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Supplementary Information for

Pattern formation and polarity sorting of driven actin filaments on lipid membranes

Alfredo Sciortino and Andreas R. Bausch*

* Andreas R. Bausch
Email: abausch@mytum.de

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Movies S1 to S10

Supplementary Information Text

Fluorescence recovery after photobleaching of the supported lipid bilayer. To estimate the diffusion coefficient of the supported lipid bilayer, we bleach a rectangular region of size approximately 11μm x 9 μm using Leica's FRAP Wizard at a Leica Leica TSC SP5 confocal (63x objective with NA 1.4) and then record its recovery. Then we fit the recovery using the method from (1). Briefly, using a Python script, the area with lowest intensity in the first frame is identified, its center is found and the 2D intensity profile is fitted with equation 11 from (1). This allows to identify the bleached region automatically despite smoothness of the bleached region and to record its intensity. The diffusion coefficient is extracted fitting the recovery curve of the bleached region using equations 12-13 from (1). To correct for photobleaching during recovery, the intensity inside the bleached region at every frame is normalized by the intensity of the 1% brightest points in the frame. See Fig. S1 for the recovery curves and Supporting Movie 1 for the experimental realization.

Line thickness analysis. To estimate the number of filaments in a stream, firstly the background of the images is set to 0 and the transverse intensity profile of several individual filaments (20 to 30 for each frame) is measured. The same is done for streams in the frame. The integral intensity of each stream is divided by the mean integral of the filaments' profile yielding an estimate of the number of filaments composing the transverse size of streams. The measurement is repeated for different frames in the steady state of the system. The profiles are measured manually using Fiji's line tool and the analysis is carried out afterwards with a Python script.

Purification of gelsolin. Gelsolin was purified from adult bovine serum (Sigma Aldrich). The freshly defrosted serum was supplied with 50 mM Tris-HCl (pH 8.0) and 0.2 mM PMSF. Then, the serum proteins were precipitated with 35% and 50% saturated ammonium sulfate and subsequently spun down by centrifugation. The pellet obtained by the second precipitation was washed twice with a buffer containing 25 mM Tris-HCl (pH 8.0) and 50% saturated ammonium sulfate and then dissolved and subsequently dialyzed against a buffer containing 25 mM Tris-HCl (pH 8.0), 45 mM NaCl and 1 mM EGTA. Gelsolin was purified by ion-exchange chromatography on a diethylaminoethyl cellulose column by washing first with the same buffer, then with a buffer containing 30 mM NaCl and finally eluted with a buffer containing 25 mM Tris-HCl (pH 8.0), 30 mM NaCl and 2 mM CaCl2.

