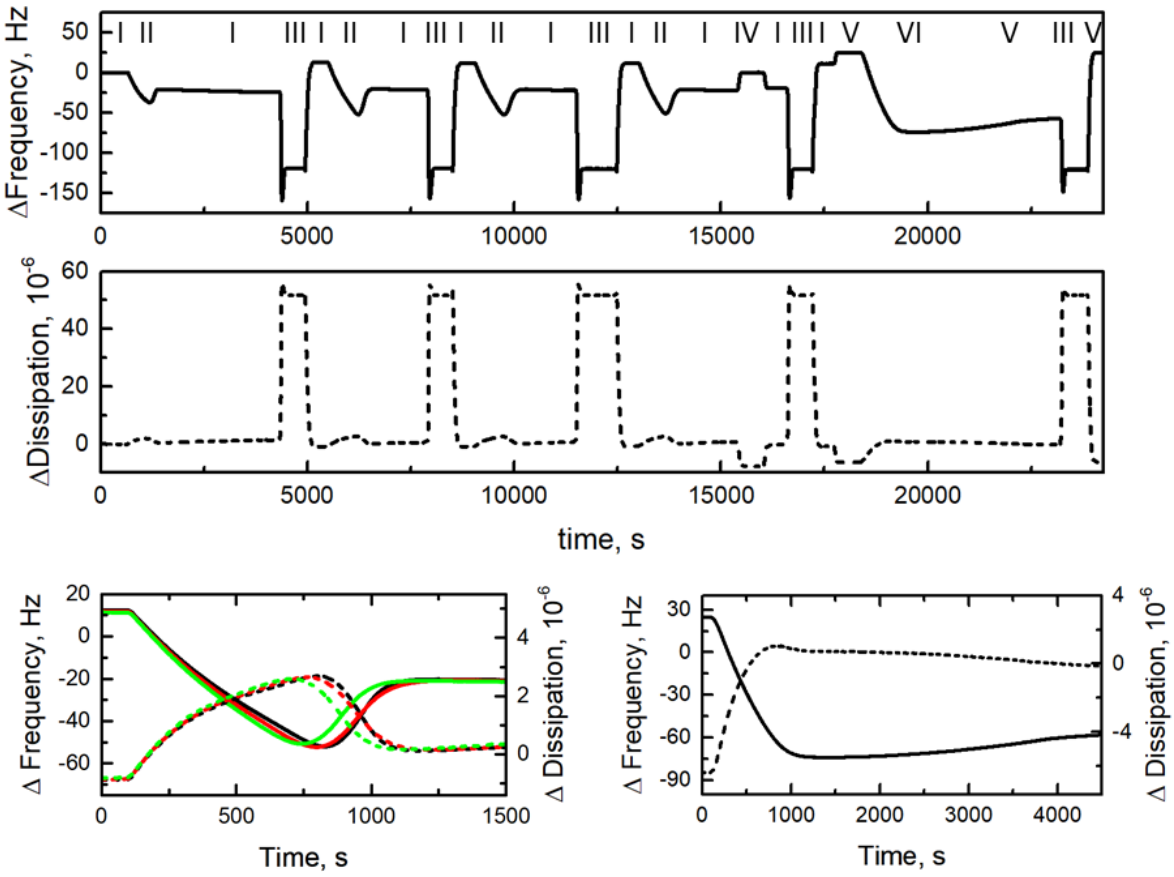

Figure S1.2. QCM-D sensorgrams of SUV solution injection on the **EG_6AC_8D** tethered substrate, i.e. **$\chi_{EG6AC8D}$=(3.6 ± 3.3) mol %**. The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively. Note the decrease in dissipation upon injection of deionized water (injection IV).

