

## SUPPLEMENTAL FIGURES

Figure S1. Bis-ANS binding to soluble Fn in guanidine hydrochloride (GdnHCl). The fluorescence intensity of Bis-ANS is detected at its peak emission wavelength (490nm) and normalized to the highest intensity measured.

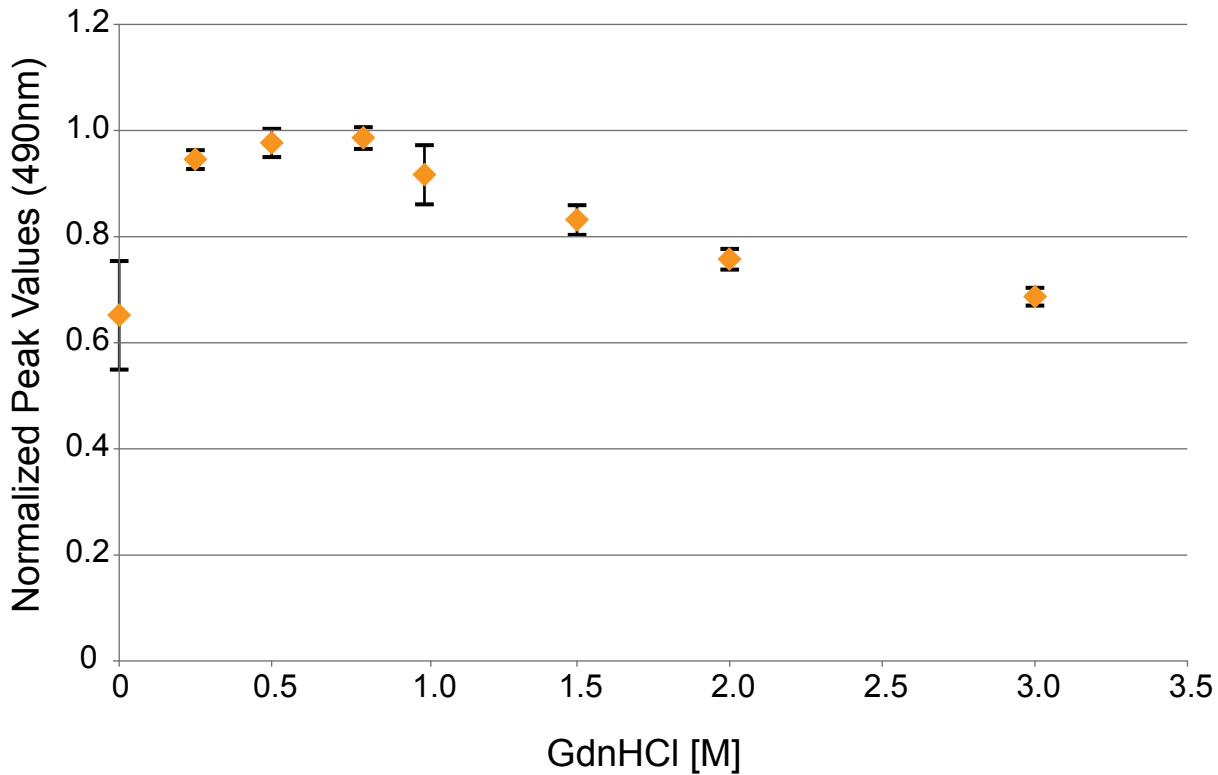
Figure S2. A star of Fn fibers stretched in a uniaxial stretcher. Under a dissecting stereoscope with a digital camera, a 0.3mg/mL droplet of Fn labeled with donors and acceptor fluorophores is used to deposit Fn fibers onto a pre-strained silicone substrate (a) such that different strains can be produced when the substrate is relaxed. After the substrate is relaxed to bring the fibers to 0% absolute strain, a transverse extension brings the fibers to an absolute 300% strain. (b) The acceptor intensity is divided by the donor intensity value ( $I_A/I_D$ ) per pixel and mapped to false color as shown. The data was analyzed as shown in Figure 1.

