Ligand Distribution and Lipid Phase Behavior in Phospholipid-Coated Microbubbles and Monolayers

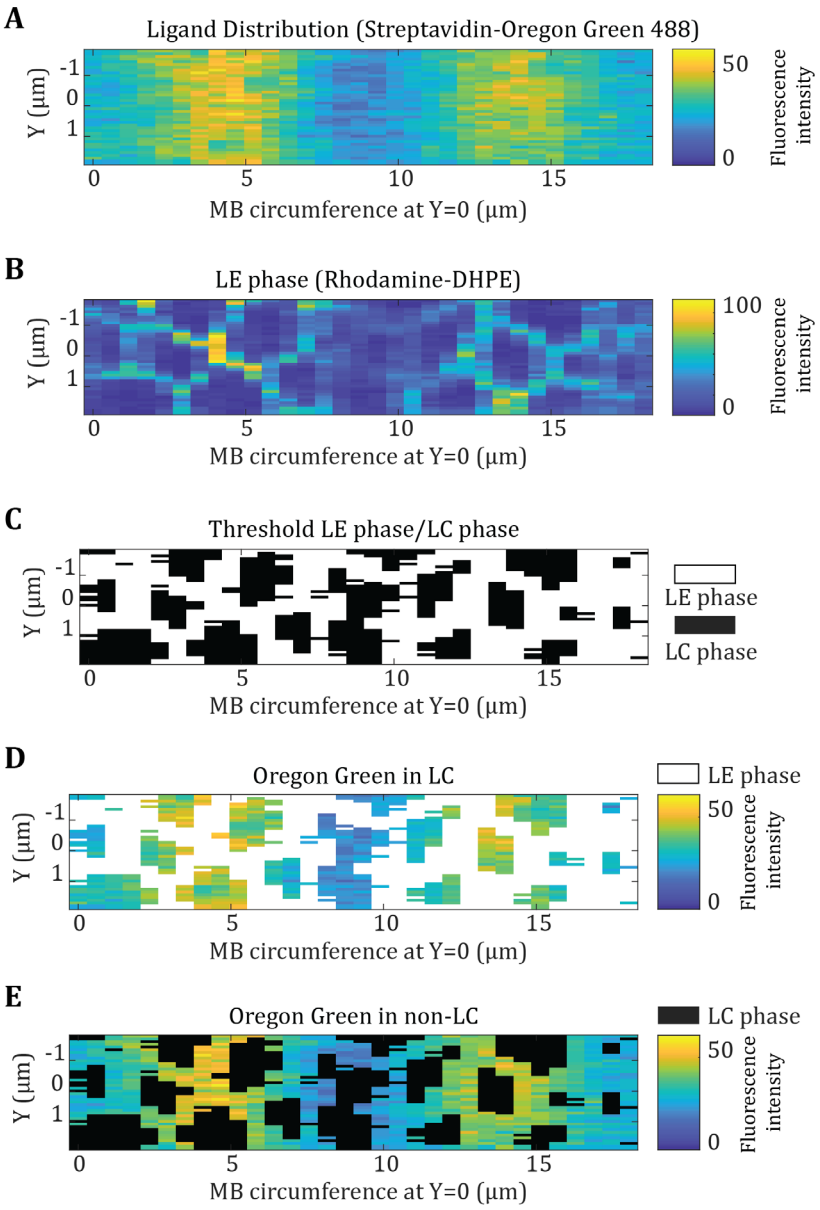
Simone A. G. Langeveld[1], Christian Schwieger[2,3], Inés Beekers[1], Jacob Blaffert[2], Tom van Rooij[1], Alfred Blume[2], Klazina Kooiman[1]

[1] Department of Biomedical Engineering, Thoraxcenter, Erasmus MC, 3000 CA Rotterdam, The Netherlands

