Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information: Peak Force Visible Microscopy

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This supporting information includes:

Supplementary Note S1. Methods and Materials.

PF-vis microscopy

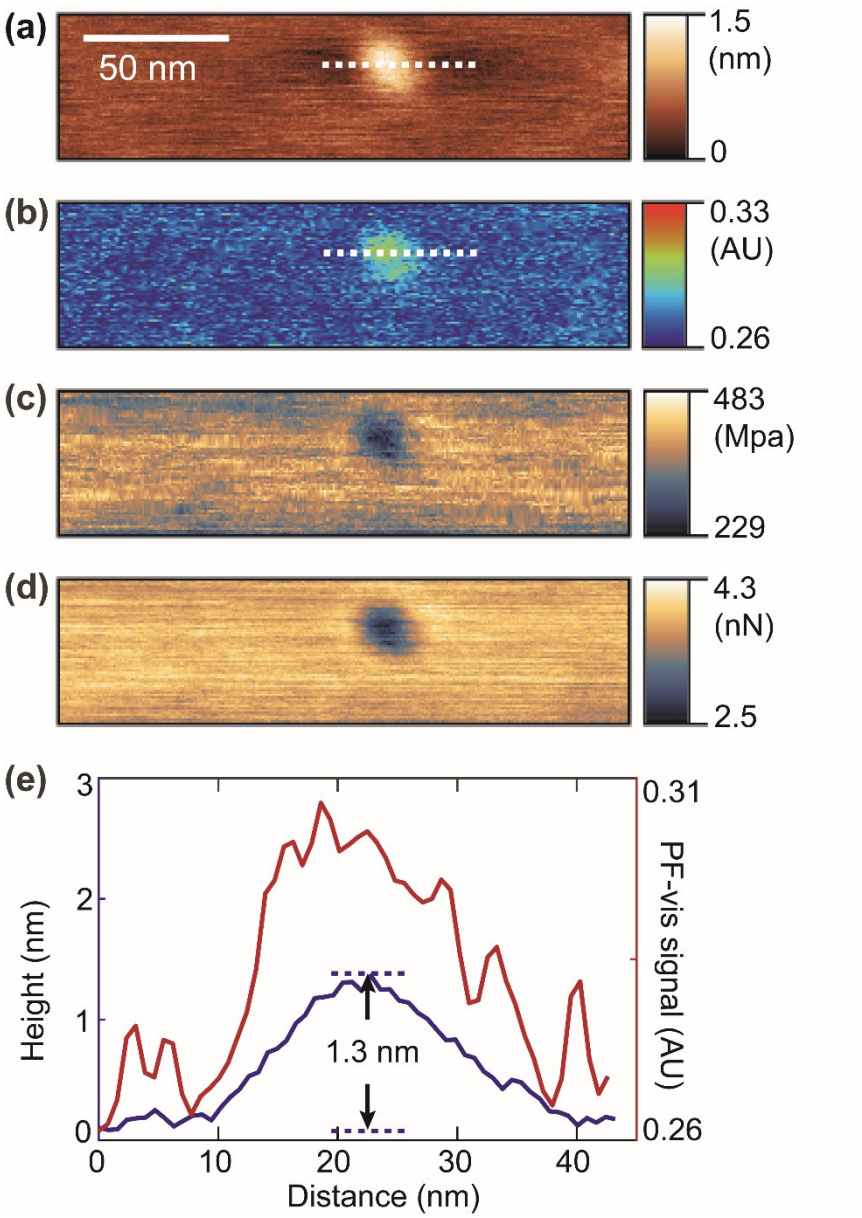
The PF-vis apparatus is identical to our previous work,[1] with the IR laser source replaced by a 532-nm pulsed laser. Pt-coated AFM probes with an end radius of around 30 nm (HQ:NSC14 Pt, Mikromasch) were used to conduct all measurements. A total of 30 vertical cantilever deflection curves (15 with the pulse and 15 without the pulse) from the PFT are averaged and used for signal processing at each pixel. This is done in real-time to provide photothermal expansion images as the AFM tip scans over the sample.

