Supporting Material to:

Influenza A Matrix Protein M1 Multimerizes upon Binding to Lipid Membranes

M. Hilsch[1], B. Goldenbogen[2], C. Sieben[1], C. T. Höfer[1], J. P. Rabe[3], E. Klipp[2], A. Herrmann[1], S. Chiantia[1]

[1] Institut für Biologie, AG Molekulare Biophysik, Humboldt-Universität zu Berlin, Germany.
[2] Institut für Biologie, AG Theoretische Biophysik, Humboldt-Universität zu Berlin, Germany.
[3] Institut für Physik, AG Physik von Makromolekülen , Humboldt-Universität zu Berlin, Germany.

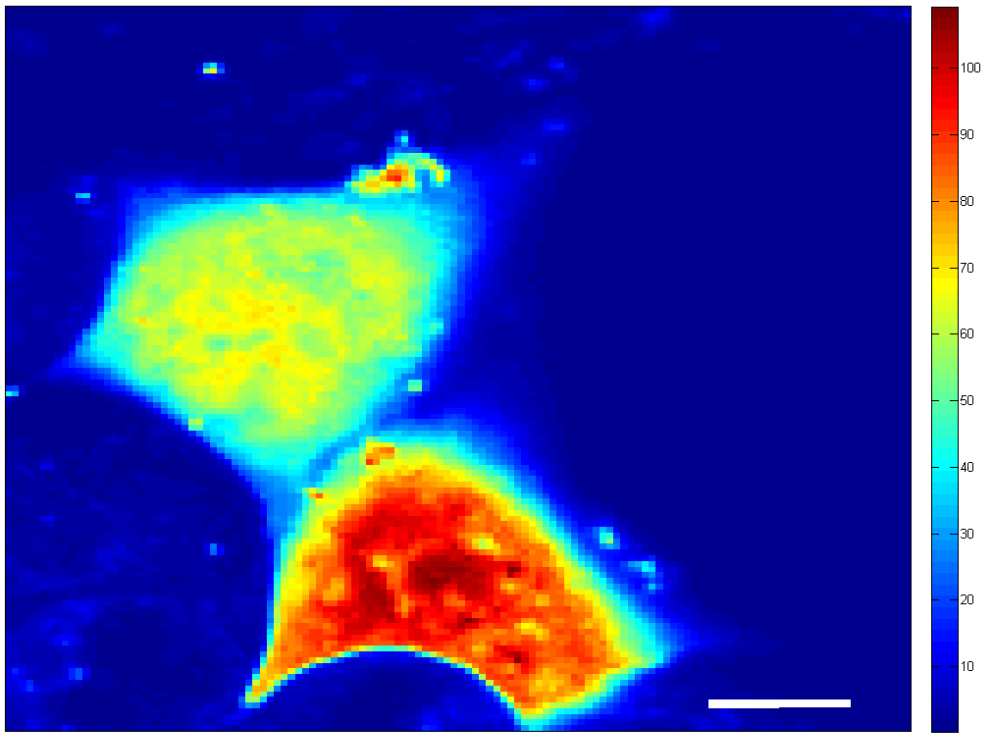

Figure S1. Confocal fluorescence imaging of soluble YFP in MDCK cells. The image shows the spatial distribution (i.e. fluorescence intensity) of YFP in two typical MDCK cells. The image is obtained as an average of 100 frames (256×256 pixels) collected over 2-3 minutes. The image was collected at 24 °C and the scale bar is 8 μm.

