Supporting Material for:

A simple supported tubulated bilayer system for evaluating protein-mediated membrane remodeling

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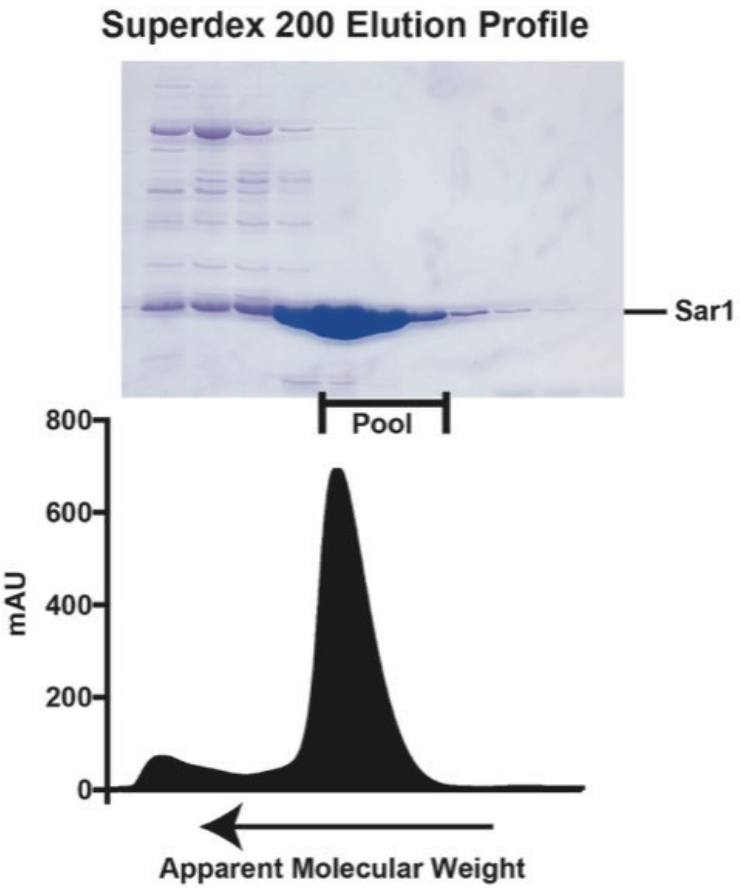

Figure S1: Purification of recombinant Sar1B using detergent-free conditions

Recombinant GST-Sar1B was expressed in BL21 DE3 *E. coli* and isolated from a detergent-free lysate by adsorption to glutathione Sepharose 4B resin. The washed resin was treated with PreScission Protease (3c Protease) to release Sar1B. Sar1B was concentrated and applied to a Superdex 200 HR10/300 GL column with running buffer. High molecular weight contaminants and aggregated Sar1B were separated from monomeric, monodisperse Sar1B. The indicated pool of Sar1B was collected, concentrated using an Amicon Ultra 15 ml centrifugal concentration device (10,000 MWCO). The concentrated protein was aliquoted, frozen, and stored at -80 ºC.