GFP-labeled VLP preparation

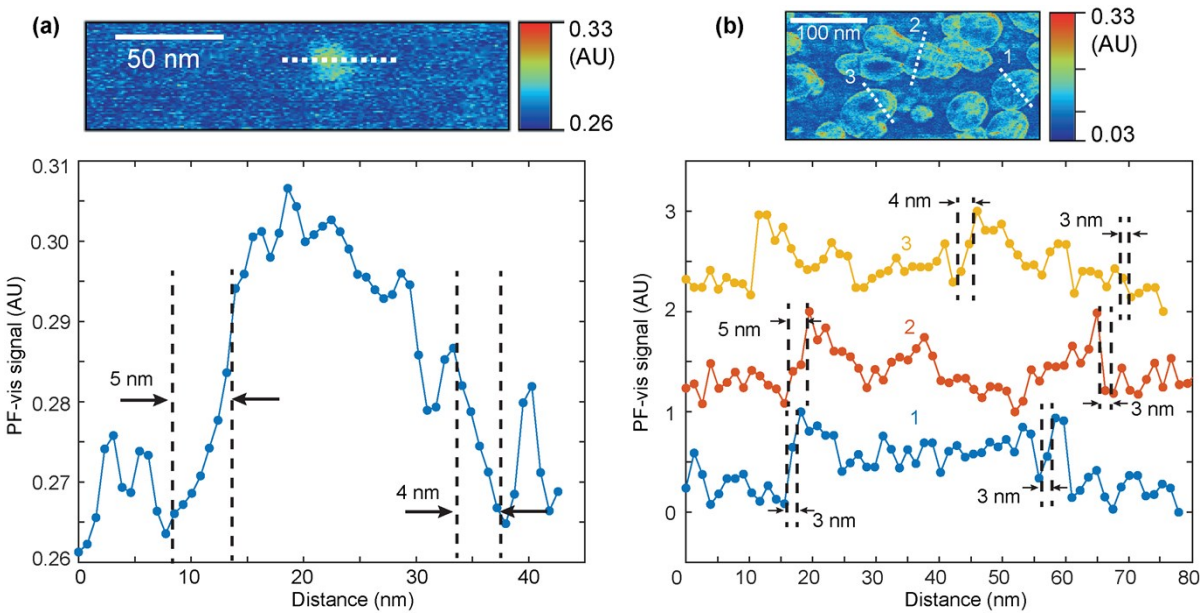
0.1mg/mL of poly-L-lysine solution was prepared in pure water. Then, a mica substrate was functionalized with ploy-L-lysine to stimulate positive charges that increase the attachment of negatively charged Ebola VLP due to its surface glycoprotein and phosphatidylserine lipid. In order to do so, 100 μL of 0.1 mg/mL of poly-L-lysine solution was added to the mica surface and incubated at room temperature for 10 minutes. The solution was removed by an air duster and the mica surface was dried. 2 μL of GFP-label VLP was then diluted in 18 μL of PBS and gently mixed by pipetting up and down. 20 μL of the diluted VLP solution was dropped on the center of the functionalized surface and incubated at room temperature for 10 minutes. The mica surface was washed with a few mL of pure water to remove the unattached VLP and then dried by the air duster. The sample can be used directly for the AFM imaging in the air. The preparation of this GFP-labeled Ebola virus glycoprotein-pseudotyped VLPs has been reported in literature.[2]