Figure S3. Pixel-by-pixel colocalization of either albumin or soluble Fn with Fn fibers. (a) FRET in Fn fibers measured as  $I_A/I_D$  is shown in false color. (b) Alexa 633-labeled BSA-633 incubated with the Fn fibers. In order to examine the differences between two normalization schemes, (c) the BSA-633 intensity was divided by the sum of the Fn donor and acceptor intensities,  $I_A+I_D$ , or (d) just the acceptor intensity,  $I_A$ , alone by illuminating by directly exciting the acceptor. Similar data (e-h) are shown for soluble Fn-633 adsorbed to Fn fibers. For the ratios  $633/(I_A+I_D)$  and  $633/(I_A)$ , the data were binned so that the mean plus or minus two standard deviations is shown within each bin that contained at least 15 data points. Scale bar = 30  $\mu\text{m}$ .

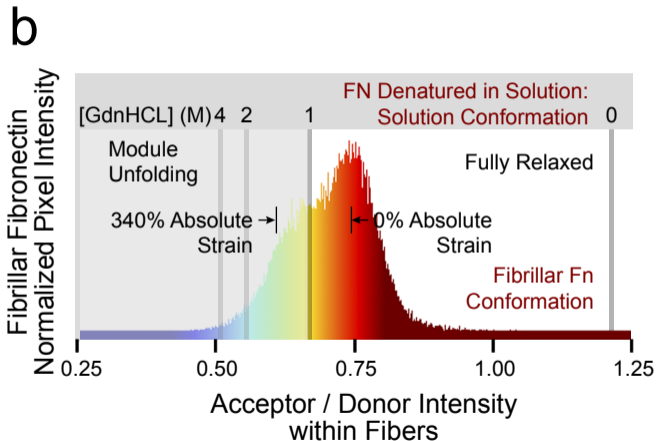
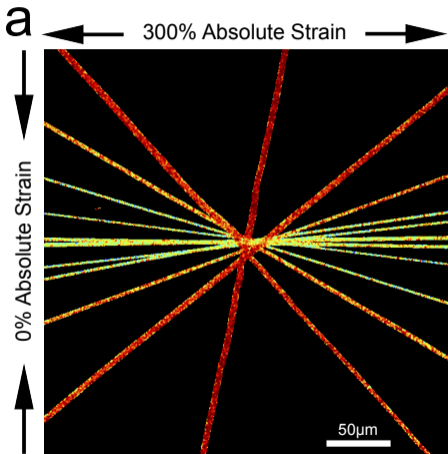
Figure S4. Data averages obtained from 10 fields of views showing strain-upregulated binding of bovine serum albumin, casein and of all proteins in whole bovine serum to Fn fibers. An explanation of how the data is displayed can be found in the caption of figure 1.

Figure S5. Data averages obtained from 10 fields showing strain-upregulated binding of soluble Fn and the 70kD Fn fragment to Fn fibers. An explanation of how the data is displayed can be found in the caption of figure 1.