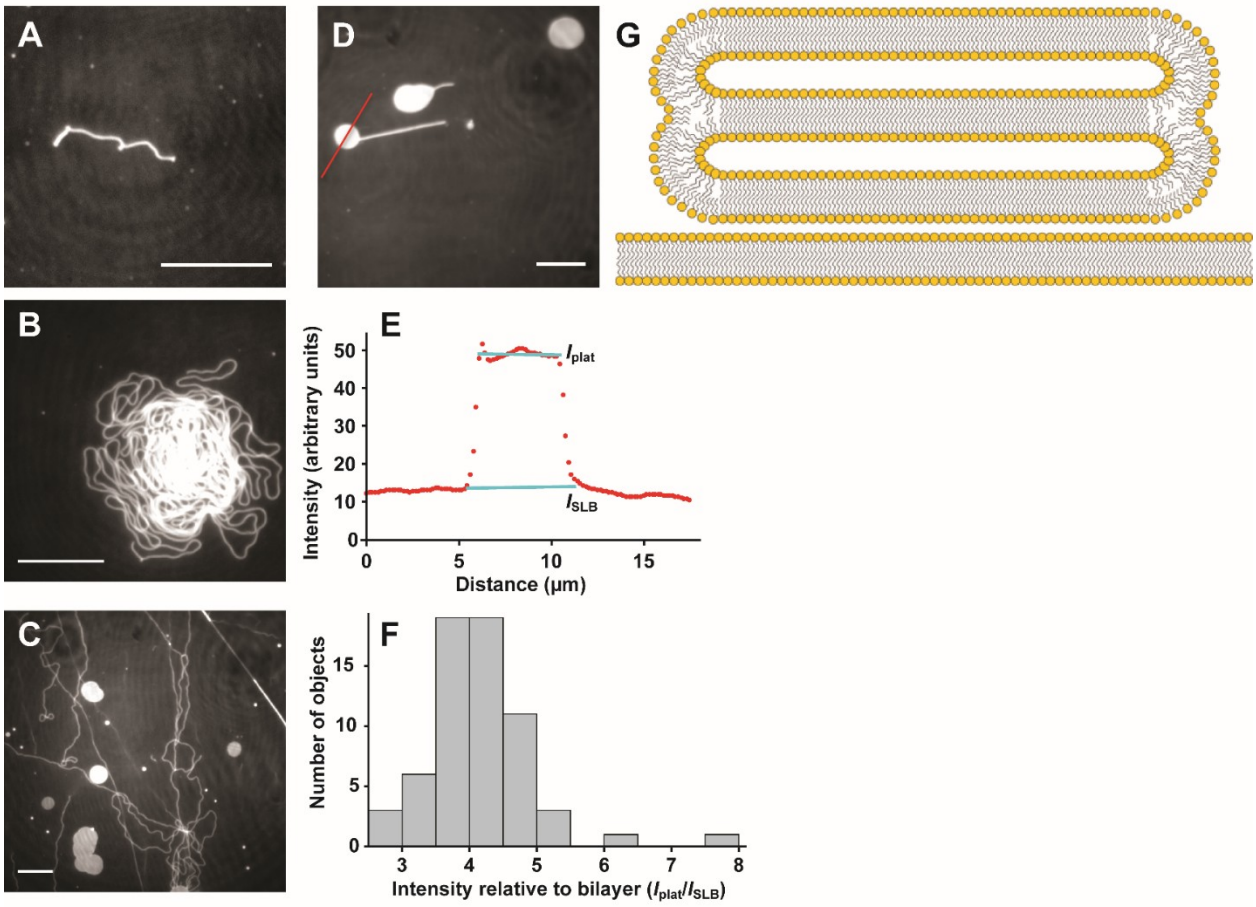

Figure S2: Representative tubules and flattened structures formed from DOPC bilayers. **A-C:** Tubules generally lay parallel to the bilayer with a variety of morphologies which were sometimes observed in the same field of view (C). Some tubules matured over tens of minutes into flattened vesicle-like structures (C-D, Figure S3). **D-F**: Quantification of fluorescence intensity profiles in order to determine morphology of the flattened vesicle-like structures. First, the LRB-DOPE fluorescence intensity profile across the structures was quantified using the Plot Profile command in ImageJ, with a linewidth of 5 pixels. A representative image used for quantification is shown in panel D, and panel E shows a quantification of the intensity profile along the red line in panel D. Second, these intensity profiles were used to calculate the average intensity of the structures (I_{plat}) relative to the underlying supported bilayer (I_{SLB}) as illustrated in panel E, using a home-written routine in *Mathematica*. The intensity ratio $I_{plat} \div I_{SLB}$ represents the number of stacked bilayers present, including the underlying SLB, assuming that the fluorescent LRB-DOPE tracer lipid partitions equally. Third, the distribution of intensity ratios $I_{plat} \div I_{SLB}$ among 63 structures are plotted in panel F. Mean ± SEM: 4.2 ± 0.1. The distribution centered on 4 suggests that most of the flattened structures contain 3 stacked bilayers on top of the SLB, while rare brighter structures may contain more stacked bilayers (intensity ratios near 6 and 8). **G**: Cross-section illustration of a hypothetical structure that would give rise to an intensity ratio of 4 (not to scale). We speculate that such structures are thermodynamically favored due to lower curvature, but there are kinetic barriers to formation of the hemifused state. Scale bars 10 μm.