OPV blend films preparation

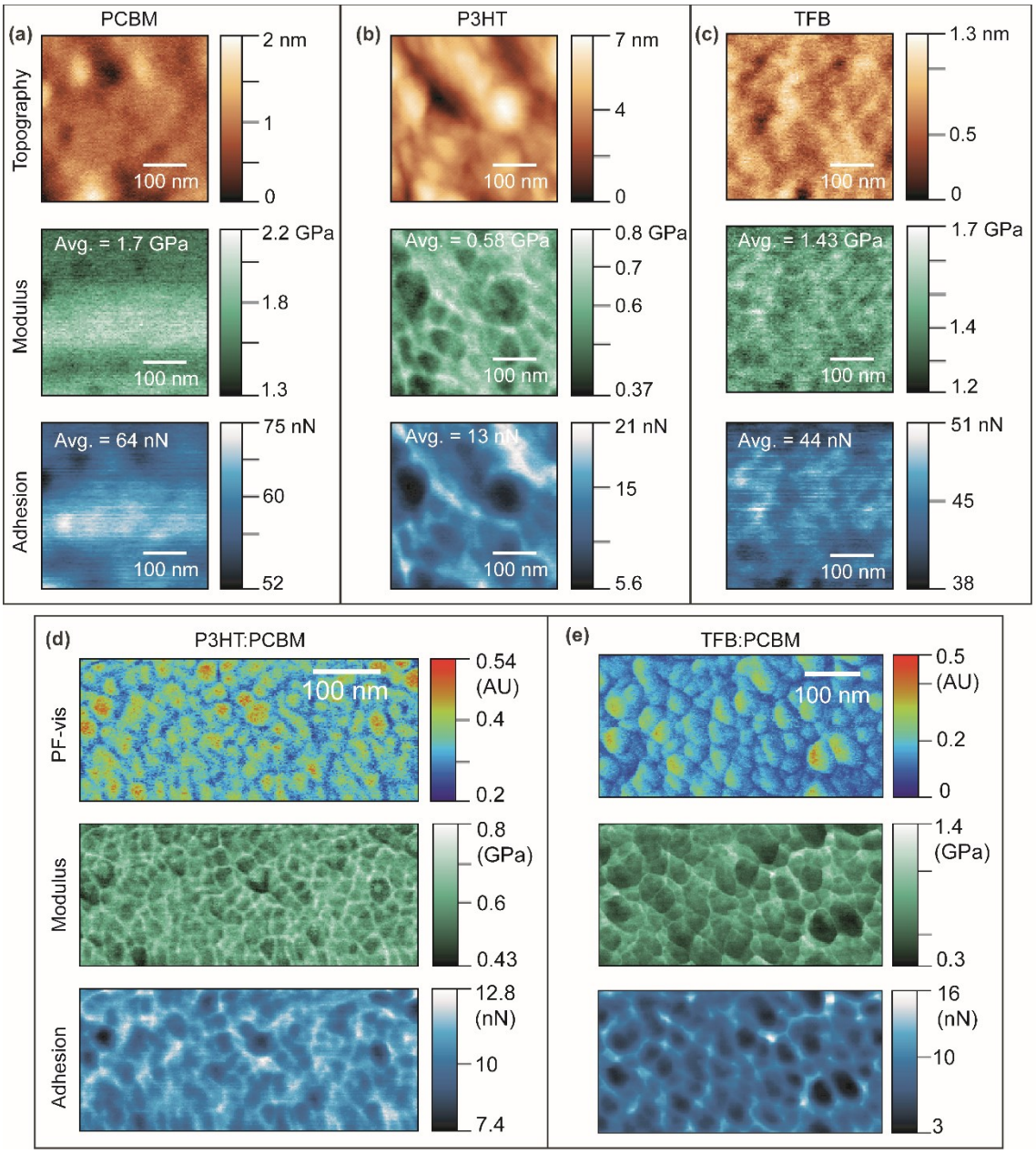
P3HT and TFB polymers were purchased from Xi'an Polymer Light Corp. PCBM was purchased from Jilin Optical and Electronic Materials CO. Solutions of 8 mg/mL P3HT:PCBM (mass ratio 1:1) and 8 mg/mL TFB:PCBM (mass ratio 1:1) were prepared in chlorobenzene (Sigma Aldrich) and then 20 μL of each was spin-cast on a 1 cm^2 gold substrate (Ted Pella) separately (1000 rpm for 1 min). P3HT:PCBM blend was then annealed in a vacuum oven at 140 °C for 30 min before use. TFB:PCBM blend was measured as cast. For measurements on homogenous films in Supplementary Fig. S3, chlorobenzene solutions of 8 mg/mL PCBM, 12 mg/mL P3HT and 11 mg/mL TFB were used. 20 μL of each of them was spin-casted on a 1 cm^2 gold substrate (Ted Pella) separately (1000 rpm for 1 min). All OPV blend samples were measured as cast.