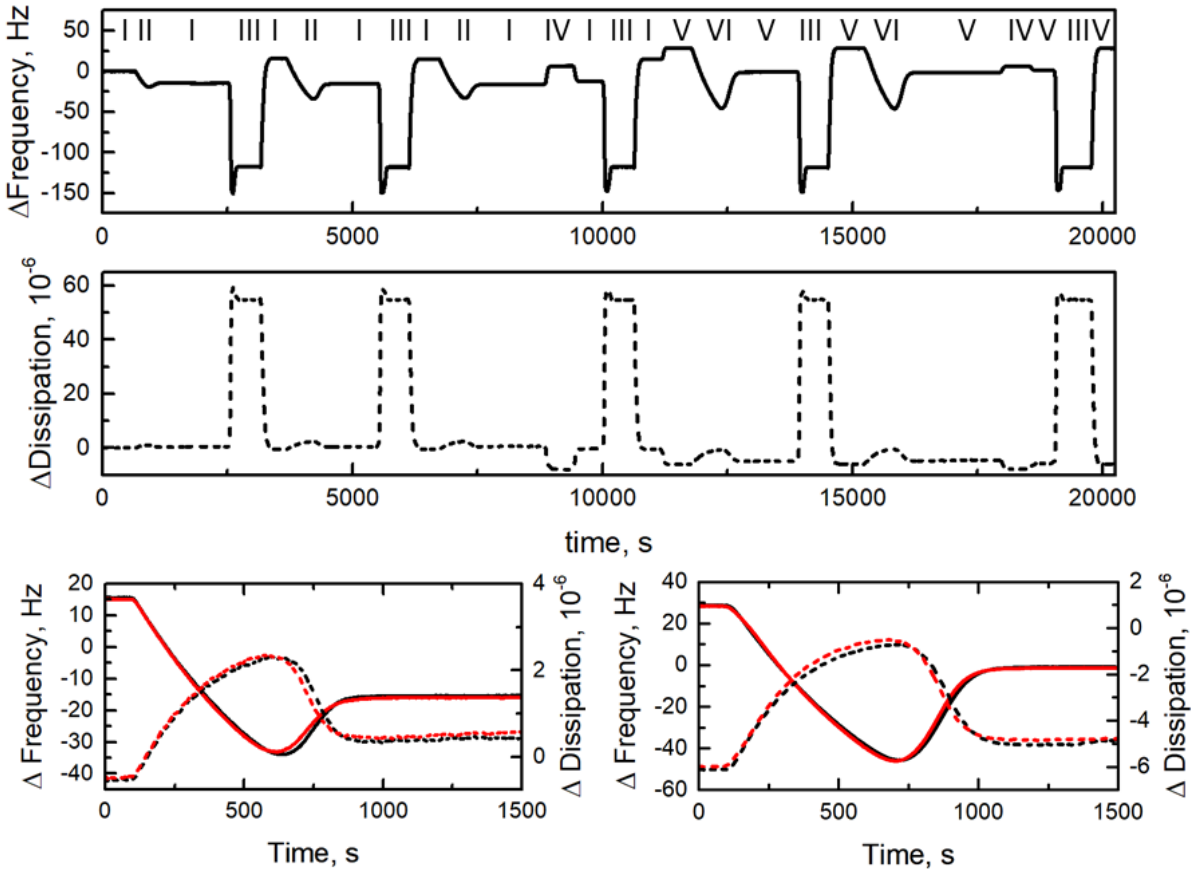

Figure S1.3. QCM-D sensorgrams of SUV solution injection on the **EG_6AC_8D** tethered substrate, i.e. **$\chi_{EG6AC8D}$=(9.7 ± 2.4) mol %.** The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively.

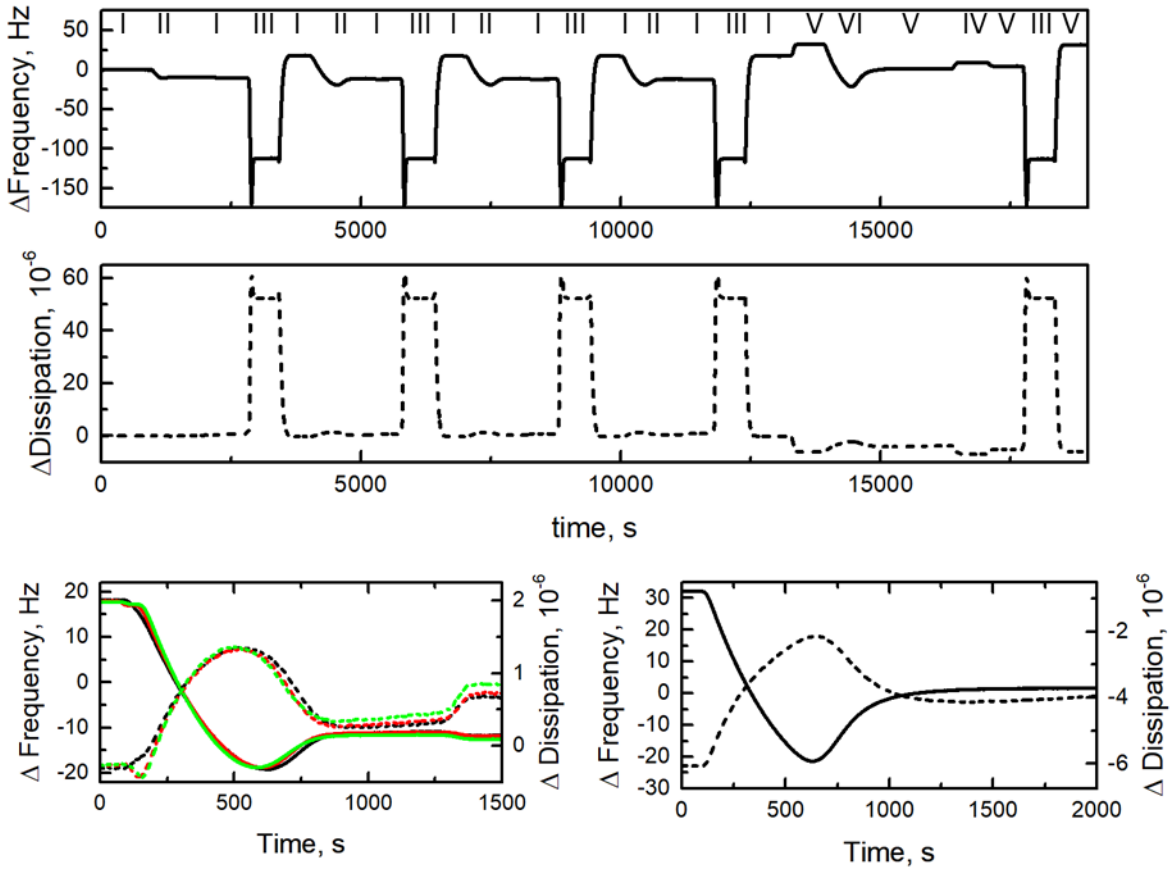

Figure S1.4. QCM-D sensorgrams of SUV solution injection on the **EG_6AC_8D** tethered substrate, i.e. **$\chi_{EG6AC8D}$=(13.3 ± 2.0) mol %.** The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively.

