

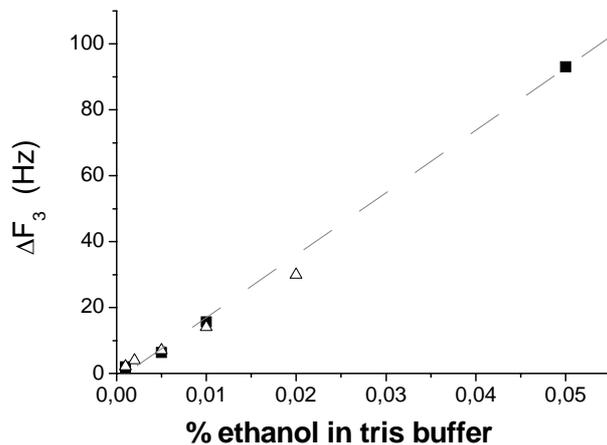
## Supplementary information:

### Effect of ethanol

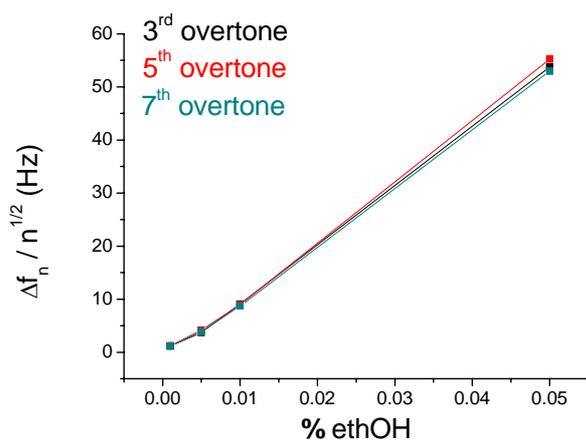
Two sets of experiments were performed. First, mixtures containing different concentrations of ethanol in Tris buffer were introduced in the cell on bare crystals (*i.e.* without supported lipid bilayer (SLB)). On QCM-D data, we observed an immediate increase of the dissipation ( $\Delta D > 0$ ) and a decrease of the frequency ( $\Delta f < 0$ ), with the amplitudes ( $|\Delta f|$  and  $|\Delta D|$ ) depending on the concentration of ethanol in the solution. Figure A displays the frequency shifts recorded at different percentages of ethanol in the buffer (filled squares). The data follow a linear increase, with a  $R^2$  of 0,999. To determine the origin of these changes, we have investigated the results obtained at different overtones (Figure B). The frequency shifts recorded at various overtones are proportional to the square root of the overtone number, which means that the shifts are solely due to changes in media properties (density, viscosity) caused by the introduction of ethanol into the bulk (Zhou, C., et al.. *Langmuir*, 2004. **20**: p. 5870-5878). The same experiments were done on a sensor crystal covered by a SLB, and we observed again an immediate dissipation increase and a frequency decrease. The amplitudes ( $|\Delta f|$  and  $\Delta D$ ) were the same as the ones recorded on the bare sensor crystals. Results for  $|\Delta f|$  are displayed in figure A (open triangles). By exchanging the ethanolized buffer with Tris NaCl buffer again, the initial values for dissipation and frequency were restored. Therefore the observed shifts, induced by the presence of ethanol, are attributed solely to changes of the bulk liquid properties (viscosity and density) and not to modifications of the mechanical properties of the lipid bilayer.

To probe the influence of ethanol concentration on the resistive properties of the bilayers, single-frequency Electrochemical Impedance Spectroscopy (sf-EIS) measurements were done at a frequency where the contribution of bilayers to the impedance signal is most significant (15 kHz in our case – cf. next paragraph). Results are displayed in figure 6, where the arrow indicates when the pure Tris NaCl buffer was replaced by a mixture of ethanol/Tris NaCl. No changes in the value of the impedance modulus or in the phase signal (data not shown) were observed. This implies that the ethanol did not induce any changes in the resistive properties of the bilayers, which would have been the case if the solvent had been disturbing the bilayers.