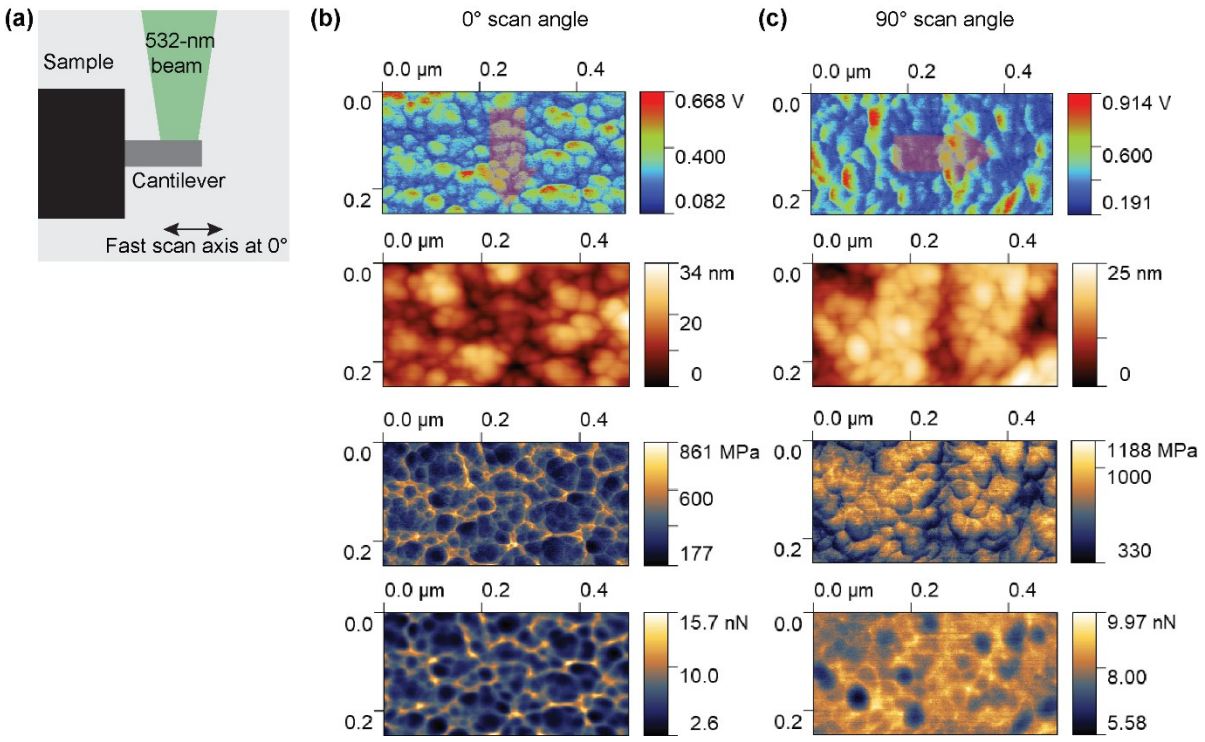
Supplementary Figure S1. PF-vis imaging of Alexa 547-labeled streptavidin. (a) AFM topography image of a Alexa 547-labeled streptavidin particle on a mica substrate. (b) PF-vis image under 532 nm illumination. (c-d) Young's modulus and adhesion obtained simultaneously with (a) and (b). (e) PF-vis signal profile (red) overlaid with the height profile (blue) across the Alexa 547-labeled streptavidin particle. The height of only 1.3 nm indicate this particle could be a single dye-labeled streptavidin molecule. The position of line cut is shown as white dashed lines in (a) and (b).



Supplementary Figure S2. Estimation of the spatial resolution from multiple imaging results. (a) The top panel shows the PF-vis image of an Alexa 547-labeled streptavidin particle. The white dashed line indicates the location where the signal profile in the bottom panel comes from. Spatial resolutions of 4-5 nm are obtained across two edges by estimating lateral distances between 90% - 10% range of the signal maximum and minimum. (b) The top panel is the same as Figure 2b in the main text. Three signal profiles plotted in the bottom panel are obtained from three locations marked in the top panel. Spatial resolutions of 3-5 nm are estimated using the same estimation method.



Supplementary Figure S3. Modulus and adhesion of P3HT, TFB, and PCBM homogeneous films and mixed blends. (a-c) Topography, Young's modulus and adhesion of spin-cast PCBM, P3HT, and TFB homogeneous films. The AFM peak force tapping condition is the same for all measurements in (a-c). The average modulus and adhesion values of PCBM, P3HT, and TFB are 1.7, 0.58, 1.43 GPa, and 64, 13, 44 nN, respectively. PCBM has the highest modulus and adhesion values among three films. (d-e) PF-vis, Young's modulus, and adhesion images of P3HT:PCBM and TFB:PCBM blend films. It is concluded that highlighted nano domains under 532 illumination in both blend films are donor domains.



Supplementary Figure S4. Scan angle dependence of PF-vis signal on TFB:PCBM blends. (a) Top-view of the cantilever position on top of the sample surface and the direction of the light illumination in PF-vis microscopy. The fast scan axis of AFM is shown by the double arrowheads. (b) PF-vis image of TFB:PCBM (from a different sample area than Figure 2b in the main text) at 0° scan angle. The illumination direction is shown by a red arrow. From the top to the bottom: PF-vis, topography, modulus, and adhesion images captured at the same time. (c) PF-vis image of TFB:PCBM (from a different sample than Figure 2b in the main text, using the same preparation conditions) at 90° scan angle. The illumination direction shown by the red arrow is changed by 90° as well. From the top to the bottom: PF-vis, topography, modulus, and adhesion images captured at the same time. In both (b) and (c), the PF-vis signal distribution is asymmetric along the illumination direction, while there is no asymmetries in other channels. The scan angle used in the main text Fig. 2 is 90°.

References

1. Wang, L. *et al.* Nanoscale simultaneous chemical and mechanical imaging via peak force infrared microscopy. *Science advances* **3**, e1700255 (2017).
2. Dragovich, M. A. *et al.* Biomechanical characterization of TIM protein-mediated Ebola virus-host cell adhesion. *Sci Rep* **9**, 267-267, doi:10.1038/s41598-018-36449-2 (2019).