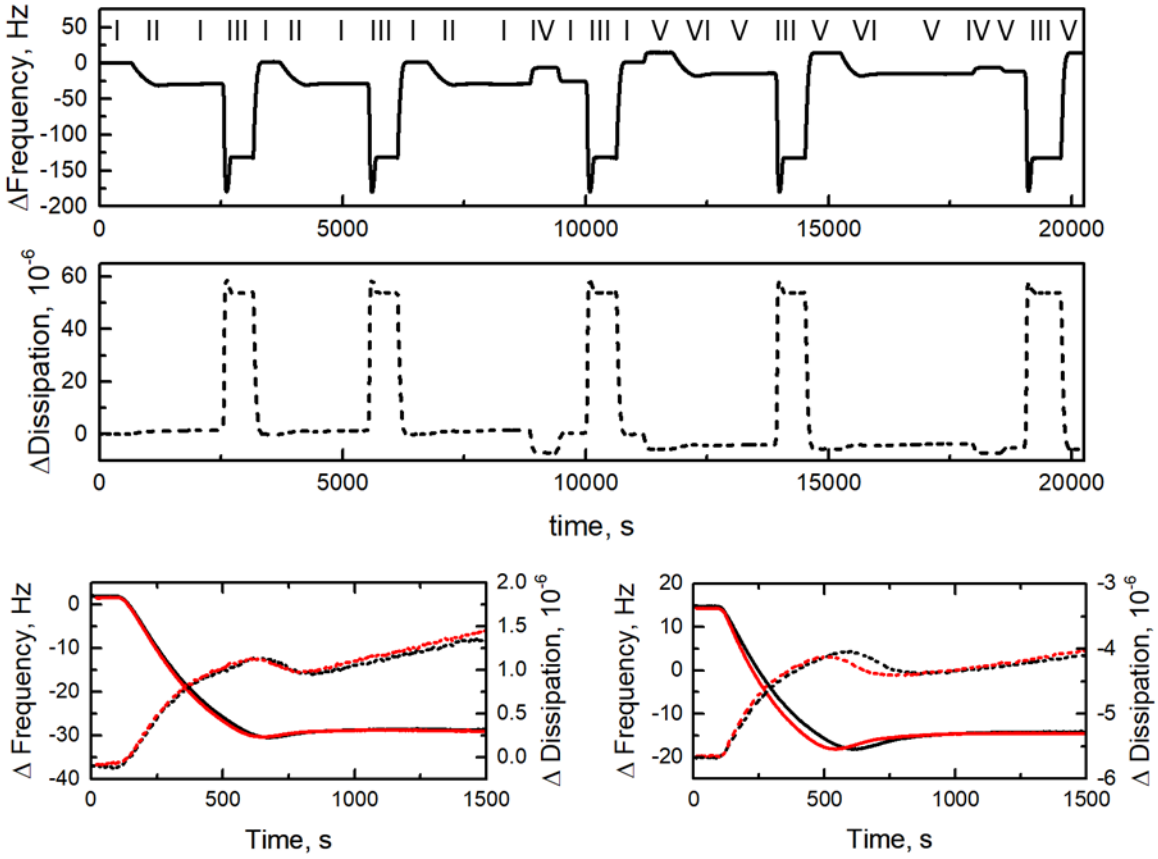

Figure S1.5. QCM-D sensorgrams of SUV solution injection on the **EG_6AC_8D** tethered substrate, i.e. **$\chi_{EG6AC8D}$=(18.1 ± 2.9) mol %.** The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively.

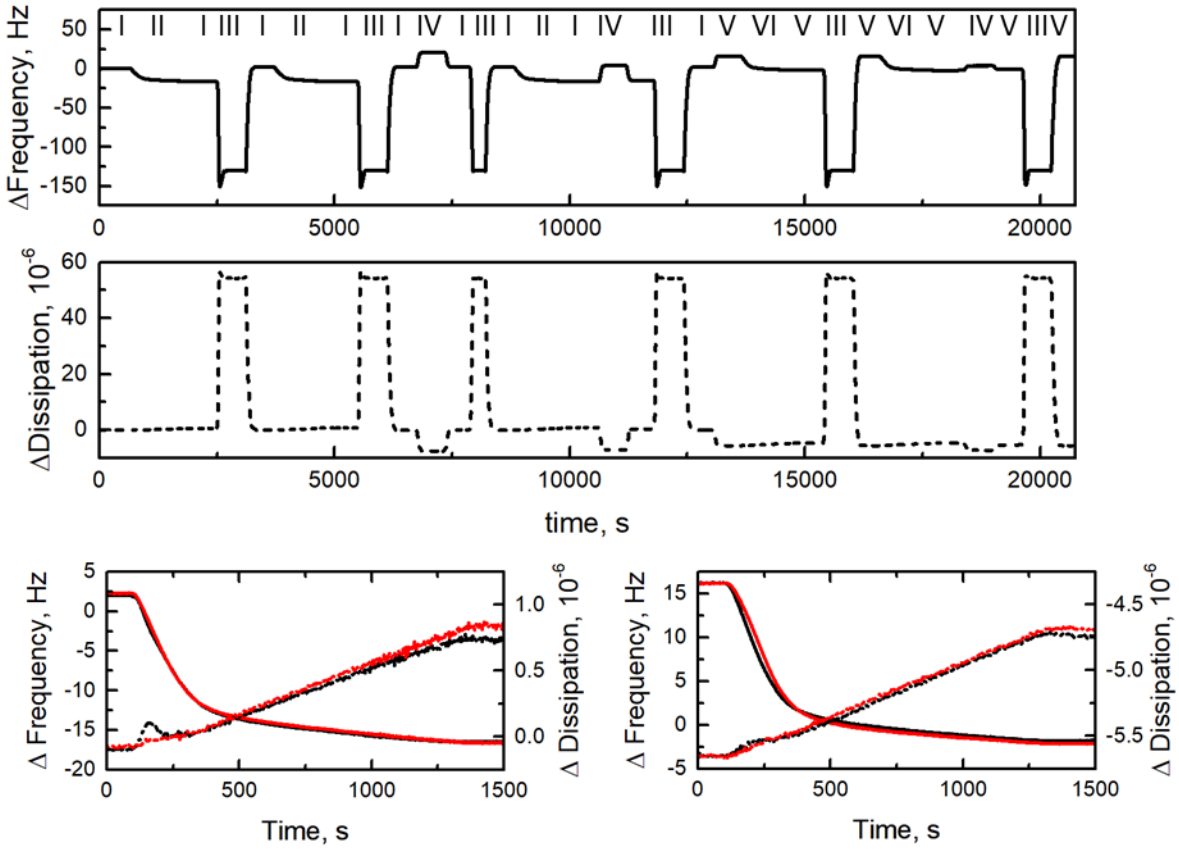

Figure S1.6. QCM-D sensorgrams of SUV solution injection on the **EG_6AC_8D** tethered substrate, i.e. **$\chi_{EG6AC8D}$=(36.7 ± 3.3) mol %.** The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively.

