Supporting Information

A Correlated Force-Optical Study on the Self-Assembly Behavior of Annexin V on Model Membranes: Effect of Dye Conjugation

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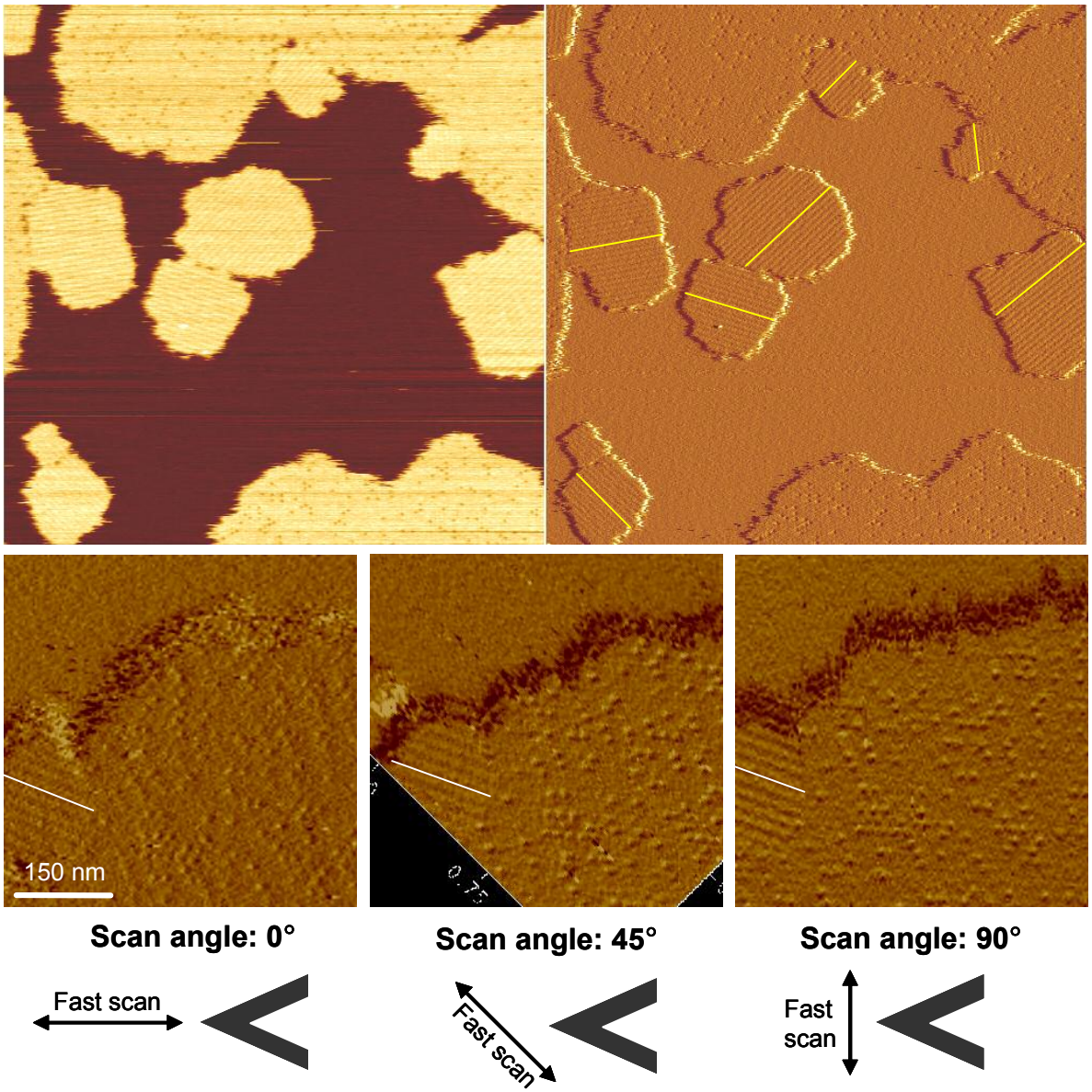
■ Experimental details.