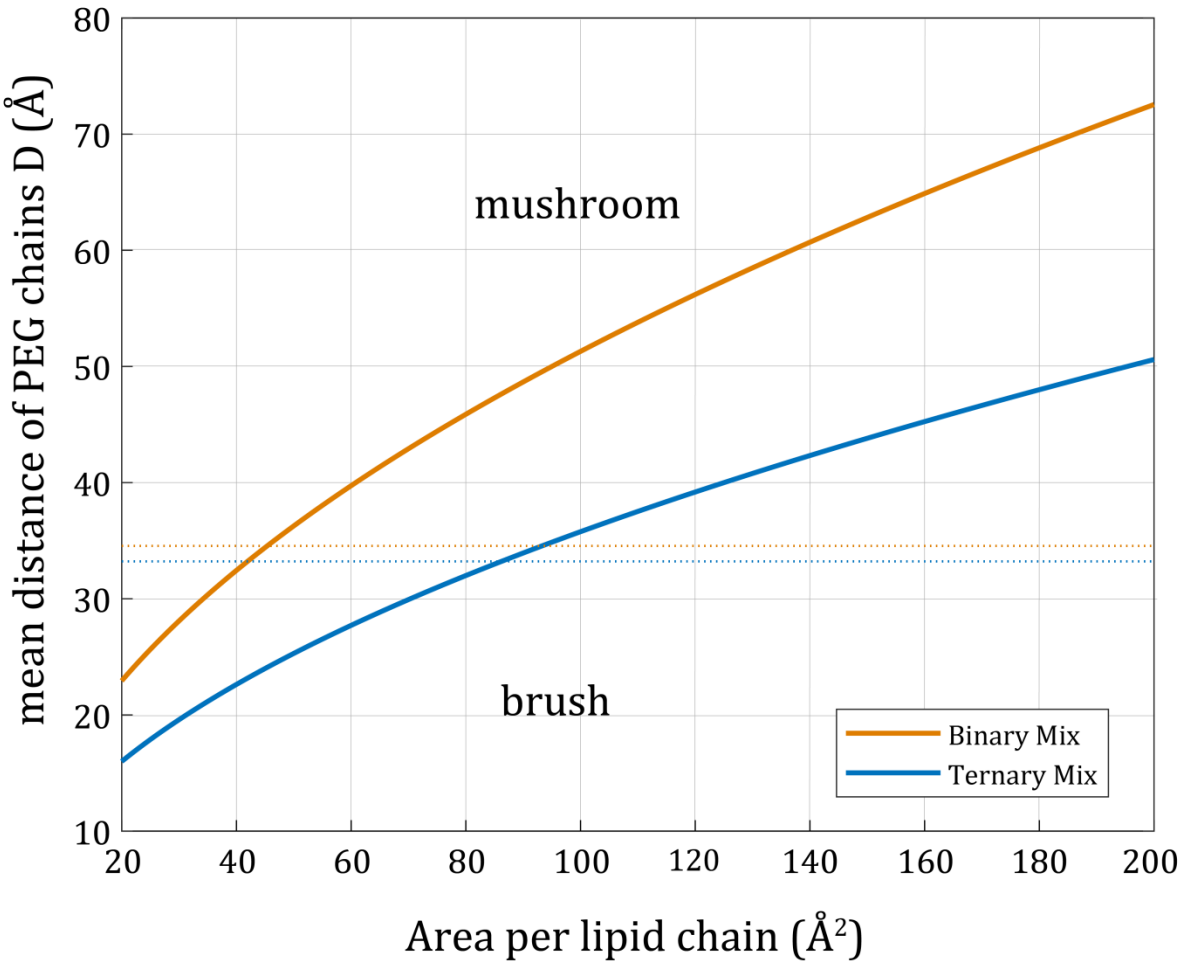
[2] Physical Chemistry, Institute of Chemistry, Martin Luther University Halle-Wittenberg, 06099 Halle (Saale), Germany

[3] Institute for Biochemistry and Biotechnology, Interdisciplinary Research Center HALOmem, Martin Luther University Halle-Wittenberg, Charles Tanford Protein Center, 06120 Halle (Saale), Germany

