Finger-like membrane protrusions are favored by heterogeneities in the actin network

Supplementary text

Estimates of biotin versus S-pVCA tetramers in the solution of liposomes

We calculate the number of biotin per volume unit in the solution containing liposomes (note that they are put in contact with 350 nM of S-pVCA i.e. 87.5 nM tetramers).

Lower estimate:

We first estimate the surface of liposomes S_v per unit volume. The volume of one field of view is 90x70x300 μm^3, the number of liposomes per field of view is 10, the average radius R of a liposome is 7 μm. Therefore, the surface of liposome per volume unit is:

$$S_v = \frac{10 \cdot 4\pi R^2}{90 \cdot 70 \cdot 300 \cdot 10^{-18}} \approx 3 \cdot 10^3 \text{ m}^{-1} \tag{1}$$

We then estimate the number of biotinylated lipid n_s per unit surface of membrane (liposome). The area of lipid head is 0.4 nm; the molar percentage of biotinylated lipids is 0.1 % (1/1000), therefore:

$$n_s = \frac{10^{-3}}{4 \cdot 10^{-19}} = 2.5 \cdot 10^{15} \text{ m}^{-2} \tag{2}$$

The number of biotin per volume unit of solution n_v reads:

$$n_v = S_v \cdot n_s = 2.5 \cdot 10^{15} \cdot 3 \cdot 10^3 \text{ m}^{-3} = 7.5 \cdot 10^{18} \text{ m}^{-3} \tag{3}$$

Concentration of biotin in solution C_{biotin}
Number of Avogadro: N_a=$6 \cdot 10^{23}$ mol^{-1}

The concentration of biotin in solution is therefore:

$$C_{biotin} = \frac{n_v}{N_a} = \frac{7.5 \cdot 10^{18}}{6 \cdot 10^{23}} = 1.25 \cdot 10^{-5} \text{ mol.m}^{-3} \tag{4}$$

$$C_{biotin} \approx 10^{-5} \text{ mol.m}^{-3} = 10^{-8} \text{ molar} = 10 \text{ nmolar}$$

Upper estimate:

We first estimate the concentration of biotinylated lipids in the lipid mix $C_{biotin-PEG}$. The total volume of lipid mix is V_{tot} = 50 μL, the biotinylated lipid concentration is $C_{mbiotin-PEG}$ = 0.1 mg.mL^{-1}, the volume of biotinylated lipid is $V_{biotin-PEG}$ = 4.8 μL and the molecular weight of biotinylated lipid is $M_{biotin-PEG}$ = 3014 g.mol^{-1}. Therefore, the concentration of biotinylated lipids reads:

$$C_{biotin-PEG} = \frac{C_{mbiotin-PEG} \cdot V_{biotin-PEG}}{M_{biotin-PEG} \cdot V_{tot}} \tag{5}$$

Given that 10 μL of lipid mix are dried and resuspended in 500 μL of internal buffer, the concentration of biotin in solution C_{biotin} is therefore:

$$C_{biotin} = \frac{C_{biotin-PEG} \cdot 10}{500} = 6.4 \cdot 10^{-8} \text{ mol.L}^{-1} \sim 65 \text{ nmolar} \tag{6}$$

In conclusion, the concentration of biotin sites in solution lies between 10 and 65 nM.

Lipid clusters in SLBs

Small lipid clusters form already in the absence of S-pVCA though at very low amounts (Fig. S1). The lipid clusters do not vary in size with the increase in S-pVCA concentration up to 1 μM, though their number increases significantly at 350 nM S-pVCA and above. At 3.5 μM S-pVCA membrane aggregation is very prominent and the clusters cannot be analyzed as individual objects.