As a result of these experiments, we conclude that the presence of ethanol in the buffer, which is required to dissolve grD, does not damage a pre-formed POPC lipid bilayer. This finding is an important prerequisite for the validity of the subsequent experiments in this study, and we consider it generally important for developing water-insoluble peptide insertion protocols for various biotechnological applications.

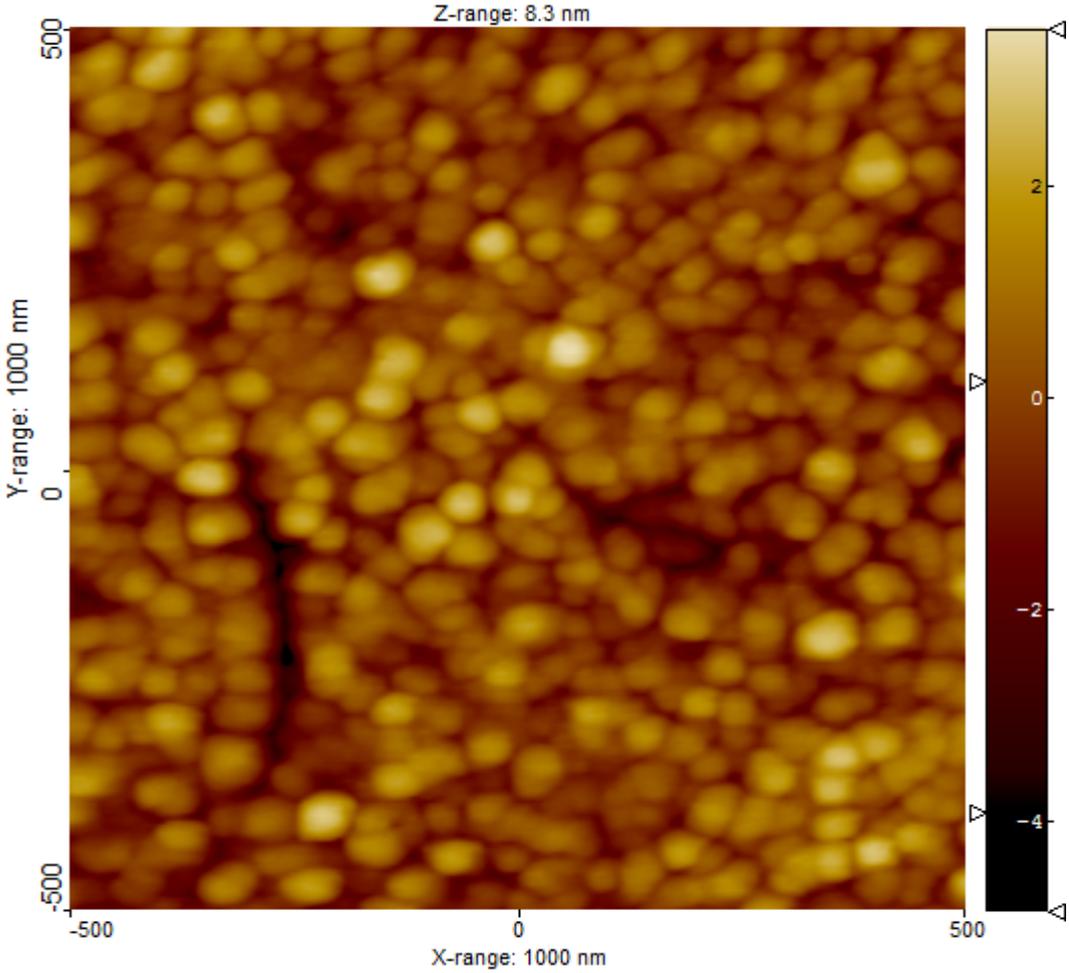


**Figure A:** frequency shifts recorded at the 3<sup>rd</sup> overtone after exposure of a bare crystal (filled squares) and a SLB-covered crystal (open triangles) to different mixtures of ethanol/Tris NaCl buffer. Data are fitted to a linear function (black line).



