

## Supplementary Information: Design of vesicle prototissues as a model for cellular tissues

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### 1 Size distribution of vesicles obtained by electroformation

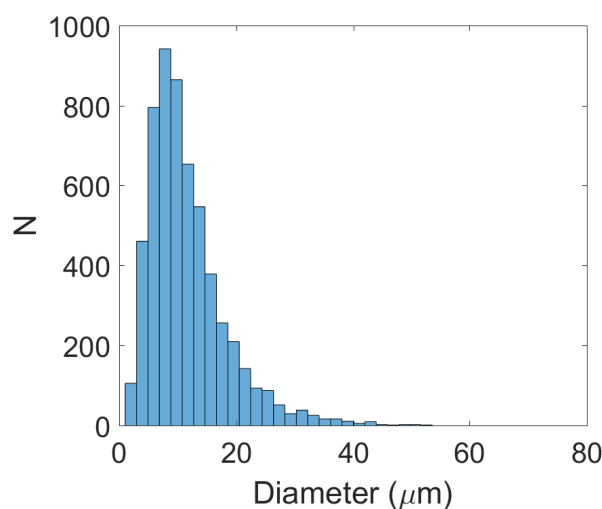


Fig. S 1: The average radius of the electroformed vesicles is  $r = 6 \pm 3 \mu\text{m}$ .

### 2 Details for DNA assembly

The DNA linkers consisted in a cholesterol-TEG anchor which enabled the grafting of the molecule to the vesicle membrane, followed by a backbone made of DNA double strand (DNAds) with a length of 43 base pairs that acted as a spacer and was ended with a 9 base pair sequence of single stranded DNA (DNAss), also called *sticky end*. The interaction of a sticky end (a) with its complementary strand (a') composed of complementary base pairs was the mechanism driving the assembly of the vesicles. We used the following DNA sequences (ds) designed by Parolini *et al.*<sup>1</sup> and were synthesized by Integrated DNA Technology:

- (a) 5-GGATGGGCATGCTCTTCCCGTTTTTTTATCACCCGCCATAGTAG A [Sticky End]-3
- (b) 5-CTACTATGGCGGGTGATAAAAAACGGGAAGAGCATGCCCATCC AAAA [Cholesterol TEG]-3

Upon arrival, the lyophilized samples were diluted in IDTE pH 8.0 1X TE Solution (IDT) at a concentration of 100  $\mu\text{M}$ , and stored at  $-20^\circ\text{C}$  for a maximum of one year. To prepare the DNA linkers for the

assembly, we first hybridized the molecule containing the DNAss-sticky end (a) with the molecule containing the cholesterol-TEG anchor (b). To hybridize the DNA strands, we diluted them to  $1.6 \mu\text{M}$  in the same TE buffer with  $100 \text{ mM NaCl}$  (Sigma Aldrich, S9888) and mixed to a ratio 1:1 sequences (a) and (b) for one assay, and (a'): (b) for the other one. A temperature ramp was performed from  $90^\circ\text{C}$  down to  $20^\circ\text{C}$  for a duration of 5h. By diminishing the temperature, the complementary base pairs of both (a or a') and (b) bond together forming the DNAds part of the molecule (a-b and a'-b). The hybridized DNA strands were stored at  $4^\circ\text{C}$  and used for a maximum of one month. To bind the hybridized DNA molecules to the membrane of the vesicles and enable the assembly of neighboring vesicles in aggregates, equal quantities of DNA complementary strands (a-b) and (a'-b) were mixed at concentrations  $32 \text{ nM}$  and  $644 \text{ nM}$  in a mix containing IDTE pH 8 buffer, a vesicle volume fraction of  $0.125\%$ , glucose  $70 \text{ mM}$ , NaCl  $80 \text{ mM}$ , and SYTO  $64.2 \mu\text{M}$  (Thermo Fisher,  $\lambda_{abs} = 599 \text{ nm}$ ,  $\lambda_{em} = 619 \text{ nm}$ ).