Supplementary Figures

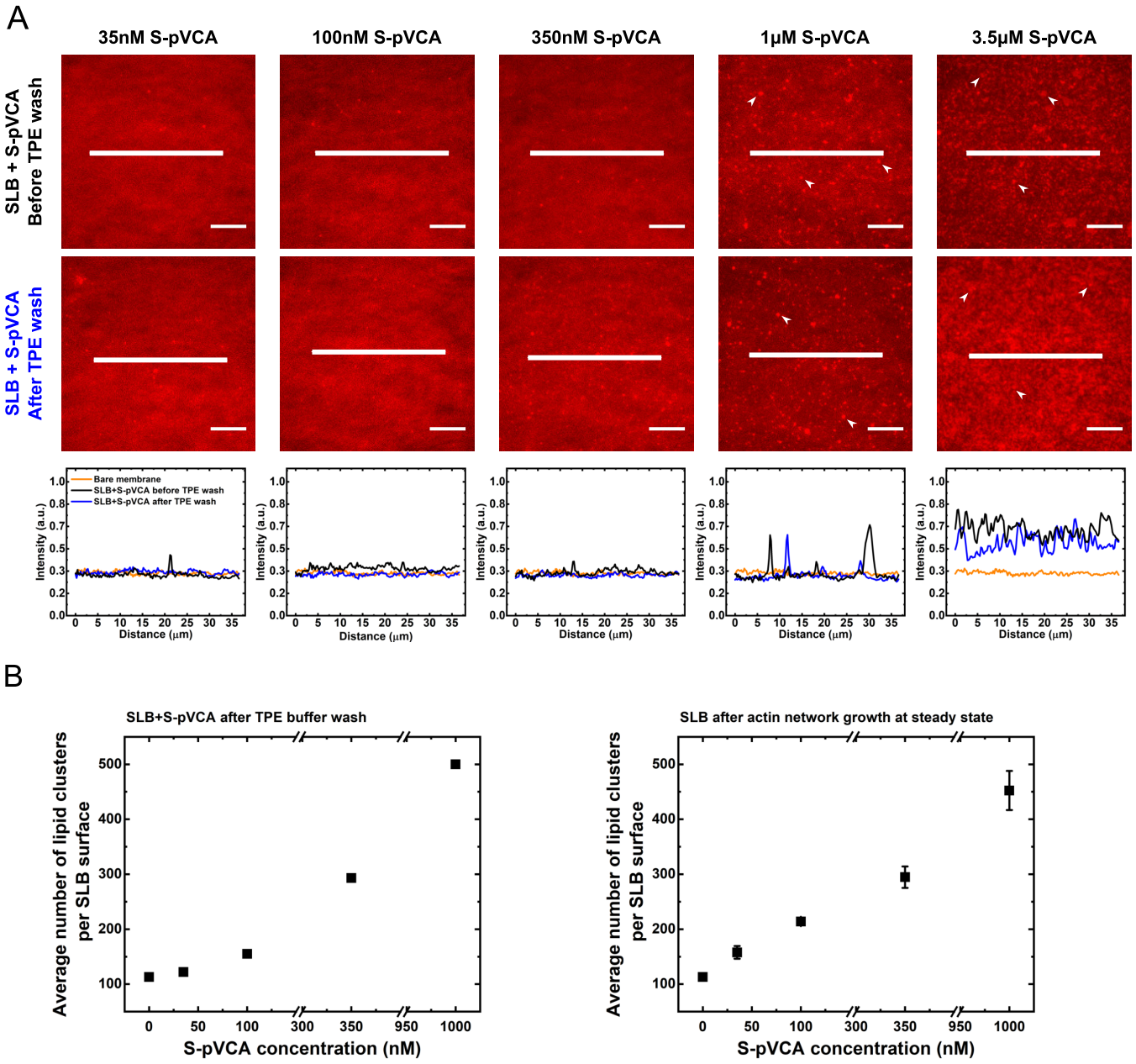

Figure S1 SLB density and structural organization at various stages of the experiment. (A) TIRFm images of the SLB at the end of S-pVCA incubation phase but before TPE buffer wash (row 1) and after TPE buffer wash to remove excess (unbound) S-pVCA (row 2). (Row 3) shown are the corresponding density (intensity) profiles along the drawn lines in rows 1,2; the density profile of the bare membrane is also given (orange line). Bars are 10 μm. Note that the intensity values are corrected for bleaching. Lipid membrane clusterization. (B) Average number of lipid clusters per SLB surface