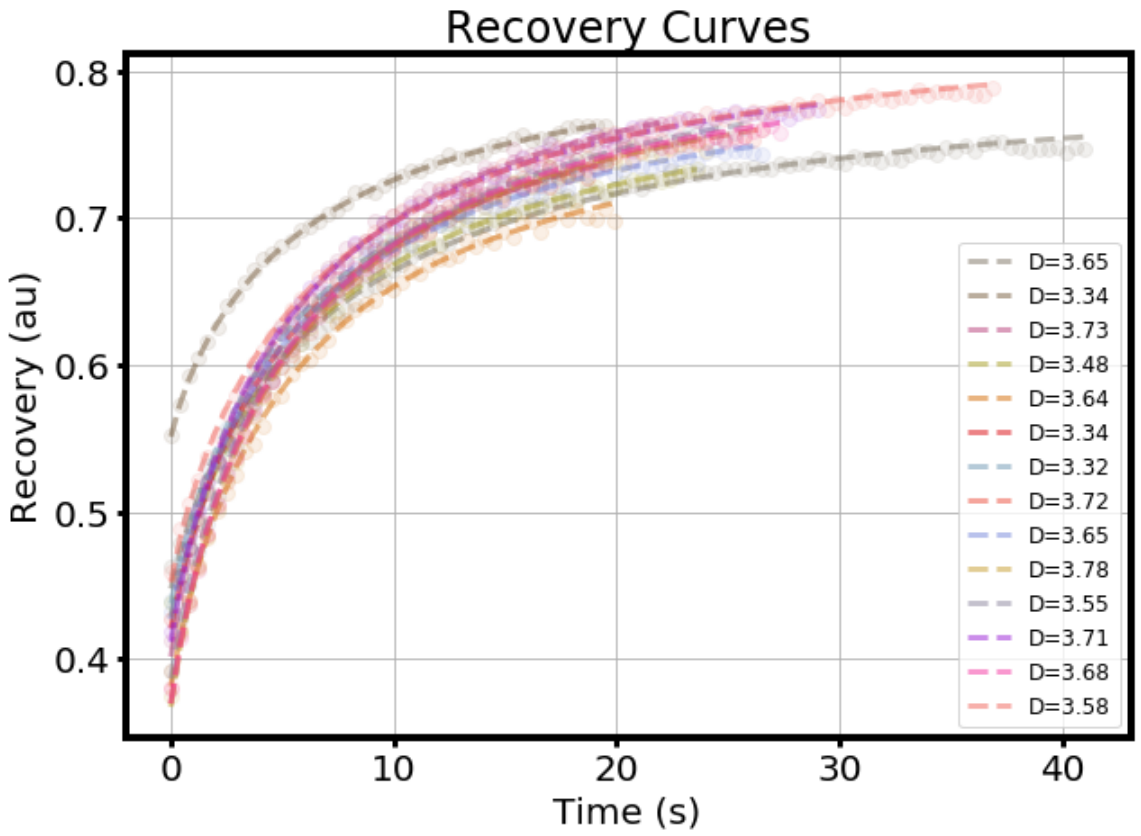

Fig. S1. FRAP recovery curve for 14 different photobleaching experiments. Dashed lines represent a fit of the recovery curve. Legend indicates the diffusion coefficient of each curve in units of µm2/s

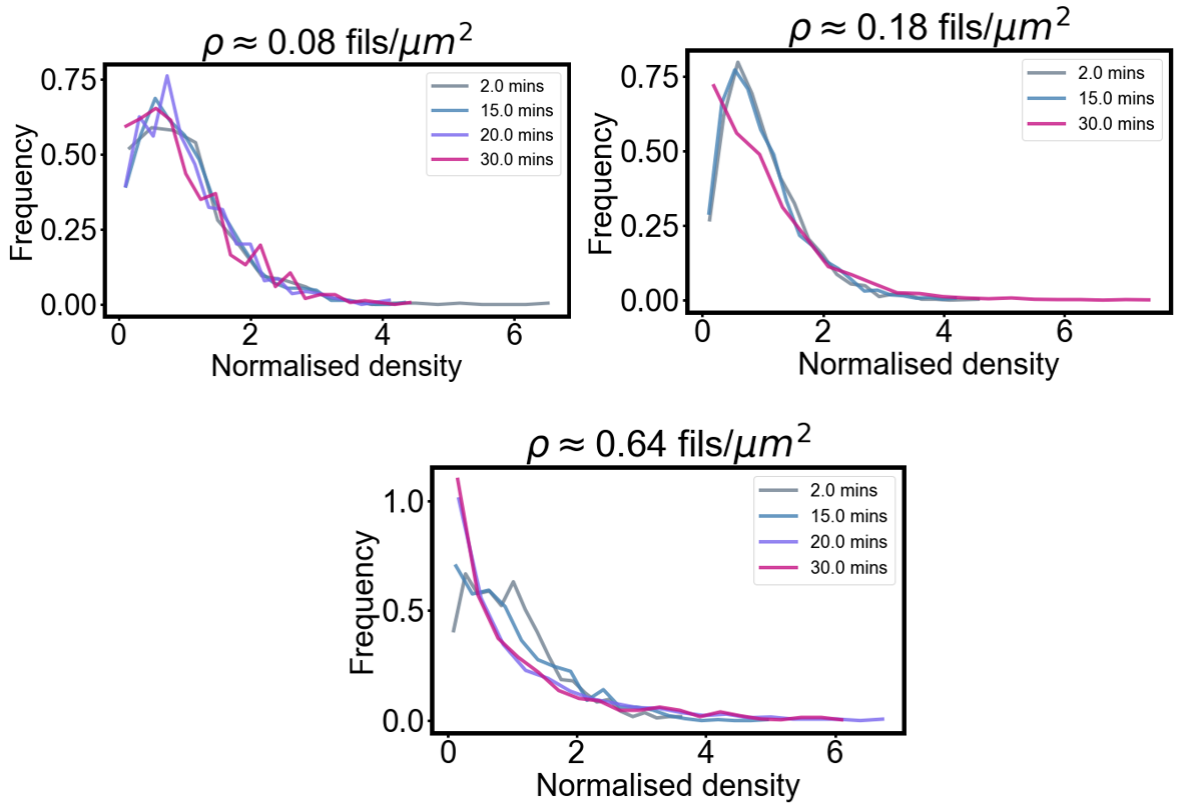

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Fig. S2 Histogram of density over time at different filaments' concentrations. As the density increases longer tail appear together with big portions of space devoid of filaments. A normalized density of one indicates the intensity of the uniform phase at each given density.