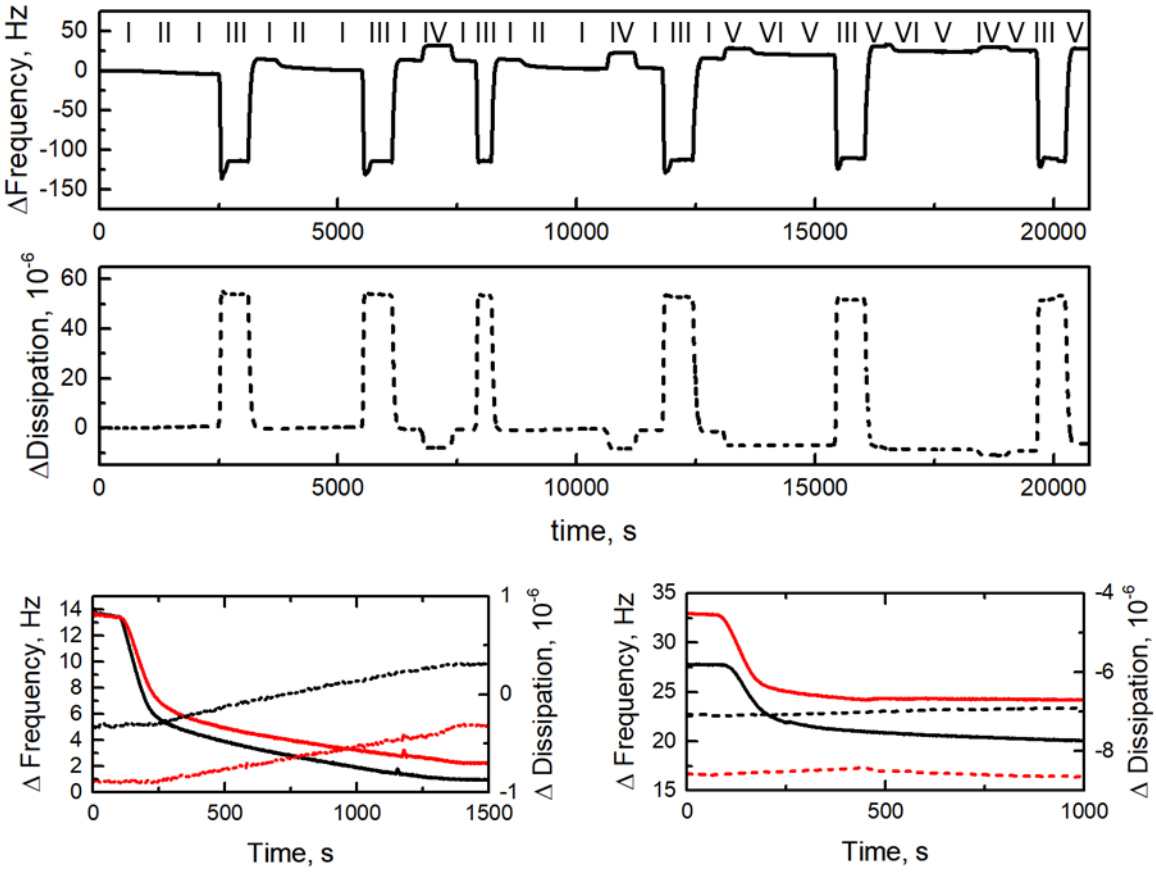

Figure S1.7. QCM-D sensorgrams of SUV solution injection on the **EG_6AC_8D** tethered substrate, i.e. **$\chi_{EG6AC8D}$=100 mol %.** The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively.

The Figures S1.8 to S1.13 show the SUV deposition and removal sensorgrams on the clustered $EG_6AC_{16}D$ tethers:

