## Supplemental Figures

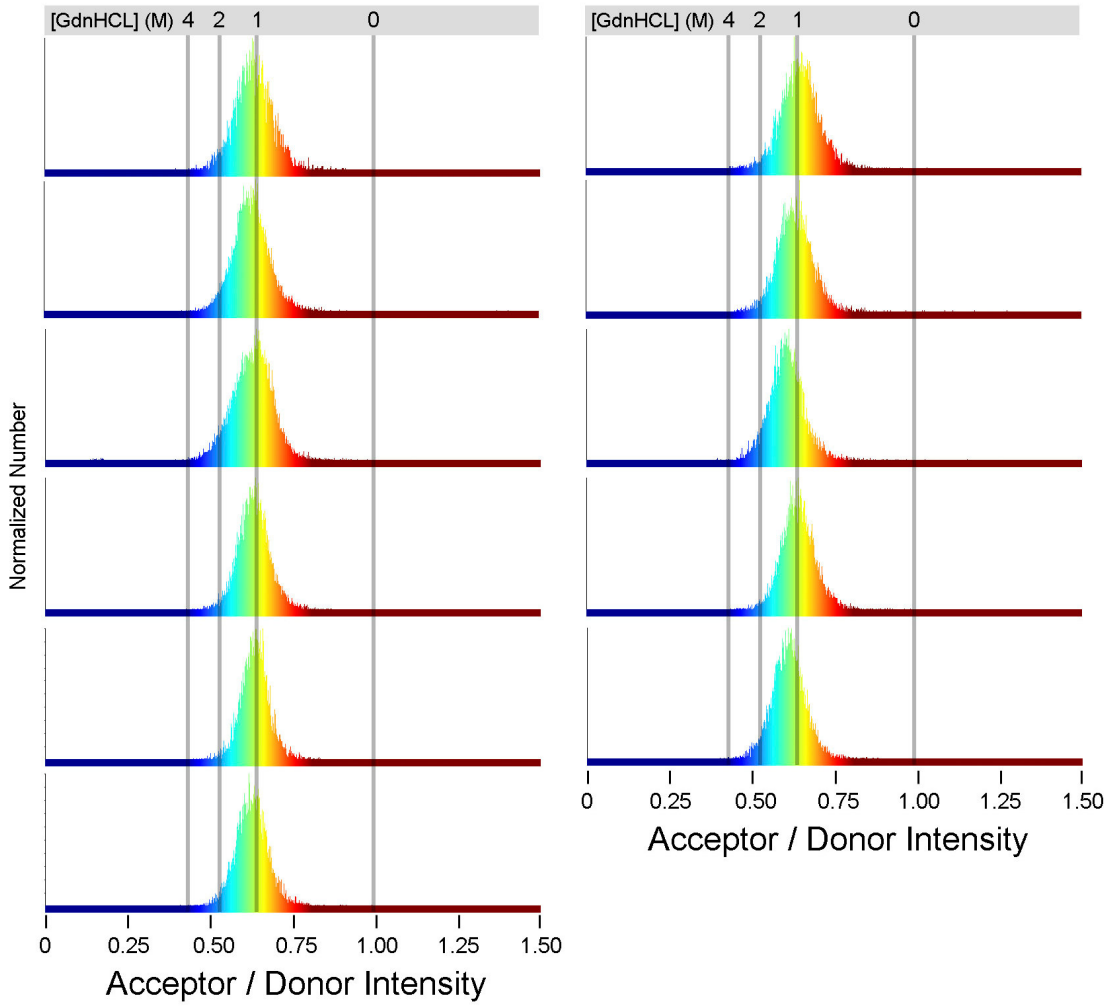


Fig S1. Acceptor / donor intensity ($I_A/I_D$) histograms of the eleven manually deposited fibronectin (Fn) fibers shown in Figure 2*B*. Fn fibers containing 5% plasma fibronectin dual-labeled with donors and acceptors (Fn-d/a - see *Methods*) are manually deposited onto silicone substrates and imaged via confocal laser scanning microscopy to capture peak intensities of both the donors and acceptors. The acceptor / donor intensities of each pixel after processing (see *Methods)* are mapped to a false color scheme and included in the displayed histograms.