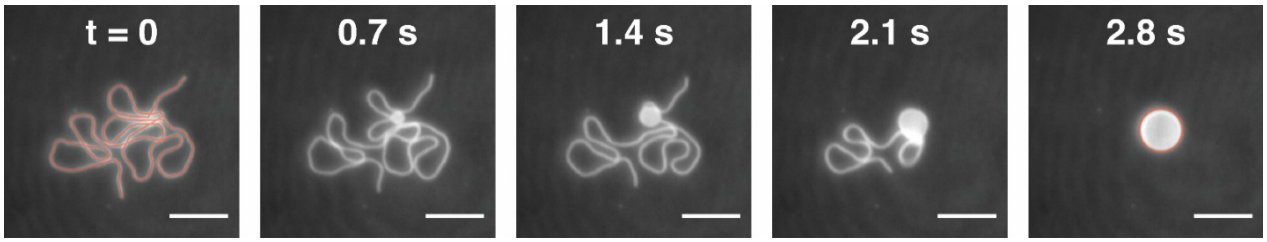

Figure S3: Maturation of DOPC tubules into flattened vesicle-like structures. Tubule width was estimated based on measuring the total length of a curved tubule before maturation (red trace, far left) and comparing to the post-maturation lateral area (red circle, far right). Assuming the tubule is unilamellar, the flattened structure consists of 3 stacked bilayers (Figure S2) and the total bilayer area in each object is constant over this timescale, the tubule diameter is estimated to be 100 ± 20 nm (n = 6, mean ± SD). The panels show still shots from a movie of one representative event. Scale bars 5 μm.

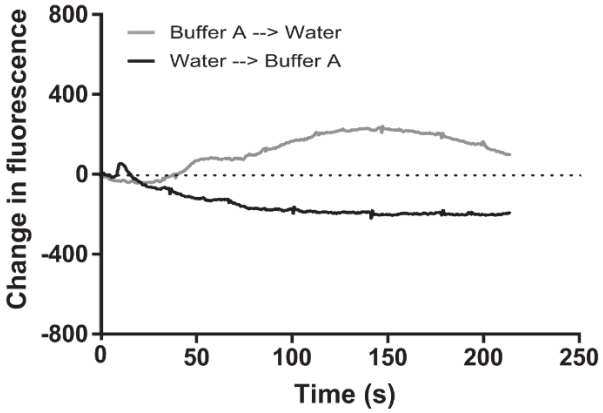

Figure S4: Physiological buffer drives bilayer expansion regardless of quantity of lipid in bilayer. SLBs (3:1 DOPC/DOPS with 0.1% LRB-DOPE) were prepared under physiological ionic strength, resulting in a lack of tubule formation upon the transition from deionized water and Buffer A. Black line indicates decrease in fluorescence intensity within the evanescent field during perfusion of Buffer A onto SLB submerged in water indicates a modest expansion of the bilayer. Grey line indicates Increase in fluorescence intensity within the evanescent field during perfusion of water onto SLB submerged in Buffer A indicates a modest retraction of the bilayer.

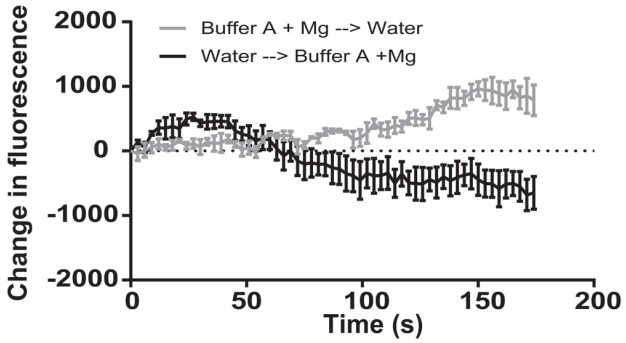

Figure S5: Physiological buffer with Mg^{2+} drives bilayer expansion
Changes in average fluorescence per unit area upon exchange of the bathing solution from Buffer A + 0.5 mM $MgCl_2$ into water (gray) or vice versa (black). A 2.4 ± 0.7 percent increase (contraction) was observed while exchanging from buffer into water, and a 2.0 ± 0.8 percent decrease (expansion) was observed while exchanging from water into buffer.

Movie S1: Movie of sample shown in Figure 1D of the main text. Scale bar: 10 μm.

Movie S2: Fluidity of a tracer lipid. The first frame shows an image of a flattened vesicle-like structure in the rhodamine channel (excitation 552 nm). Subsequent frames show the same view in the DiD channel (excitation 640 nm). The SLB under the structures is intact, as indicated by the ability of individual DiD molecules to diffuse. Some of the DiD molecules are clearly in the underlying bilayer, as they cross over the boundary of the flattened vesicle-like structure. Conditions: 99.9% DOPC, 0.1% LRB-DOPE, 100 ppb DiD, imaged in Buffer A. Scale bar: 10 μm.

Movie S3: Movie of sample shown in Figure 3A of the main text. From a scratched SLB initially bathed in water, Buffer A was introduced at t = 0. Scale bar: 10 μm.

Movie S4: Movie of sample shown in Figure 4D of the main text. Scale bar: 10 μm.