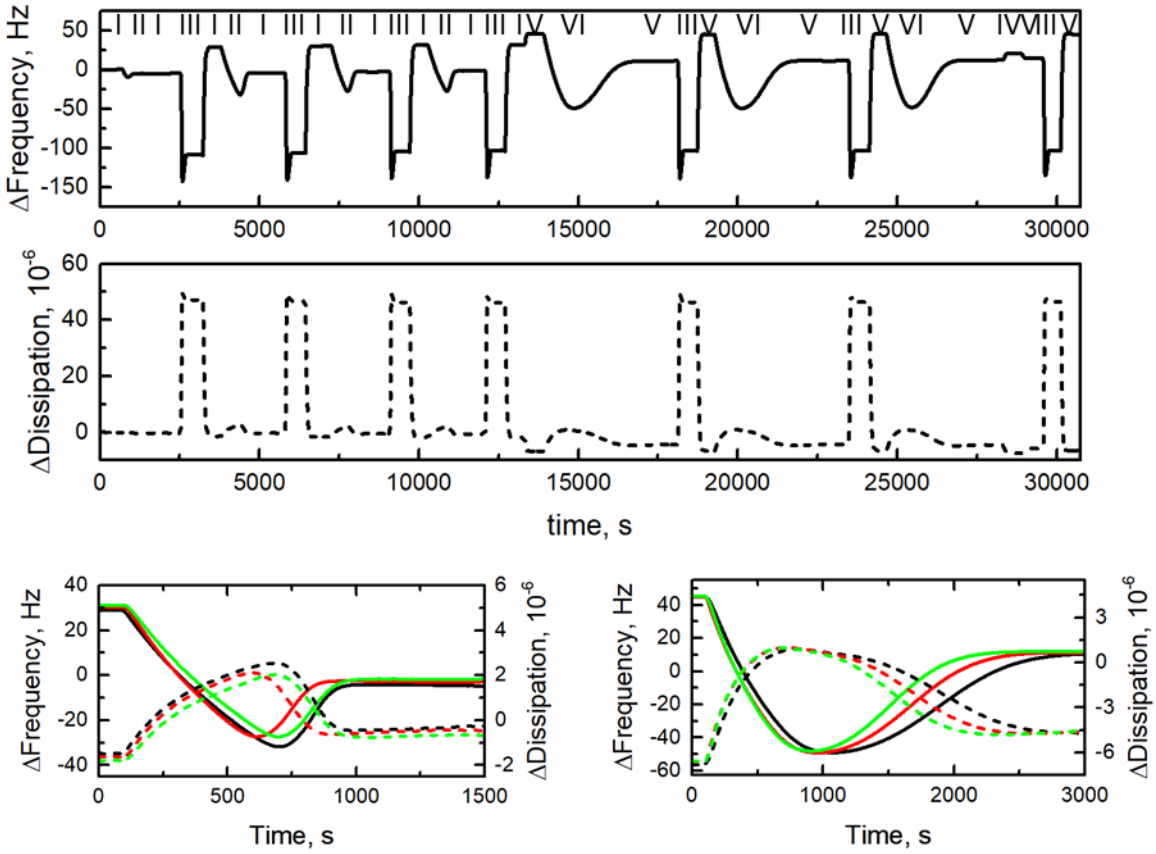

Figure S1.8. QCM-D sensorgrams of SUV solution injection on the **$EG_6AC_{16}D$** tethered substrate, i.e. **$\chi_{EG6AC16D}$=2.9 ± 2.1 mol %.** The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively.

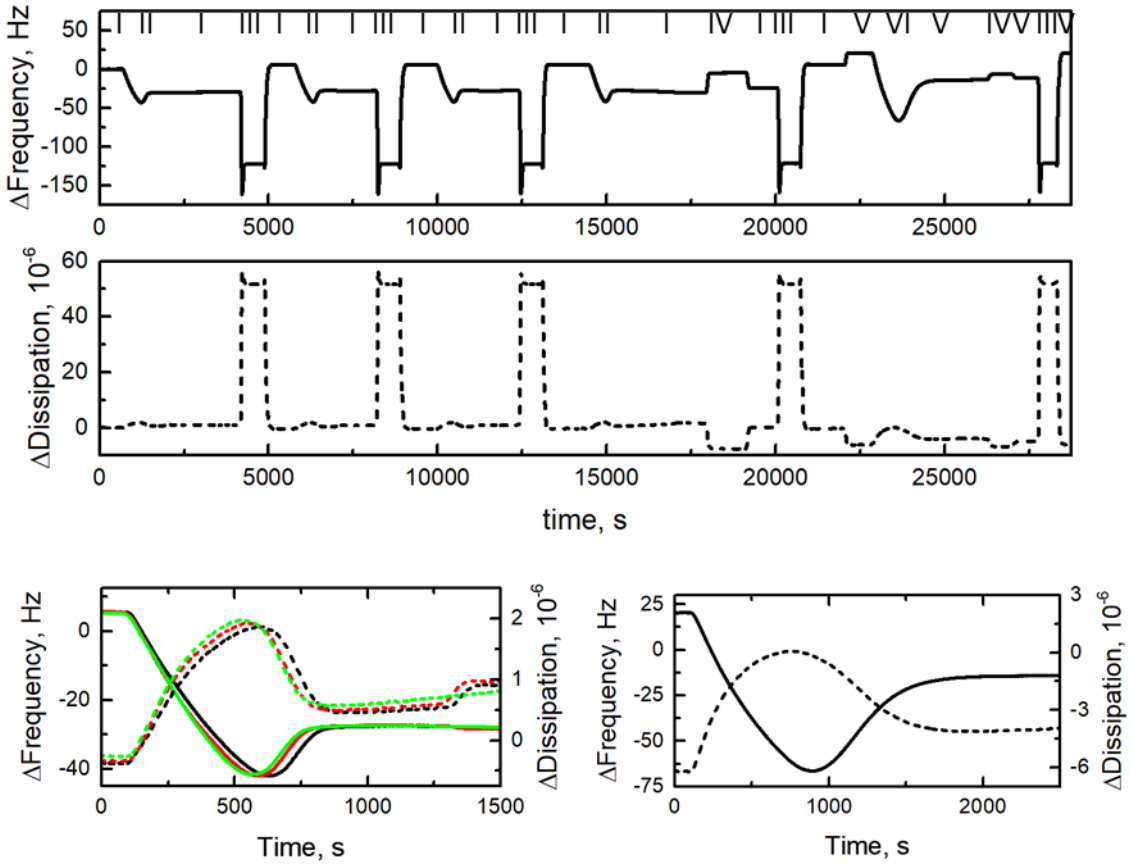

Figure S1.9. QCM-D sensorgrams of SUV solution injection on the **$EG_6AC_{16}D$** tethered substrate, i.e. **$\chi_{EG6AC16D}$=3.5 ± 2.1 mol %.** The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively.