as a function of S-pVCA concentrations for two conditions: (left) after S-pVCA incubation and after TPE buffer wash, (right) at the end of the polymerization process (at steady state, t= 30 min). Conditions: the actin polymerization solution and membrane lipid composition are the same as in Fig. 1. The lipid membrane is labeled with TexasRed.

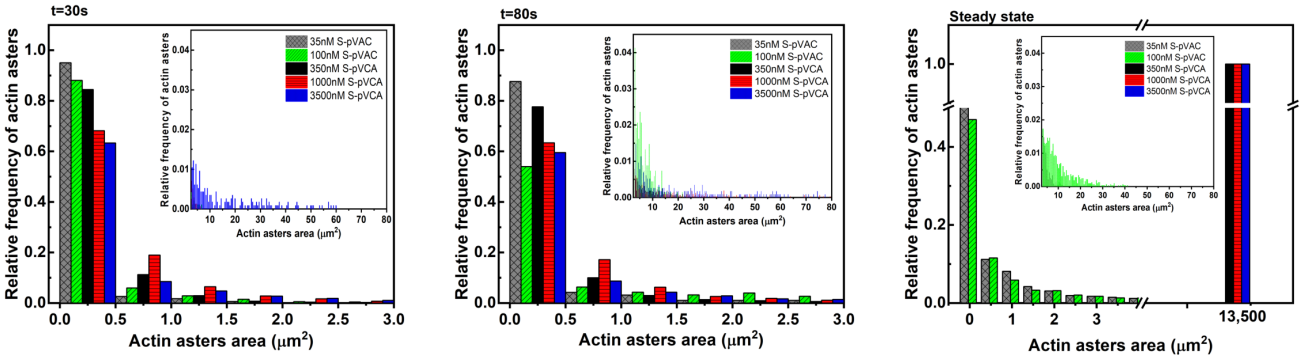

Figure S2 Distribution of actin asters area during actin network growth (at t = 30 and 80 s) and at steady state (t = 30 min). Main figures: Zoom-in of the distribution at the low and high asters area. Insets: Distribution at intermediate actin asters area.

