

## SUPPLEMENTARY MATERIAL

### Dexamethasone and Dexamethasone phosphate: Effect on DMPC membrane models.

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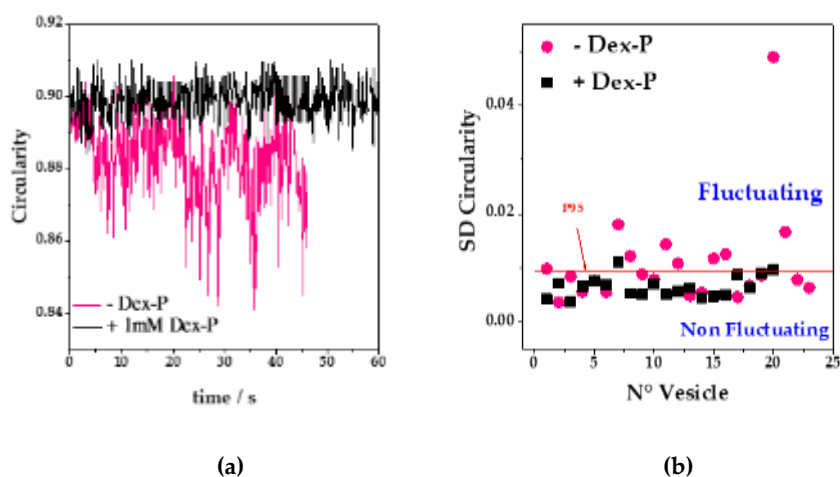
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*Giant Unilamellar vesicles (GUVs), fluctuation analysis.*

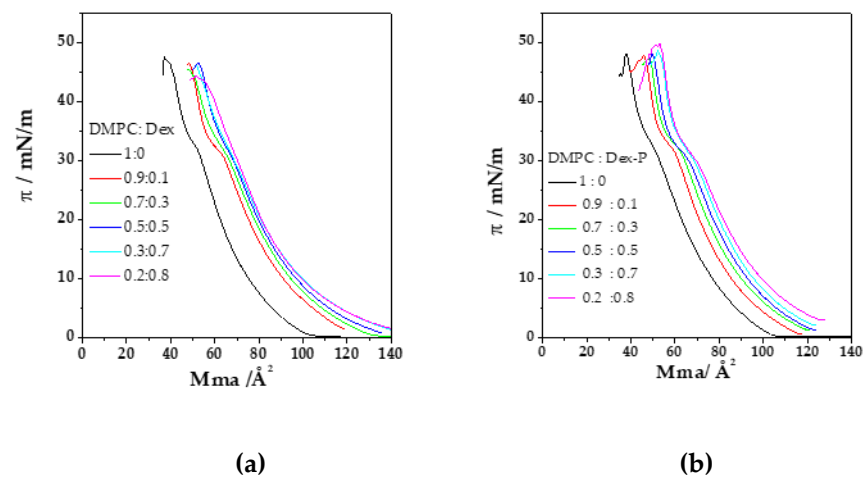


**Figure S1:** (a) Demonstrative image of Circularity a function of the time for a fluctuating (pink) and non- fluctuating (black) vesicle. (b) Standard deviation of  $\bar{C}$  ( $SD\bar{C}$ ) as function of vesicle number. In absence (pink) and presence (black) of Dex-P 1 mM. The straight line corresponds to the P95<sup>th</sup> value used to classify the vesicles in fluctuating or non-fluctuating.

Condition	P(95) G1	
- Dex-P	0.018	
+ Dex-P	0.0087	Less fluctuating population
-Dex	0.01041	Less fluctuating population
+ Dex	0.01276	