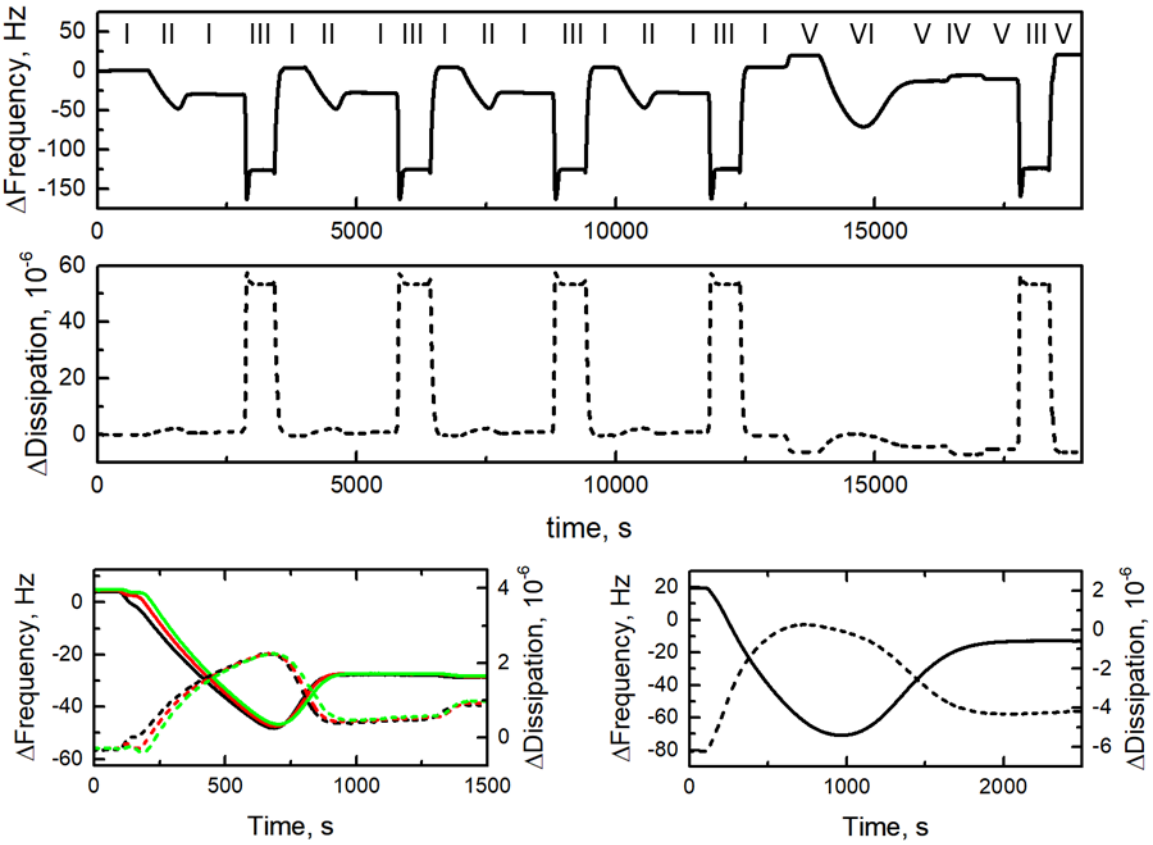

Figure S1.10. QCM-D sensorgrams of SUV solution injection on the **$EG_6AC_{16}D$** tethered substrate, i.e. **$\chi_{EG6AC16D}$=5.6 ± 3.6 mol %.** The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively.

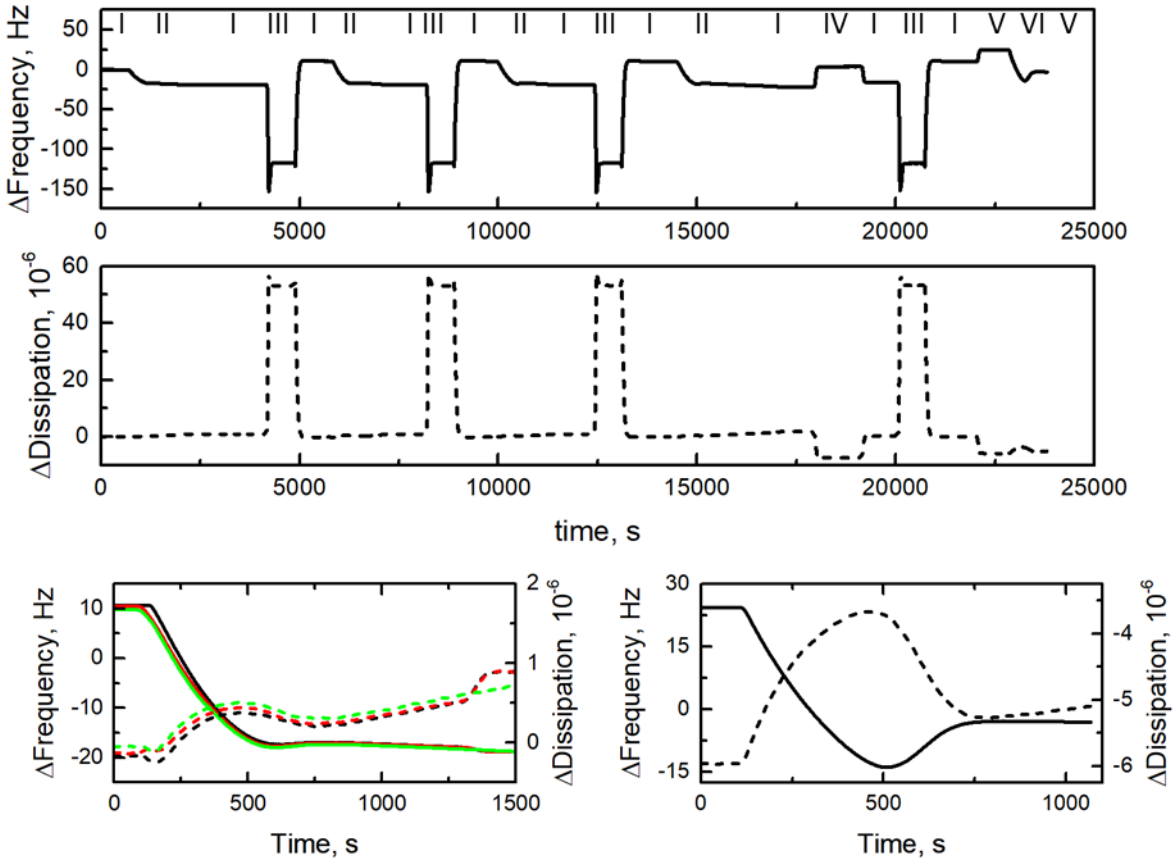

Figure S1.11. QCM-D sensorgrams of SUV solution injection on the **$EG_6AC_{16}D$** tethered substrate, i.e. **$\chi_{EG6AC16D}$=6.2 ± 2.1 mol %.** The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively.