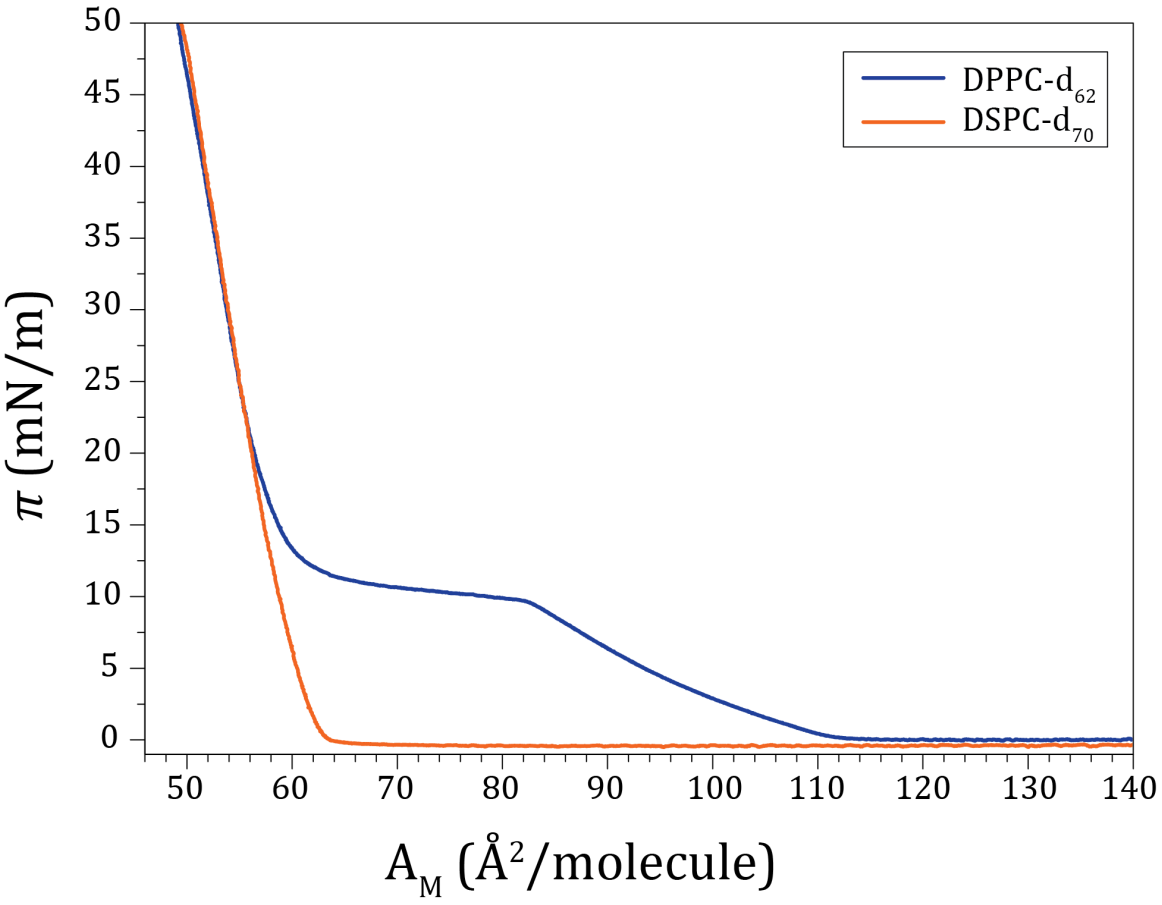
Table of contents



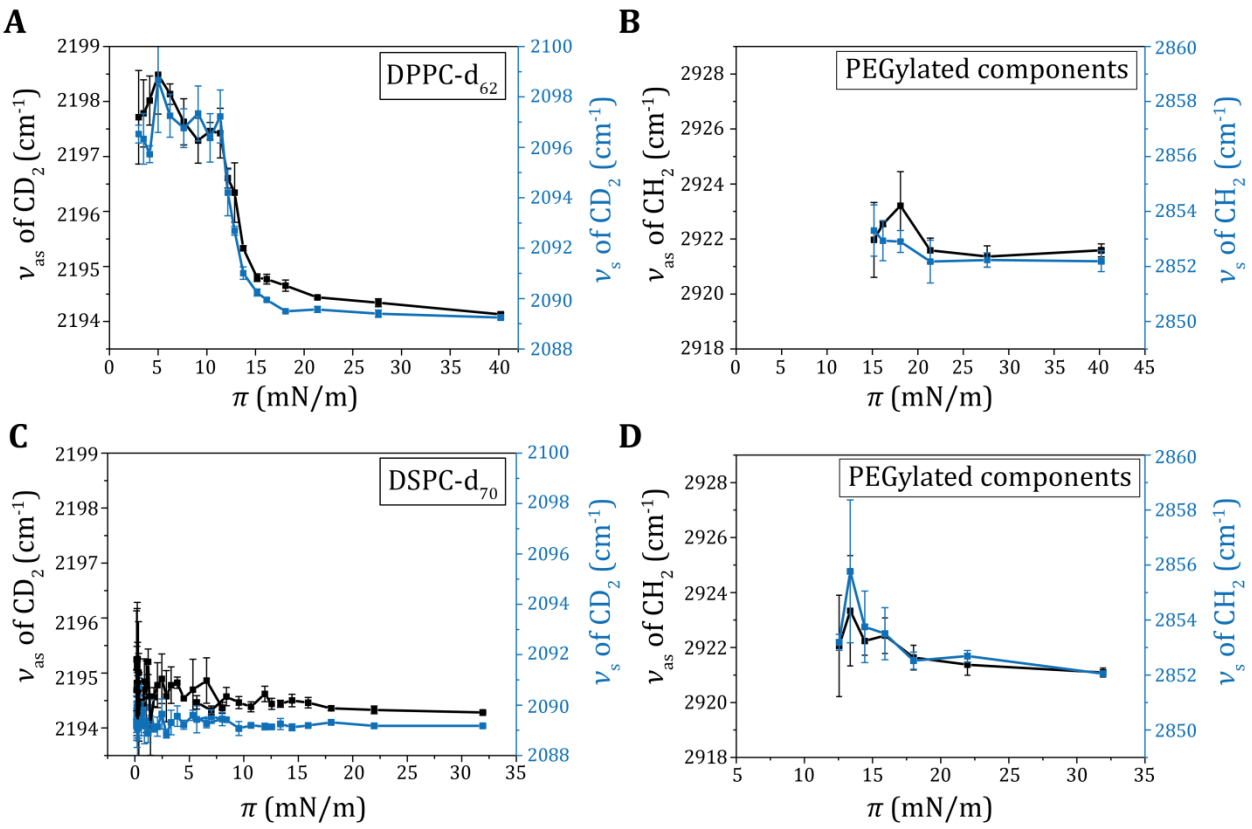
Supplemental Figure 1. Typical example of 2D heatmaps of an indirect DSPC microbubble with a diameter of 5.7 µm. Mean fluorescence pixel intensity (I_{part}) of A) the ligand (Streptavidin-Oregon Green 488) and B) the LE phase (Rhodamine-DHPE). C) Thresholded image with LC phase area in black and inter-domain region in white. Masked image of ligand fluorescence intensity in D) LC phase area (median 34.26) and E) inter-domain region (median 34.13, colocalization ratio 1.00).