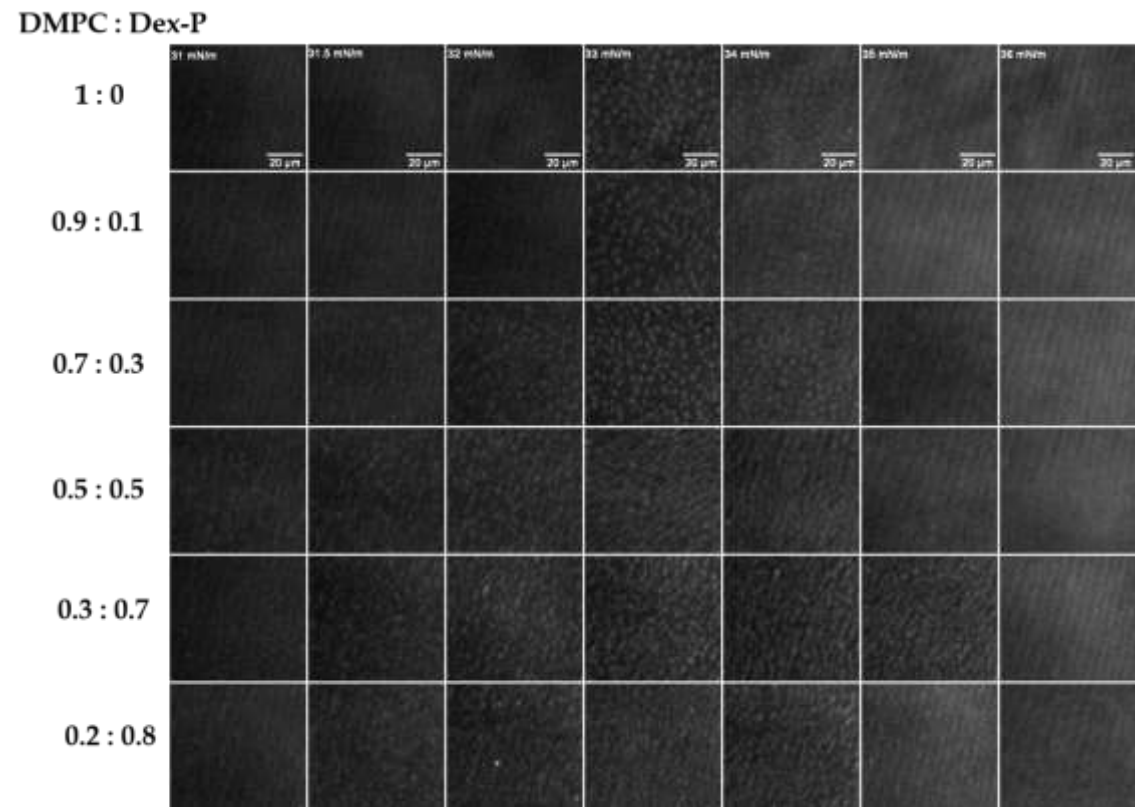
**Table S1:** Calculated P95th for each condition. The lower value of P95 correspond to the less fluctuating population and is the limit above which the vesicle is consider fluctuating.

Langmuir Isotherm:



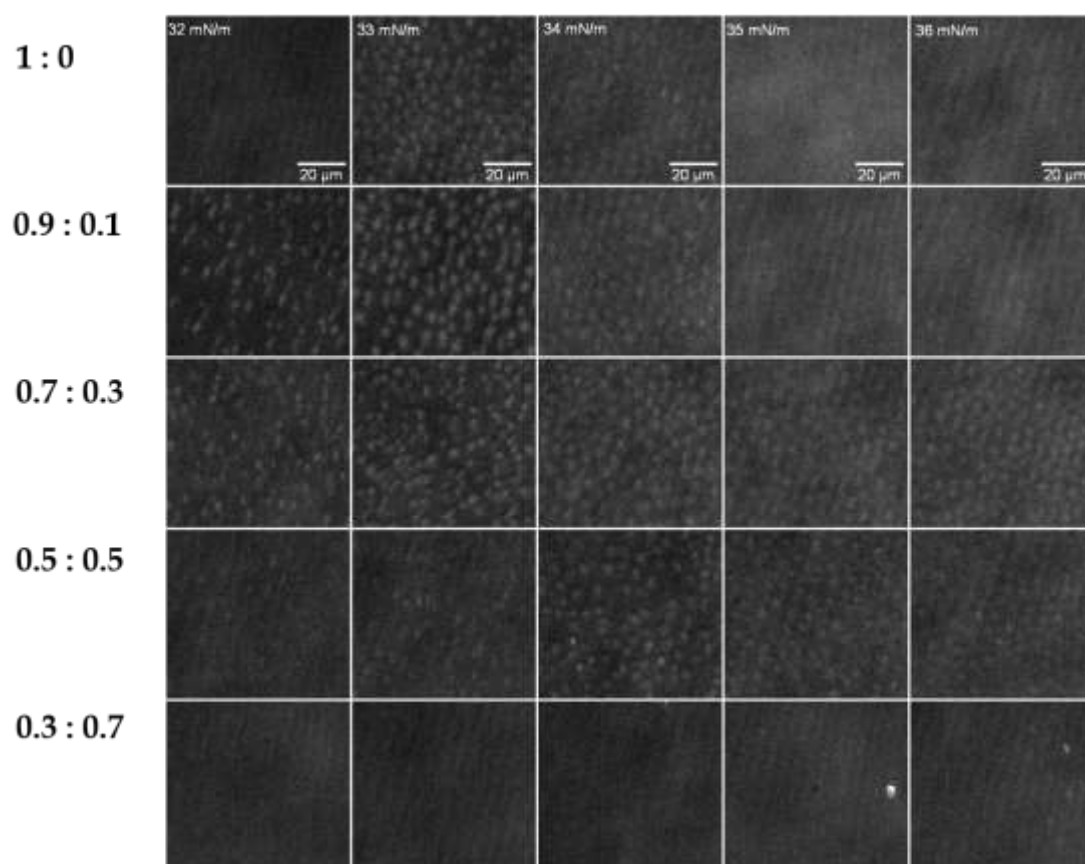
**Figure S2:** Surface pressure – lipid average molecular area compression isotherm for different mixtures DMPC: X were X: (a) Dex and (b) Dex-P. Inset: Compressibility coefficient as function of the surface pressure. DMPC : X molar fraction; 1: 0 (black), 0.9:0.10(red), 0.7:0.3 (green), 0.5:0.5 (blue), 0.30:0.7 (cyan) and 0.2:0.8 (magenta). **Experimental conditions:** 150 mM NaCl, Compression rate 5 mm/min, T: 15° C.