Annexin V (A5), annexin V FITC (A5-FITC) and annexin V Cy3 (A5-Cy3) were purchased from Sigma-Aldrich. A second annexin V-Cy3 conjugate was purchased from BioVision, Inc. Both A5-Cy3 conjugates were filtered through 100 kDa membranes prior to use (Sigma-Aldrich, cat no. M1161). 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC) and 1,2-Dioleoyl-sn-Glycero-3-[Phospho-L-Serine] (DOPS) were purchased from Avanti Polar Lipids, Inc. and used as received. Liposomes were prepared by mixing lipids at a DOPC:DOPS molar ratio of 60:40% in chloroform, evaporating to dryness under N_2, drying in vacuo for one hour, rehydrating in buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM Ca^{2+}, pH 7.2), and finally subjecting to bath sonication for 10-20 minutes until clear. Final concentration of all as-prepared liposomes suspensions was 1 mg/mL. Supported lipid bilayers (SLBs) were prepared by incubating a 2X-diluted (0.5 mg/mL) liposome suspension on freshly cleaved 22 mm mica squares or 9 mm discs (Grade V1, Ted Pella) for 1 hour at RT followed by 5 rinses with buffer. For growth of annexin domains, the appropriate A5 was added directly to the bilayer sample and incubated at RT for 1-24 hours until domains were observed. For co-incubation studies, a buffer solution containing equal molar concentrations of A5-FITC and A5-Cy3 was added to the bilayer sample. Final total annexin concentration in all samples was ~5-10 μg/mL. Samples were kept in humidified containers during incubation to prevent evaporation and changes in buffer volume.

For correlated imaging, we used a force-optical hybrid set-up that includes an Olympus IX70 microscope, a Veeco Dimension 3100 AFM head, Ar^+ (488 nm) and cw Nd-YAG (532) lasers, and all necessary optics for total internal reflection (TIR) excitation and detection. Samples were excited in TIR mode using 488-nm excitation for A5-FITC, 532-nm excitation for A5-Cy3, and an appropriate set of notch and emission filters. Images were captured on a color digital camera (Olympus C-5060) as well as an EMCCD (Andor iXon). Fluorescence spectra were acquired with a 100 μm pinhole and custom spectrometer using a typical dwell time of 150 ms.

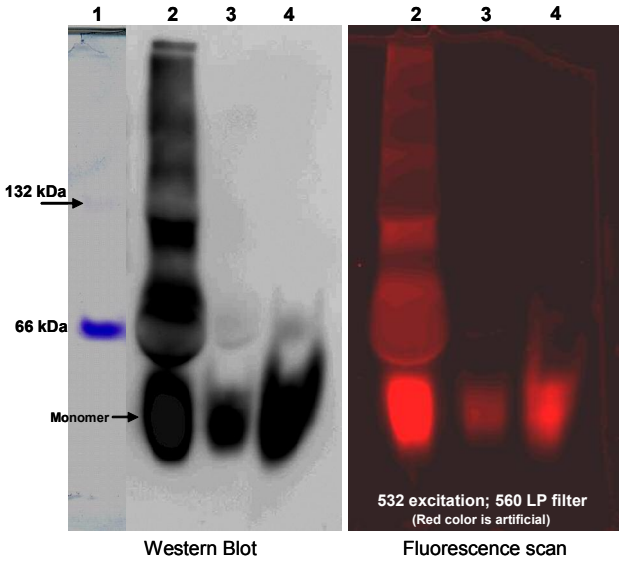
High resolution AFM images were obtained on a Veeco Multimode head with J scanner. All AFM images were acquired in contact mode using the 200 μm triangular lever on Olympus OMCL-TR400PSA-1 silicon nitride tips cleaned in 5% detergent, rinsed in water, EtOH, and dried with N_2. High resolution imaging scan rates for scans under 1 μm were typically 3-5 Hz. Set point value was maintained at low interaction forces and adjusted during scanning to compensate for drift. Images were flattened to zeroth or first order, and low-pass filtered if necessary to remove noise.