### 3 Fluorescence intensity of vesicle membranes

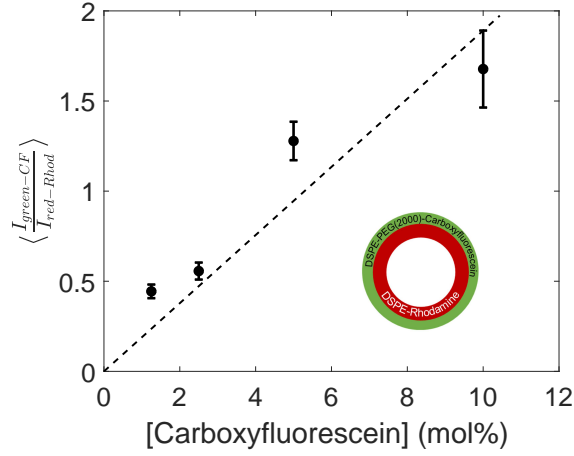


Fig. S 2: Quantification of the fluorescence intensity of vesicles containing a varying content of green fluorophore Carboxyfluorescein (from 1.25 to 10 % molar) and a constant content of DSPE-Rhodamine (red) of 1 %. The fluorescence intensity is reported as the ratio of the green over the red intensities. A linear fit is represented with a dashed line ( $\frac{I_{green-CF}}{I_{red-Rhod}} = 0.19[CF]$ ). The number of vesicles analyzed for all conditions was set to  $N=20$ . The displayed values are the average, with the standard error as the error bars.

### 4 Calculation of contact angle for vesicle doublets

In order to calculate the contact angle of vesicle doublets two circles were manually fitted to the vesicles forming each doublet, from which the position of their centroids ( $x_1, y_1$  and  $x_2, y_2$ ) as well as their radii ( $R_1, R_2$ ) were identified (a sketch of a vesicle doublet is shown in Fig. 3). The distance between the centroids of the circles is  $d$ . The contact angle ( $\theta$ ) between the two overlapping circles was computed according to:

$$x = x_2 - x_1 ; y = y_2 - y_1 \quad (1)$$

$$d = \sqrt{x^2 + y^2} \quad (2)$$

$$h = \frac{1}{2d} \sqrt{4d^2 R_1^2 - (d^2 - R_2^2 + R_1^2)^2} \quad (3)$$

$$\theta_1 = \arcsin\left(\frac{h}{R_1}\right) ; \theta_2 = \arcsin\left(\frac{h}{R_2}\right) \quad (4)$$

$$\theta = \frac{\theta_1 + \theta_2}{2} \quad (5)$$

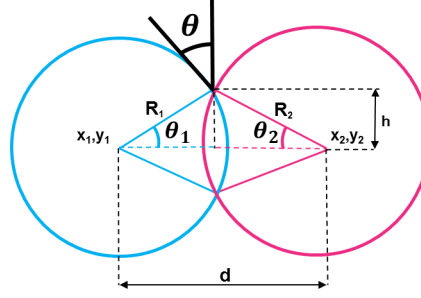


Fig. S 3: Sketch of a vesicle doublet.

## 5 Contact angle of vesicle doublets

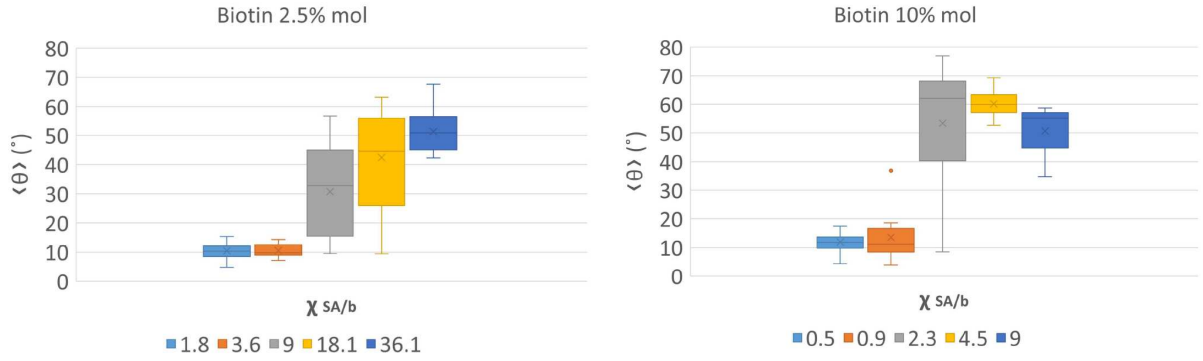


Fig. S 4: Contact angle ( $\theta$ ) of vesicle doublets, displayed in Fig. 3A, represented in the form of box-plots. Contact angle as a function of X, and for 2.5 % (left) and 10 % (right) mol biotin contents. The contact angle was computed by averaging the two contact angles of the doublet. The number of doublets analyzed for all conditions was set to  $N = 20 \pm 4$ .

## References

- [1] L. Parolini, B. M. Mognetti, J. Kotar, E. Eiser, P. Cicuta and L. Di Michele, *Nature communications*, 2015, **6**, 1–10.