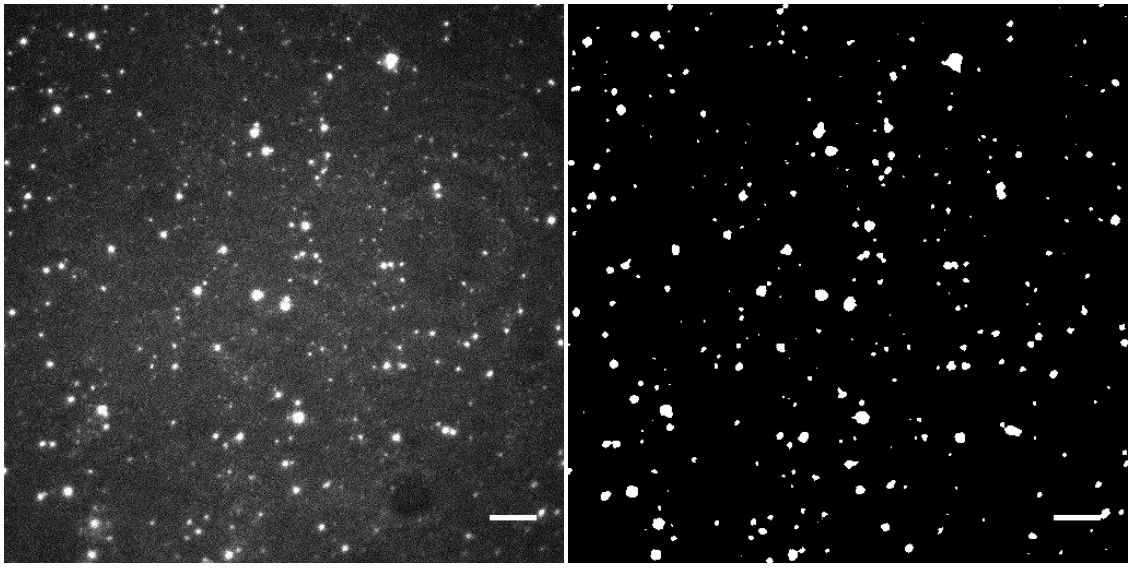

Figure S3 Image processing prior actin asters number and area analysis. Full frame original raw image (117x117 $\mu\mathrm{m}^2$) (left) and processed image (right) showing the asters formed on the SLB surface. Image processing includes suppression of background fluorescence by applying a Gaussian filter ($\sigma = 15$) to the image and subtracting pixel by pixel the Gaussian filtered image from the original image. High frequency noise is then removed by applying a median filter (3,3) to background subtracted image. The actin polymerization solution and membrane lipid composition are the same as in Fig. 1. The concentration of S-pVCA is 35 nM. Bars are 10 $\mu\mathrm{m}$.

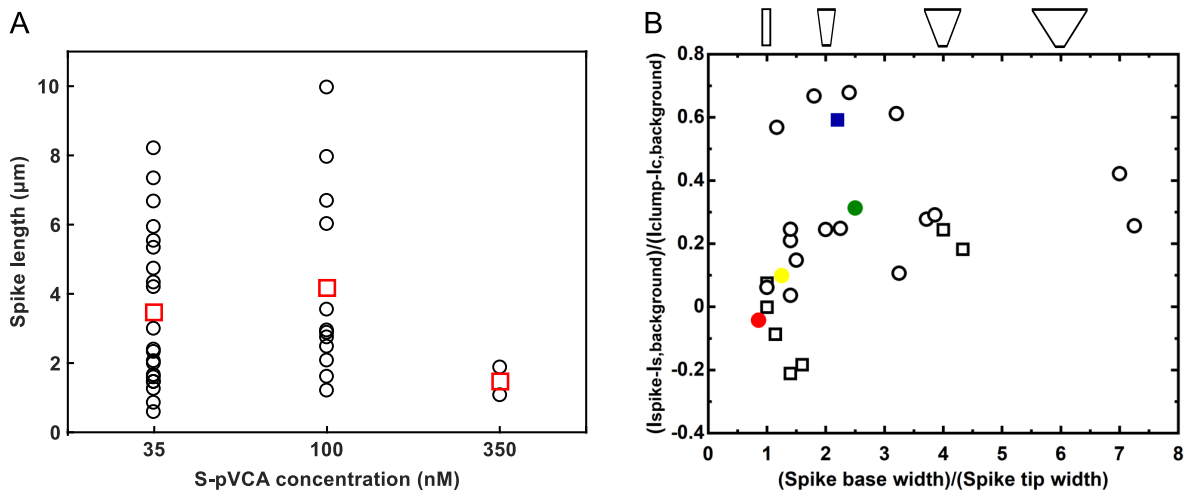

Figure S4 (A) Spike length as a function of S-pVCA concentration. Each circle represents one spike and red square corresponds to the mean length in each concentration condition. (B) Top: schematic representations of spike shape. Bottom: Arp2/3 complex fluorescence intensity values as a function of the ratio $\frac{\text{spike base width}}{\text{spike tip width}}$. The colored points (green, blue, red, yellow) refer to rows in Fig. 4A. Squares (resp. circles) refer to 35 nM (resp. 100 nM) S-pVCA. Note that a different batch of S-pVCA is used for these experiments compared to main figures. Due to a difference in protein activity, spikes are obtained in the 100 nM condition for actin and profilin set to 1 μM. N=25 liposomes, n = 27 spikes.