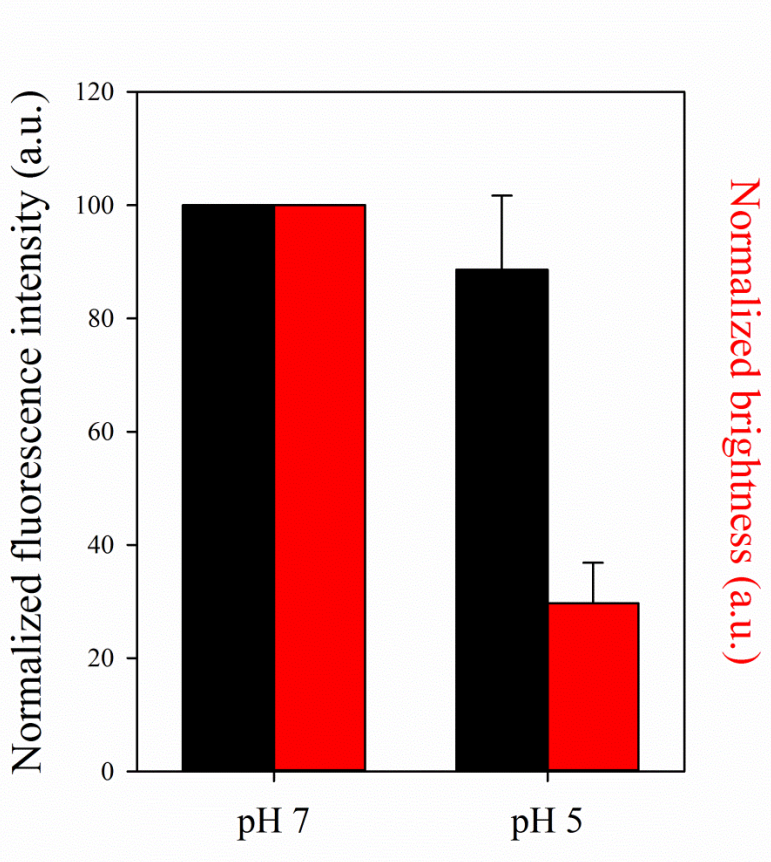

Figure S2. Decreasing pH from 7 to 5 decreases M1 multimerization. In order to measure the effect of decreasing pH on M1-lipid interaction, we let 50 nM M1 bind to a 40 mol% bPS membrane as described in the main text. After removing the unbound protein we measured the total fluorescence intensity (black, pH 7) and the brightness (red, pH 7) using RICS. The intensity value is proportional to the amount of bound M1. Both intensity and brightness values were normalized to 100. The same measurements were repeated after decreasing the pH from 7 to 5 using 6 µL (in a 200 µL total volume sample) of a 2 M pH 5 Na-acetate buffer. After 10 minutes and without further washing, we measured an only marginal decrease in the amount of bound protein (black, pH 5) and a ~3-fold decrease in M1 multimerization (red, pH 5).

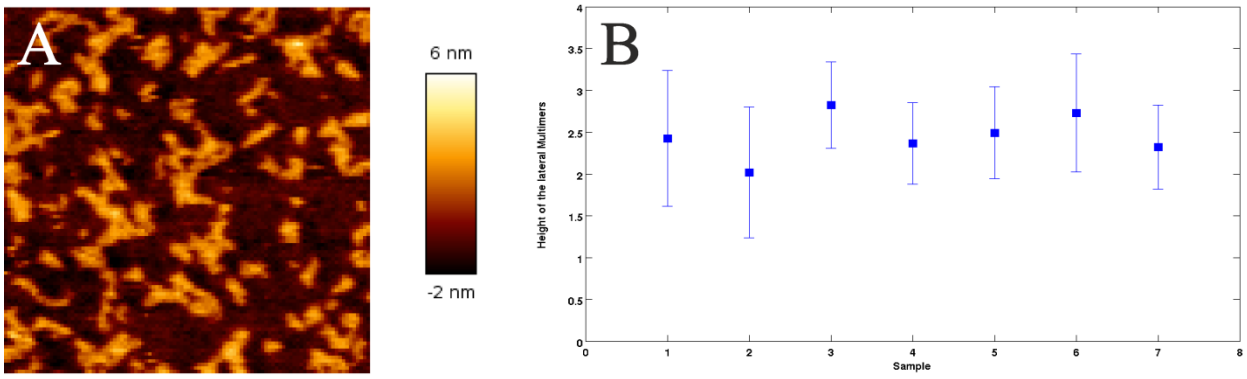

Figure S3. M1 oligomers on the surface of a lipid membrane. Panel A shows the AFM topographical image of the surface of a typical SLB with 40 mol% bPS incubated with 10 nM M1. The image is 2×2 µm² in size (digital zoom of Fig. 5 A). Panel B shows the quantification of the height measured for the M1 oligomers depicted in panel A. More specifically, each point represents the average height of the protein features measured in seven independent sample preparations. Height quantification was performed by fitting the pixel height frequency histogram (see e.g. Fig. S5) with two Gaussian functions: One for the main peak centered around 0 nm (representing the membrane surface) and one for the peak centered around 2 nm (representing the protein structures). The average height of the M1 oligomers was then defined as the center height value of the second peak. The error bars were calculated as: $\sqrt{\sigma_1^2 - \sigma_2^2}$, where σ_1 and σ_2 are the width of the first and second peak, respectively.

