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**Supporting Material**

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***Supplementary Material for the manuscript***

# Profilin interaction with phosphatidylinositol (4,5) bisphosphate destabilize the membrane of giant unilamellar vesicles

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1. **Preparation of Giant Unilamellar Vesicles**
2. **Purification of human profilin I**
3. **Fluorescence lifetime measurement setup details**

## 1. Preparation of Giant Unilamellar Vesicles:

3 µl of a 0.2 mg/ml lipid stock solution were dried on the chamber platinum electrodes and the residual solvent was removed under vacuum. The chamber temperature was set at 45°C. 300µl of a 200mM sucrose solution were added to each well of the chamber and the electric field was applied using a function generator. A signal at 1.5V at 10Hz was applied for 90 minutes and then when required, the frequency was reduced to 1Hz for 15min in order to detach the GUV from the platinum wires. The samples were collected and 50 µl of the solution containing the GUVs were added to 250 µl of 200 mM glucose solution in each of the microscopy chambers (Lab-tek Brand Products, Naperville IL).

## 2. Purification of human profilin I:

Cells were lysed either using 5 ml/g of wet cells of BugBuster HT reagent (Novagen, 70922-5 ) or by sonication (Branson B-30) in 50 mM Tris pH 7.5 with 0.2 mM EDTA and PMSF. The cell lysate was then loaded on a poly-L proline affinity column and after washing with 4 M Urea, profilin was eluted using 7 M urea in Tris buffer pH 7.5. The purified profilin was renatured by extensive dialysis in 50 mM Tris buffer pH 7.5 and concentrated with 5 kDa cut-off Amicon Ultra centrifugal filter (Millipore, Cat # UFC900524). Just before the experiments, the concentrated profilin was extensively dialyzed against 200 mM glucose solution in order to avoid osmotic shock when adding to the GUV. The same protocol was used to express and purify the cysteine mutant of profilin.

## 3. Fluorescence lifetime measurement setup details:

The laser is operating at a repetition rate of 19.46 MHz and the emitted pulses have a width of 10 ps. Wavelength selection is achieved using a heat-absorbing filter of Schott KG1 glass (Filter 03FHA023 from Melles Griot, Carlsbad, CA, USA), which absorbs in the 800-2500 nm range, followed by a double grating monochromator DH10 (with 1200 g/mm gratings; ISA-Jobin Yvon, Edison, NY, USA). The detector is a small and fast head-on PMT, R7400U-04 from Hamamatsu (Iwata City, Japan). The PMT output signal enters a Becker & Hickl ACA-4-35dB amplifier. The amplifier output is connected (by a cable of adjusted length) to a Becker & Hickl (B&H) time-correlated single photon counting device SPC-144. Timing synchronization is provided by partial reflection of the excitation light onto a DET10A high speed silicon photodiode from Thorlabs (Newton, NJ, USA). The output signal of this photodiode is directed via cable of matched length to a signal inverter. After the inverter, the signal is connected to the synchronization port of the SPC-144. The fluorometer utilizes a cuvette holder integrated in a Koala box from ISS (Champaign, IL, USA). Along the emission light-path the Koala box allows the insertion of the desired emission filters (appropriate to the excitation and emission wavelength range) in order to prevent scattered excitation light from reaching the detector. Data acquisition control is provided by Becker & Hickl software.