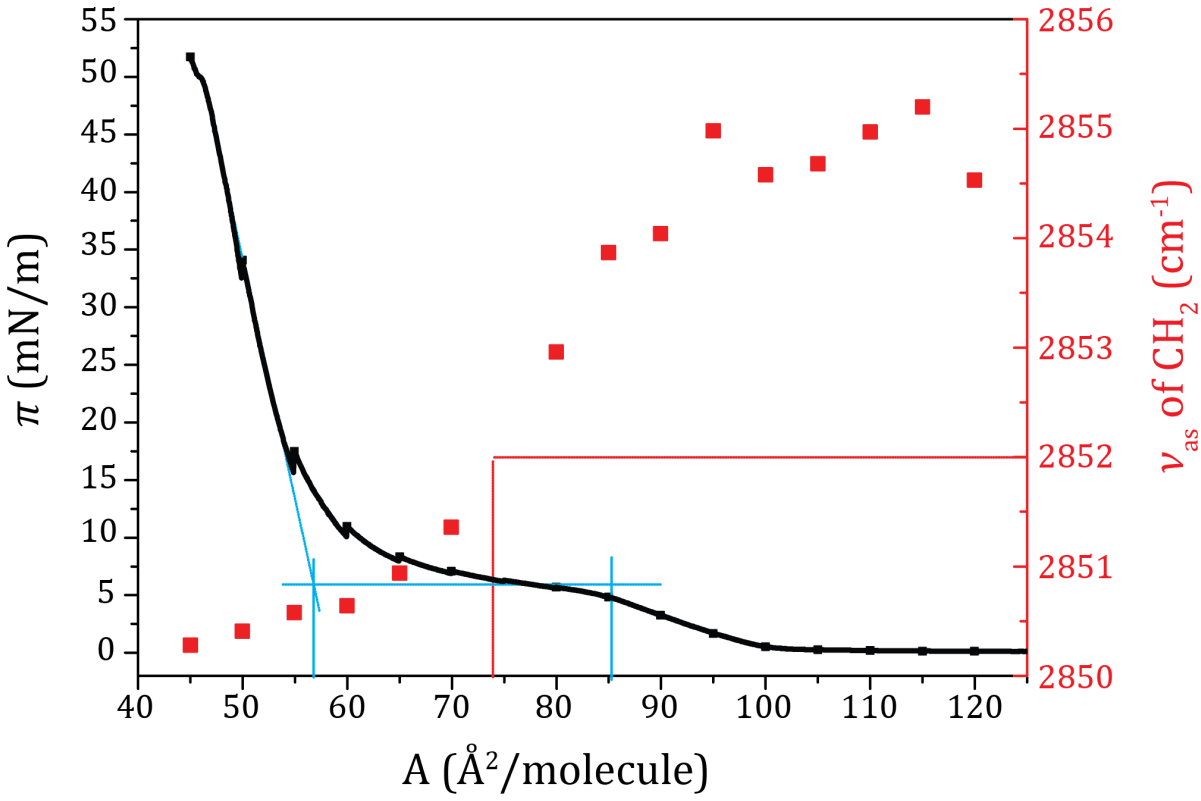
Supplemental Figure 2. Theoretical calculation of the mushroom to brush transition for the binary and ternary mixture. The solid lines are the calculations using Equation 2, $D = \sqrt{\frac{A_{lipid}}{m}}$ from Abou-Saleh et al.[1], where D is the mean distance between PEG chains, A_{lipid} the area occupied per lipid chain, and m the mole fraction of PEG / lipid chain. For the binary mixture (orange solid line), an m of 0.038, i.e. 7.6 mol% PEG / (2*100) lipid chains, was used while this was 0.0781 for the ternary mixture (15 mol% PEG / (2*85 + 2*7 + 1*8) lipid chains) (blue solid line). The dotted lines show the Flory radius calculated from Equation 1, $R_F = aN^{3/5}$ from Abou-Saleh et al.[1], where R_F is the Flory radius of the grafted PEG, a the size of the monomer (=0.35 nm), and N is the number of monomers per chain. For the binary mixture (orange dotted line), an N set to 2000 / 44 (mean molecular weight of the Polymer chain / monomer molecular weight), while for the ternary mixture (blue dotted line), which contains PEG chains of slightly different length a weighted average of N = 44.24 was used (8/15 * 40 + 7/15*(2000/44)). The mushroom to brush transition is predicted where dotted and solid lines intersect, i.e. where the mean PEG chain distance becomes smaller than the Flory radius.