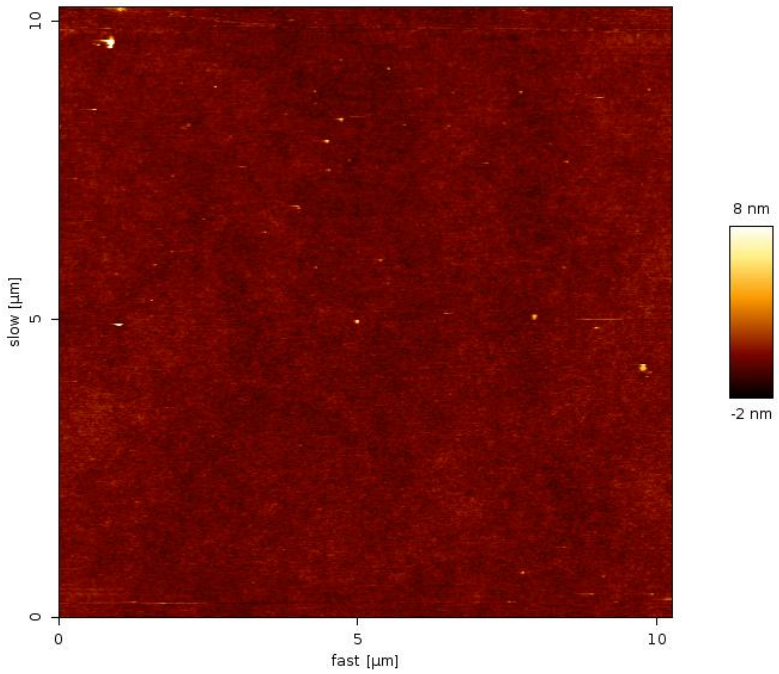

Figure S4. AFM imaging of a typical SLB containing 40 mol% bPS, without any protein. This measurement was performed in conditions comparable to those used to acquire the images shown in Fig. 5, A and B (see main text). The surface of the bilayers appears in general flat, with only few exceptions.

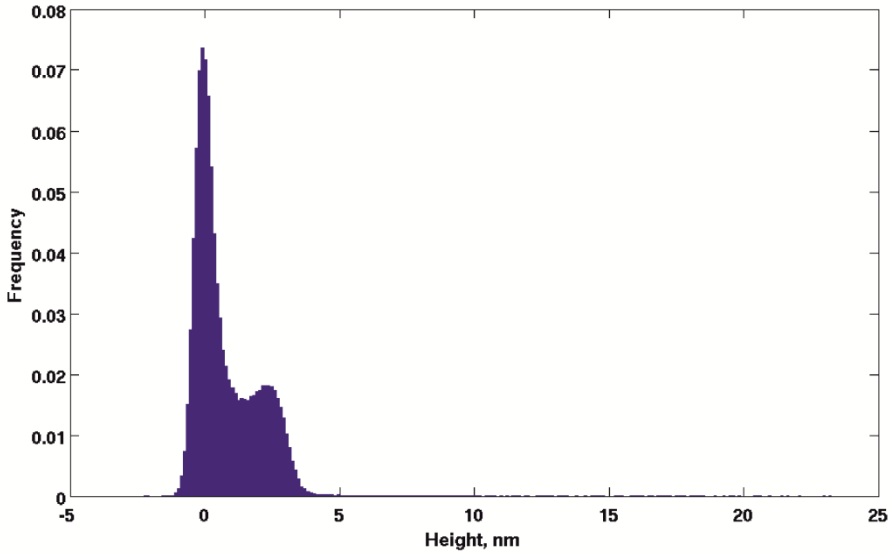

Figure S5. Histogram of pixel height observation frequencies in a typical sample, after incubation with 10 nM M1. The histogram shows a peak around 0 nm, corresponding to the pixels belonging to the SLB surface, and a peak around 2 nm, corresponding to pixels belonging to the M1 structures shown in Figs. S3 A and 5 A.