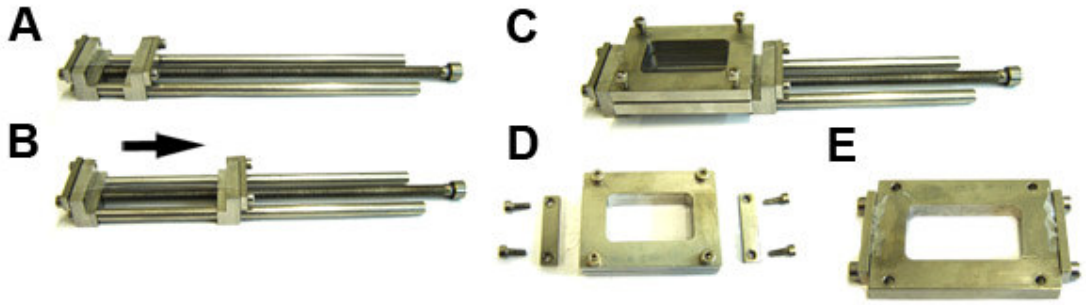


Fig S2. One-dimensional strain device: (A) A stainless-steel strain device was custom-built to hold a transparent silicone sheet and (B) apply defined amounts of strain in one-dimension. (C) For microscopic imaging of a strained sample, two steel rectangular clamps are fastened together with screws and the sheet is removed from the strain device. (D) In order for a microscope objective to physically contact the substrate for imaging, the silicone sheet must next be fastened to the top rectangle by screwing metal clamps to the side that hold the sheet in place and (E) the bottom rectangle is removed.

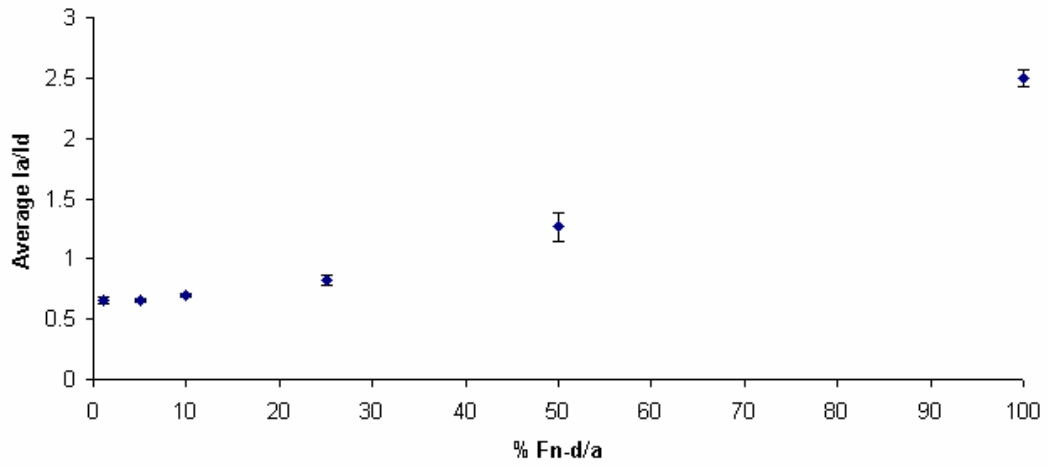


Fig S3. Average Acceptor / Donor intensity (Ia/Id) versus percent of fibronectin (Fn) labeled with acceptor and donor fluorophores (Fn-d/a) mixed with unlabeled Fn (Fn-u). The average Ia/Id ratio ± standard deviation from at least 10 manually deposited Fn fibers for each of the six data points containing the indicated percent of Fn-d/a mixed with Fn-u are shown. Fibers were deposited onto silicone sheets for imaging as described in *Methods*. The plot justifies the use of 5% Fn-d/a in experiments with manually deposited Fn fibers to prevent inter-molecular FRET.