

Supplementary Figure 1. Fluorescence emission spectra of fibronectin labeled with AlexaFluor 488 carboxylic acid (donor) and AlexaFluor 546 maleimide (acceptor) molecules on random amines and free cysteines (FnIII₇ and FnIII₁₅). The labeled fibronectin was subjected to series denaturation using guanidine hydrochloride in phosphate buffer and excited near the donor absorption wavelength ($\lambda_{\text{max}} = 495$ nm). Unconjugated AlexaFluor 488 emission maximum is 520 nm, AlexaFluor 546 ($\lambda_{\text{max}} = 554$) emission maximum is 570 nm. (a) The spectra were normalized to the peak donor emission. FRET efficiency is basically eliminated at 6 M guanidine hydrochloride. The spectral data was averaged over 5 labeled protein samples prepared and tested on separate days. The remaining acceptor emission at 6.0 M guanidine hydrochloride is due to intermolecular forces. (b) Calibration curve of fibronectin denaturation.