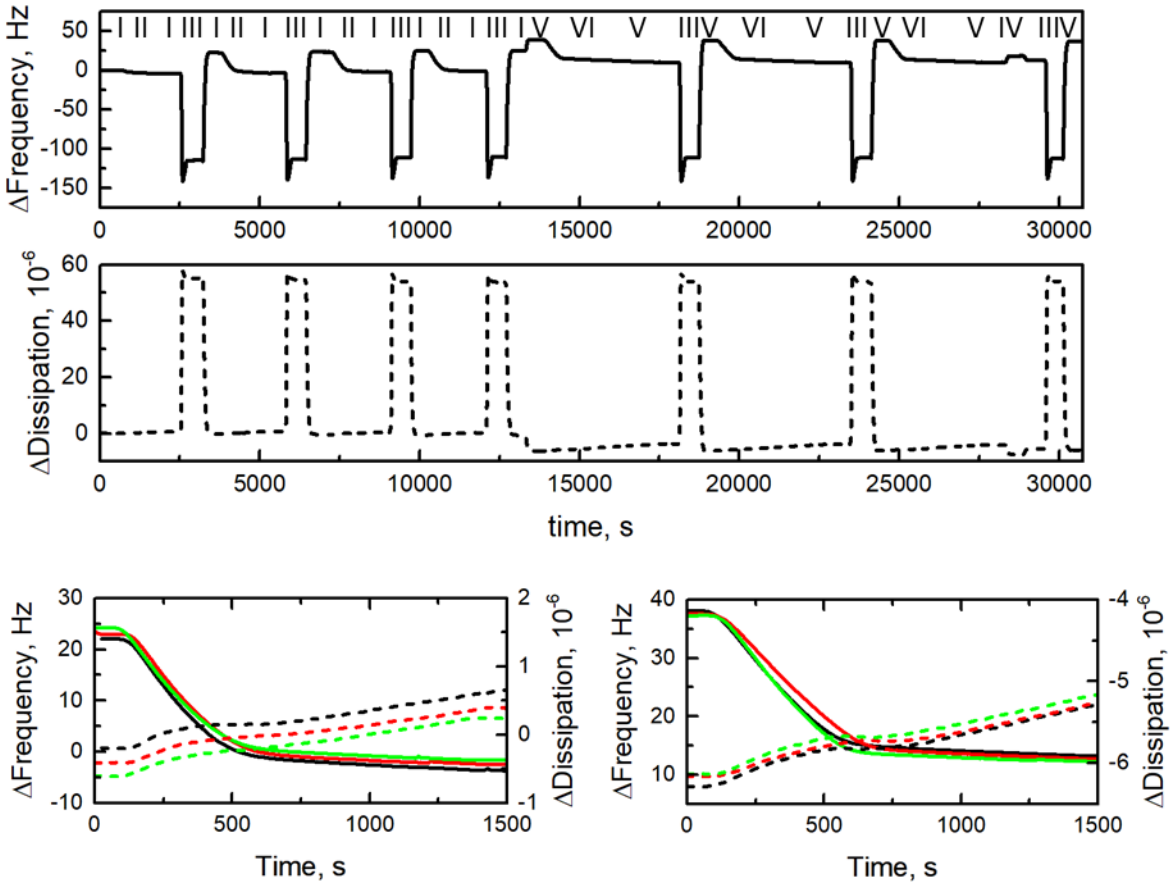

Figure S1.12. QCM-D sensorgrams of SUV solution injection on the **$EG_6AC_{16}D$** tethered substrate, i.e. **$\chi_{EG6AC16D}$=10.0 ± 3.0 mol %.** The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) and neutral (isotonic solution) osmotic pressure are presented in the bottom left and bottom right panels, respectively.

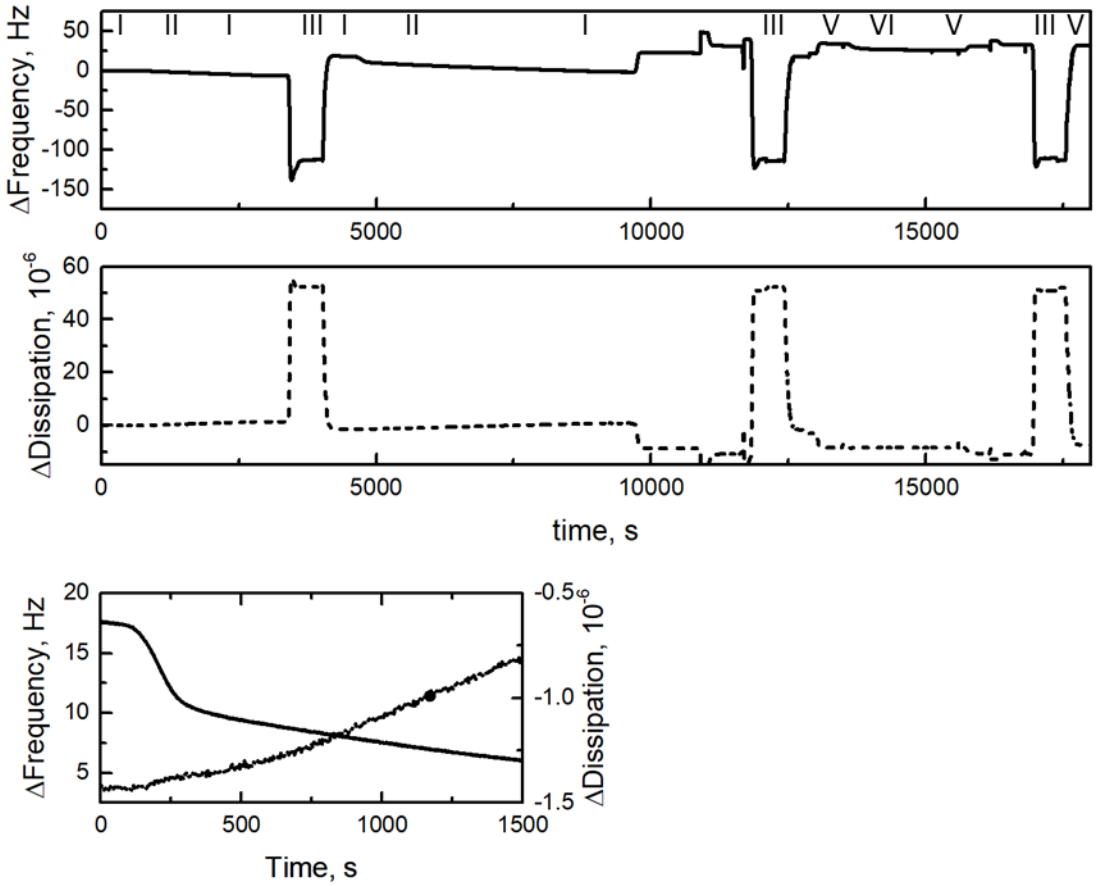

Figure S1.13. QCM-D sensorgrams of SUV solution injection on the **$EG_6AC_{16}D$** tethered substrate, i.e. **$\chi_{EG6AC16D}$=100 mol %.** The injection sequence is marked in Roman numerals above the frequency sensorgram and described above. The overlays of vesicle injection at the positive (SUVs in hypertonic buffer) in the bottom left panel, respectively.