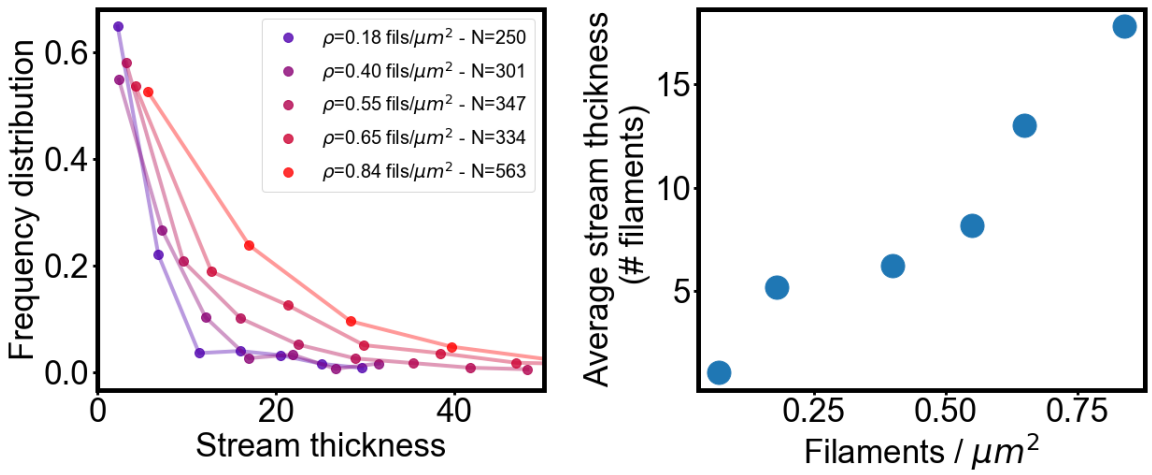

Fig. S3 Streams‘ thickness distribution and mean value in terms of number of filaments at different surface concentrations. N indicates the number of streams analyzed.

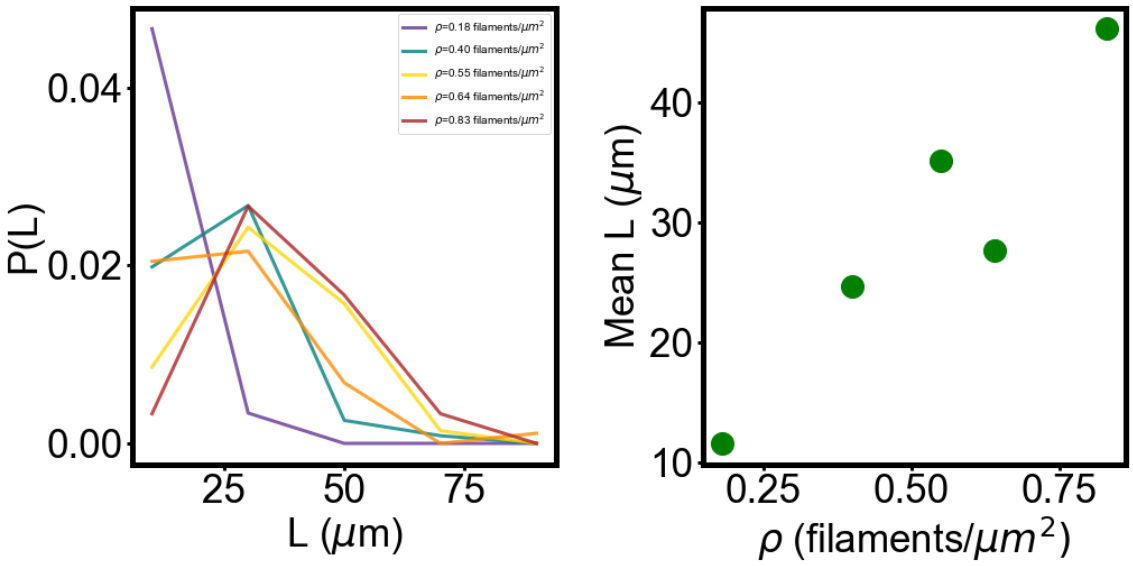

Fig. S4 Length and mean length of streams as the density increases.

Movie S1 (separate file). Fluorescence recovery after photobleaching experiment on a supported lipid bilayer. Scale bar is 10 μm.

Movie S2 (separate file). Actin filaments moving on a supported lipid bilayer.

Movie S3 (separate file). Example of a collision between filaments on a supported lipid bilayer. Scale bar is 10 μm.

Movie S4 (separate file). Formation of streams and vortices at 0.55 filaments/μm2. Scale bar is 10 μm.

Movie S5 (separate file). Formation of streams and vortices at 0.83 filaments/μm2. Scale bar is 10 μm.

Movie S6 (separate file). Close up of streams and vortex formation at filaments/μm2 (left, scale bar is 5 μm) and filaments/μm2 (right, scale bar is 10 μm).

Movie S7 (separate file). Formation of aggregated structures at high density.

Movie S8 (separate file). Formation of nematic defects and comet-like streams,

Movie S9 (separate file). Example of FRAP experiment at 0.83 filaments/μm2.

Movie S10 (separate file). Example of FRAP experiment at high density.

SI References

1. N. W. Goehring, D. Chowdhury, A. A. Hyman, S. W. Grill, FRAP analysis of membrane-associated proteins: Lateral diffusion and membrane-cytoplasmic exchange. *Biophys. J.* **99**, 2443–2452 (2010).