■ **Multi-angle AFM scanning of corrugated A5-FITC domains.**



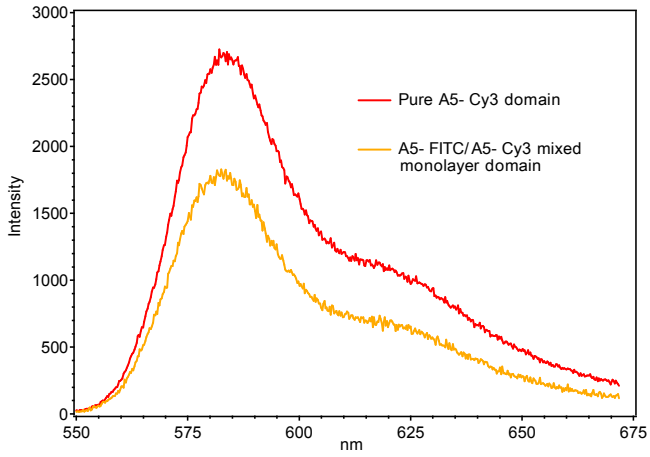
Supporting Figure 1. Multi-angle AFM scanning of A5-FITC domains. Top: One of many AFM images showing unusual corrugated domains with stripes at random angles relative to the scan direction (1.9 x 1.9 μm scan size). Bottom: Multi-angle scan of the same region at 0°, 45°, and 90° degrees scan angles. Constant stripes in the corrugated domain appear at all three angles.

■ Native PAGE of A5-Cy3 conjugates.



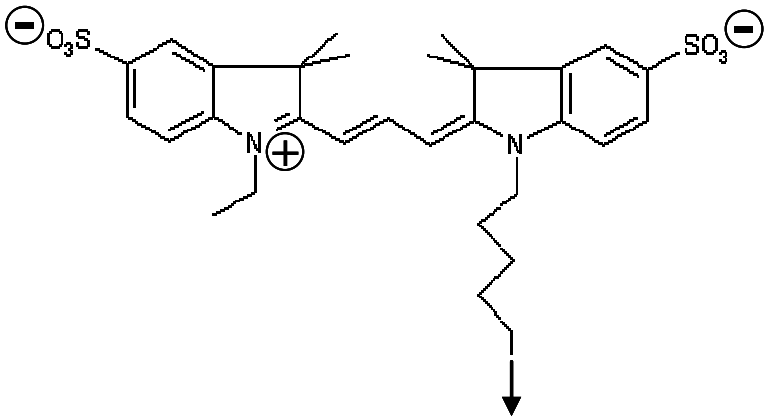
Supporting Figure 2. Two separate native PAGE gels of A5-Cy3 samples imaged by Western Blot and fluorescence scanning. Lanes: 1) 66 + 132 kDa marker, 2) unfiltered BioVision A5-Cy3 conjugate, 3) BioVision A5-Cy3 conjugate after filtration through 100 kDa membrane, 4) Sigma-Aldrich A5-Cy3 conjugate after filtration through 100 kDa membrane.

■ Emission spectra of A5-FITC/A5-Cy3 co-incubated monolayer domains.



Supporting Figure 3. Cy3 emission from pure A5-Cy3 monolayer domains and A5-FITC/A5-Cy3 mixed monolayer domains showing a decrease in Cy3 emission in the mixed domains due to dilution of A5-Cy3 with A5-FITC.

■ **Chemical structures Cy3 dye molecule.**



Supporting Figure 4. Chemical structure of Cy3 dye molecule. Arrow indicates location and direction of attachment of dye to protein.