Brewster Angle Microscopy:

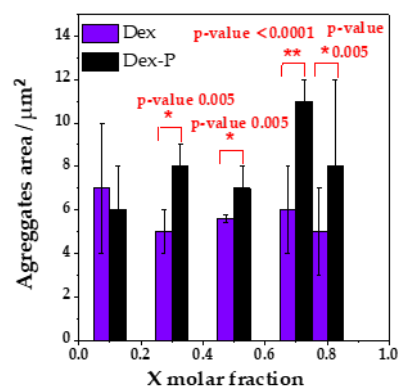


**Figure S3:** Brewster angle images at different surface pressures for DMP: Dex-P, the images correspond to the Langmuir isotherm shown in Figure 2b. For a better visualization the images brightness were adjusted from 0-255 to 8-60.

## DMPC : Dex



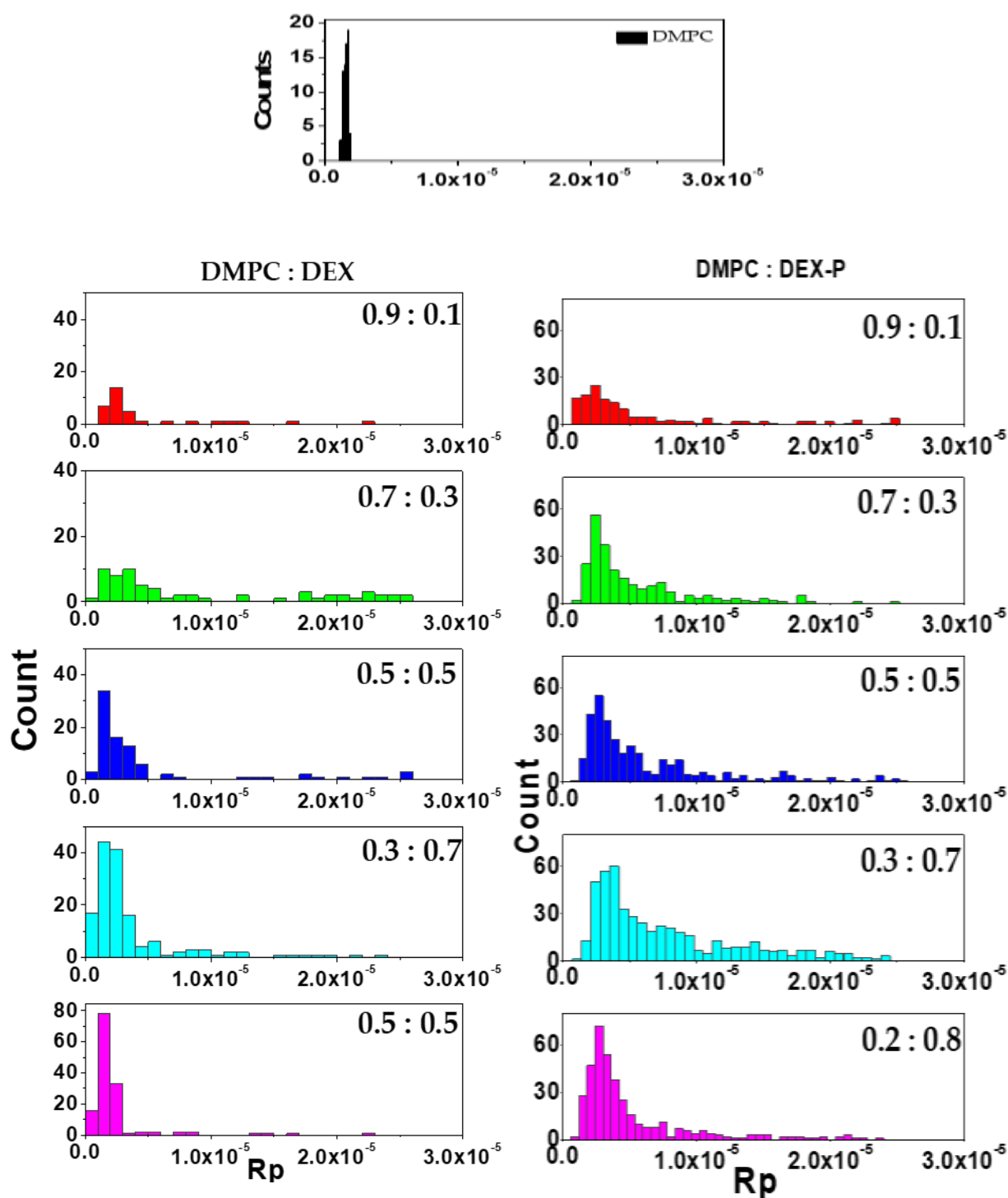
**Figure S4:** Brewster angle images at different surface pressures for DMPC: Dex, the images correspond to the Langmuir isotherm shown in Figure 2a. For a better visualization the images brightness were adjusted from 0-255 to 8-60.



**Figure S5:** Aggregates area ( $\mu\text{m}^2$ ) as function of the drug molar fraction X (X: Dex or Dex-P). The values were obtained from BAM images shown in Fig 3a-b, as an average of the different pressures (isn't an aggregate size tendency on pressure), three images for pressure and two independent experiments at each molar fraction. An independent t-test was done at fixed molar fraction to evaluate if there is a significant difference between the aggregates area obtained for Dex and Dex-P. The significance level for the test was  $\alpha = 0.05$ . \*, \*\* there is a significative difference between Dex and Dex-P aggregate area.

Dex molar fraction	Pearson coefficient	p-value	Dex-P molar fraction	Pearson coefficient	p-value
0.1	-0.15	0.32	0.1	-0.22	0.22
0.3	0.19	0.21	0.3	-0.15	0.23
0.5	0.11	0.09	0.5	0.08	0.49
0.7	0.10	0.09	0.7	0.12	0.16
0.8	0.02	0.72	0.8	0.13	0.09

**Table S2:** Pearson coefficient and associated p-value, for the correlation analysis of aggregate reflectivity vs lateral surface pressure, at all drug molar fraction for Dex and Dex-P. The significance level for the test was  $\alpha = 0.05$ . In all cases p-value  $> \alpha$ . There is not statical sufficient evidence to reject the non-linear association between  $R_p$  and the lateral pressure.



**Figure S6:** Absolute frequency a function of the reflectivity, for the aggregates present on BAM images. (a) DMPC  $R_p$  in the FC phase; DMPC:X for X: (b) DEX and (c) DEX-P. From top to down increasing X molar fraction. Bin size  $0.7 \times 10^{-7}$ . The  $R_p$  value is independent of the surface pressure. The values were obtained from BAM images shown in Fig 4a-b. **Experimental conditions:** 150 mM NaCl, scan rate 5 mm/min, T: 15° C.