2. Summary of tBLM formation at a positive osmotic pressure on substrates containing preadsorbed SOPC lipids

The panels in the Figure S2 are composed from the raw sensorgrams presented in the Section 1 above.

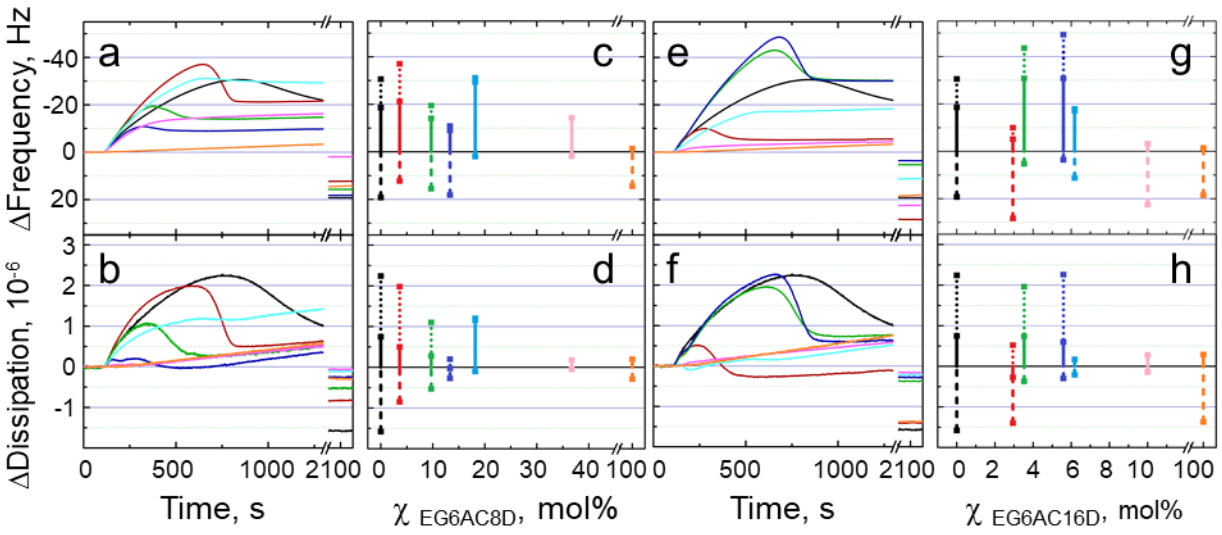

Figure S2. Analysis of tBLM formation on the mixed SAMs containing different surface molar densities of the EG_6AC_8D (a-d) and $EG_6AC_{16}D$ (e-h) tethers. All SAMs were incubated with SOPC lipid solution prior the SOPC SUV injection. The frequency (c, g) and dissipation (d, h) signal amplitude (dotted line), and equilibrium (solid line) values together with the signal values obtained after the EtOH washing step (dashed line) are plotted as function of the tether surface molar density. The color coding of the sensorgrams is the same as in the corresponding bar chart.

3. Summary of tBLM formation at a neutral osmotic pressure

The panels in the Figure S3 are composed from the raw sensorgrams presented in the Section 1 above.

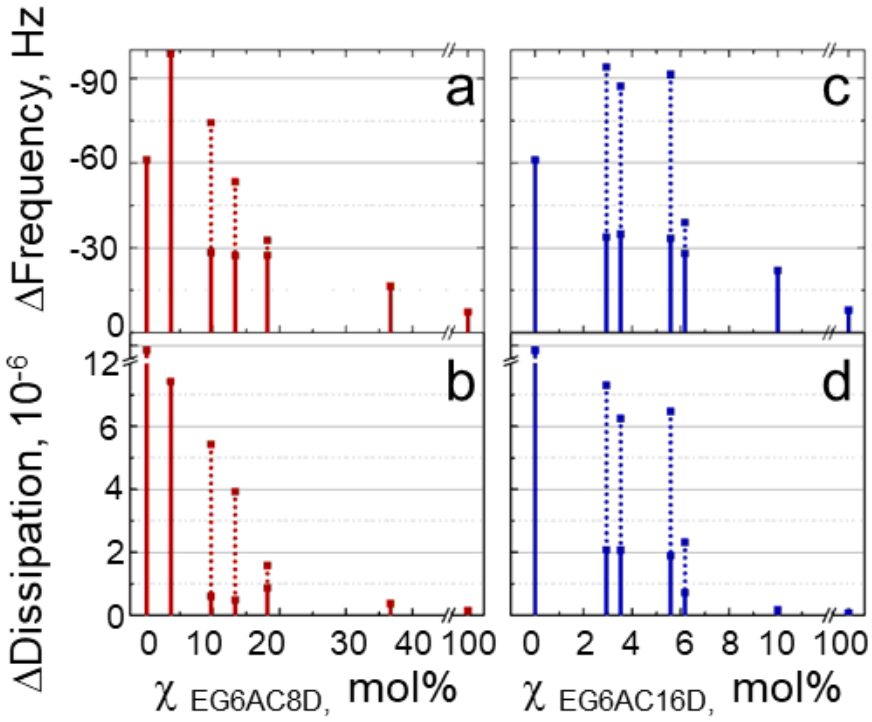

Figure S3. Analysis of lipid tBLM formation at neutral osmotic pressure (isotonic solution) conditions on the EG_6AC_8D (red) and $EG_6AC_{16}D$ (blue) tethered surfaces, respectively. The frequency (a, c) and dissipation (b, d) signal amplitude (dotted line), and equilibrium (solid line) values are plotted as function of the tether surface molar density.