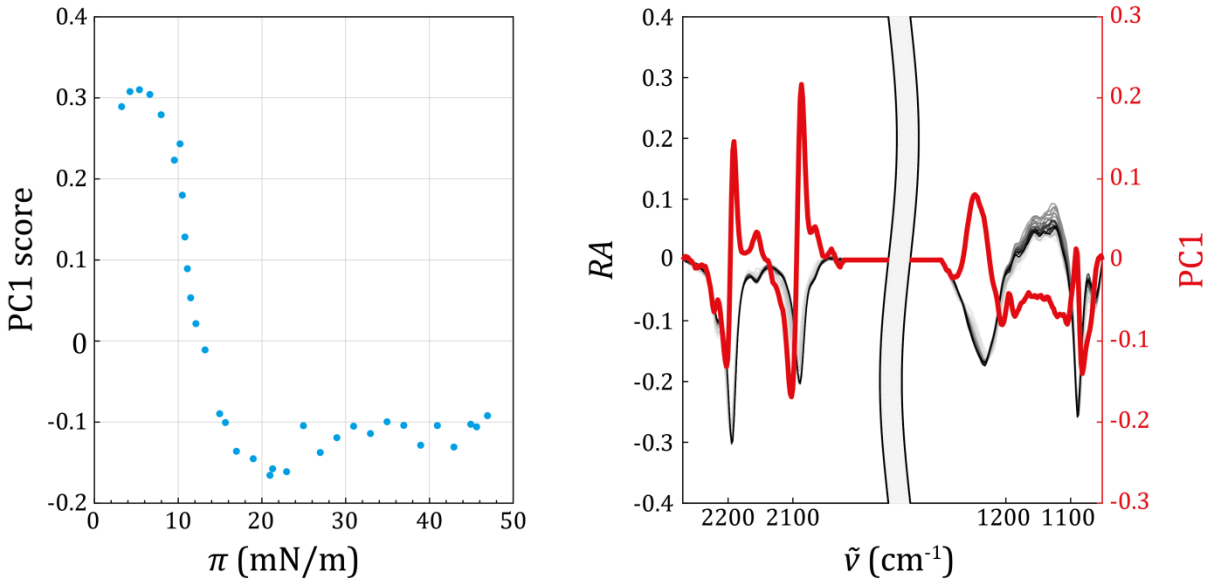
Supplemental Figure 3. Compression isotherms of pure DPPC-d_{62} and DSPC-d_{70} at the air/water interface. Graph of surface pressure (π) as a function of the area per molecule (A_M). Experiments performed at 20 °C.



Supplemental Figure 4. Wavenumbers ($\tilde{\nu}$) of antisymmetric (ν_{as}) and symmetric (ν_s) CD_2 (A, C) and CH_2 (B, D) stretching vibration of the ternary mixtures DPPC-d_{62} / DSPE-PEG2000 / PEG40-stearate (A, B) and DSPC-d_{70} / DSPE-PEG2000 / PEG40-stearate (C, D) (84.8:7.0:8.2 mol%) as function of the surface pressure (π). Note the difference in *x*-axis of 3A, 3B, 3C, and 3D.



Supplemental Figure 5. Compression of a pure DPPC monolayer with simultaneous recording of the compression isotherm (black line, left *y*-axis) and IRRA spectra. Wavenumbers of antisymmetric CH_2 stretching vibrations (ν_{as} of CH_2) are shown as red symbols (right y-axis). The blue perpendicular lines indicate the limits of the LE to LC phase transition plateau. The phase transition pressure is marked with a blue horizontal line at ca. 6 mN/m. The position of ν_{as} at 2852 cm^{-1} (marked with a red line) is indicative for a partly ordered monolayer state with LE and LC phase co-existence, where the majority of the lipids is still in the LE state and the minority in the LC state. The LE/LC ratio as estimated from the position in the transition plateau was approximately 1.5 (or 60 % of the lipids are in LE, 40% are in LC).



Supplemental Figure 6. Principal component analysis of IRRA spectra recorded during the compression of a pure DPPC-d_{62} monolayer. IRRA spectra were simultaneously analyzed in the range of the headgroup vibrations (1050 – 1300 cm^{-1}) and the CD_2 stretching vibrations (2020 – 2270 cm^{-1}) after separate vector normalization in the two respective ranges. The left panel shows the PC1 scores as function of the surface pressure (π). The right panel shows the reflection absorption (*RA*) as a function of the wavenumber (ν) for different surface pressures (low – light grey, to high – dark grey, left *y*-axis), and the PC1 as a function of the wavenumbers ($\tilde{\nu}$) (red line, right *y*-axis).

References

1. Abou-Saleh, R. H.; Swain, M.; Evans, S. D.; Thomson, N. H., Poly(ethylene glycol) Lipid-Shelled Microbubbles: Abundance, Stability, and Mechanical Properties. *Langmuir* **2014,** *30* (19), 5557-5563.