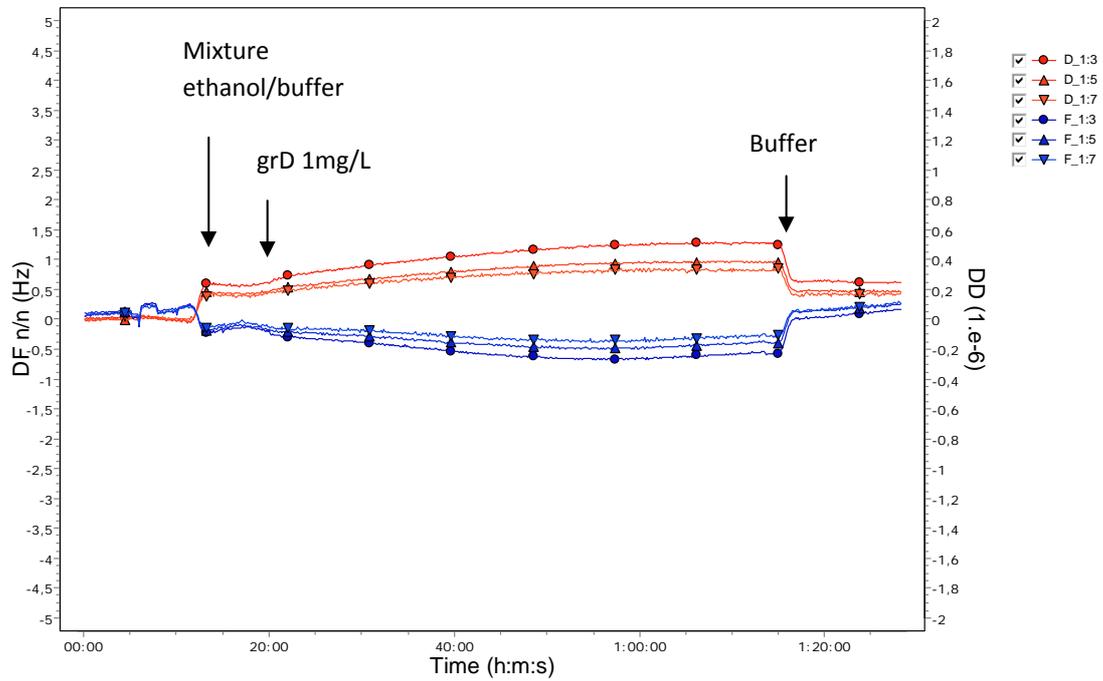
**Figure B:** frequency shifts divided by the square root of the number of overtone versus concentration of ethanol in the buffer for the 3<sup>rd</sup> (black), 5<sup>th</sup> (red) and 7<sup>th</sup> (cyan) overtone as recorded on a bare crystal.

**Topography of SiO<sub>2</sub> coated substrates**



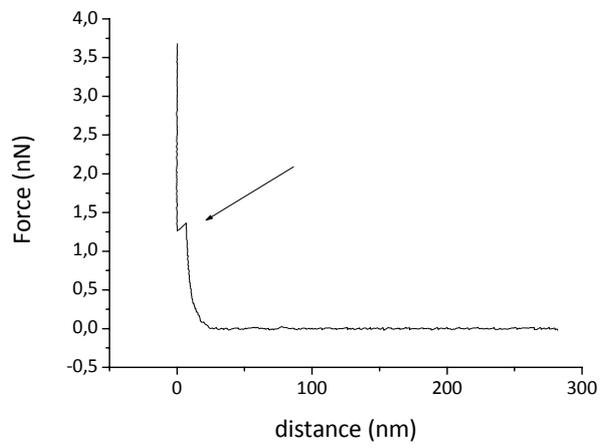
**Figure C:** Topography image of a crystal coated with a 5nm layer SiO<sub>2</sub> by e-beam evaporation. The RMS roughness is about 1 nm. The presence of defects in the layer can be observed.

## Influence of grD at 1 mg/L on QCM-D signal



**Figure D:** normalized  $\Delta f$  and  $\Delta D$  recorded while exposing a SLB to a grD concentration of 1 mg/L.

## Lipid bilayer probing by force spectroscopy



**Figure E:** Characteristic force curve obtained by AFM after bilayer formation. The arrow is pointing to a kink occurring when the AFM tip penetrates through the lipid bilayer.