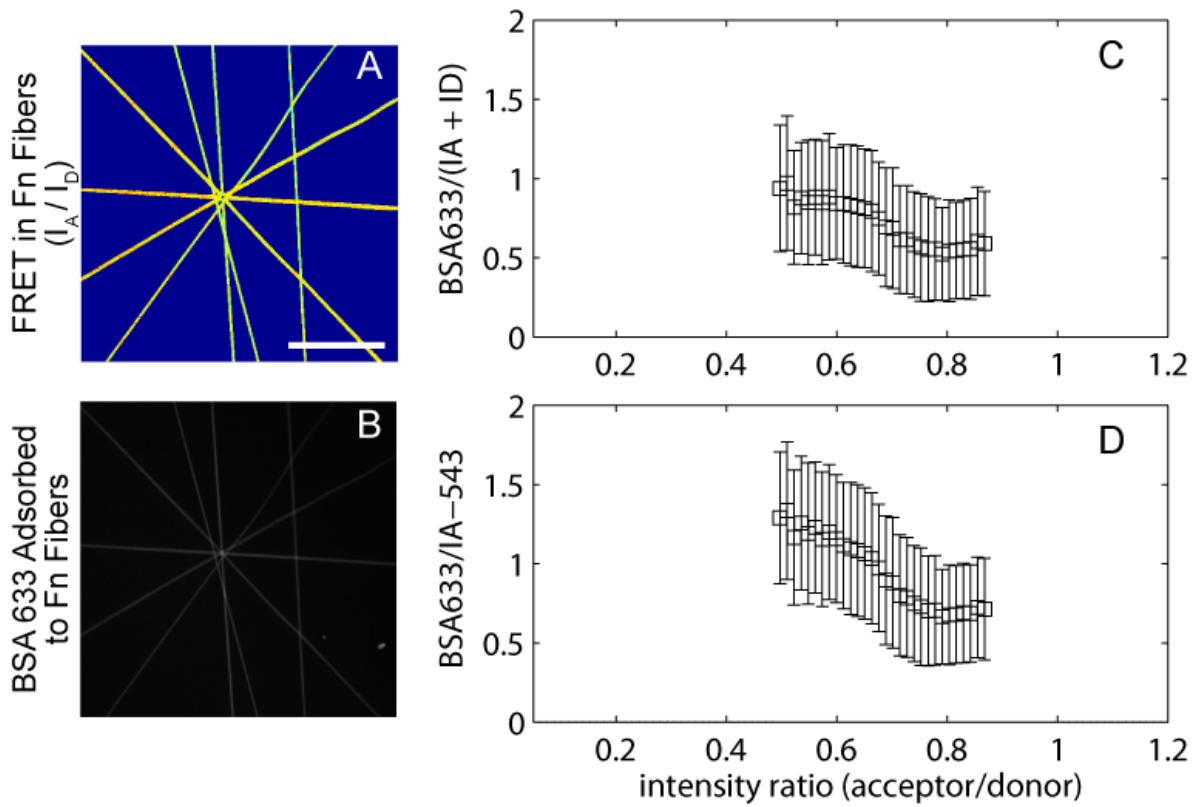
Figure S1



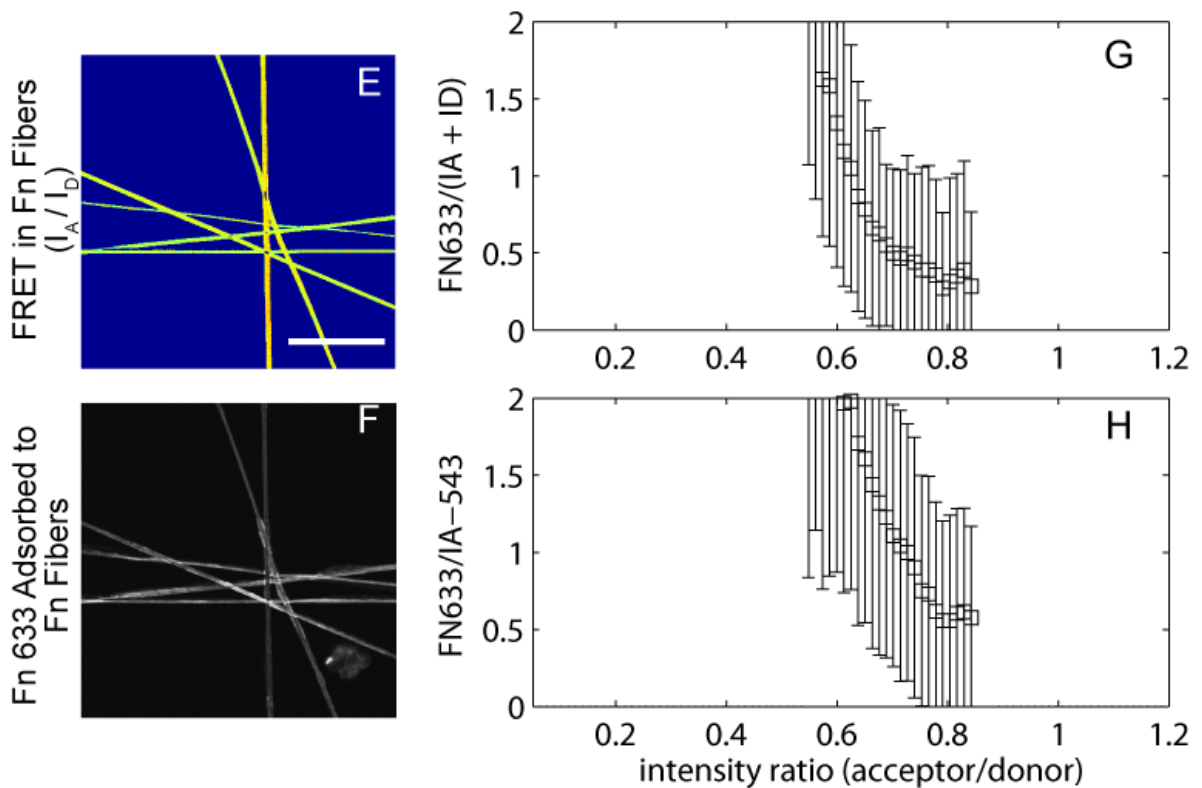
# Figure S2



### albumin colocalization to fibronectin fibers

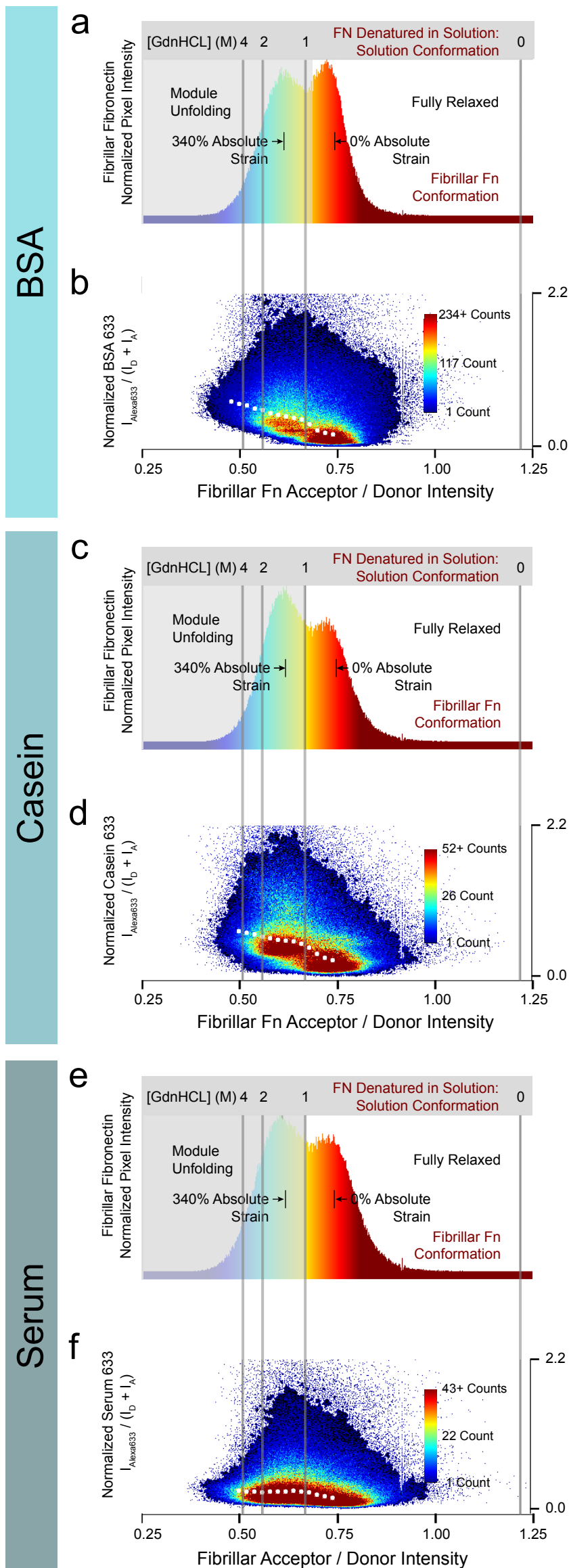


### soluble fibronectin colocalization to fibronectin fibers



# Figure S4

## Composite of Amphipathic protein binding



# Figure S5

Composite of Fn and Fn fragment binding

