Supporting information

Origin of the Nonadhesive Properties of Fibrinogen Matrices Probed by Force Spectroscopy

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Figure S1. Kinetics of fibrinogen adsorption on mica. Different concentrations of ¹²⁵I-labeled fibrinogen (0.25-3 μ g/ml) were incubated with freshly cleaved mica for various times at 37 °C. Excess fibrinogen was removed by rinsing mica with water and bound fibrinogen was determined in a γ counter.



Figure S2. Force map showing the interaction between an unmodified AFM tip and a substrate prepared by adsorption of 0.6 μ g/ml fibrinogen.



Figure S3. Dependence of adhesion forces observed between the AFM tip and adsorbed fibrinogen on substrate contact (dwell) time. The dwell time, which is the total time span from the moment when the tip contacted the sample during the approach the first time until it left the sample during retraction was calculated as previously described¹. Adhesive surfaces were prepared by adsorption of 0.7, 1.5 and 20 μ g/ml fibrinogen and adhesion forces were determined for contact times of the tip with the surfaces between 0.02 and 0.51 s.

(1) Fuhrmann, A., Schoening, J. C., Anselmetti, D., Staiger, D., and Ros, R. Quantitative analysis of single molecule RNA-protein interaction, *Biophysical Journal* **2009**, *96*, 5030-5039.



Figure S4. 2D histograms generated from the force-distance curves obtained on surfaces prepared by adsorption of 5 and 20 μ g/ml fibrinogen (red, high frequency; blue, low frequency). Fg, fibrinogen.



Figure S5. The 1D and 2D histograms generated from the force-distance curves obtained on surfaces prepared by a consecutive addition of 0.6 and 0.9 μ g/ml fibrinogen, in the presence of NHS or BS³.

Table S1

Molecular extensions and compliance (dl/dF) of fibrinogen matrices determined from forceextension curves at 50 pN applied tensile force.

| Concentration, µg/ml | <i>l</i> , nm | d <i>l</i> /d <i>F</i> , nm/pN |
|-------------------------|---------------|--------------------------------|
| BS^3 type 1 | 0.1±0.1 | 0.005 ± 0.001 |
| BS ³ type 2 | 2.8±0.5 | 0.190 ± 0.021 |
| NHS type 1 | 0.2±0.1 | 0.008 ± 0.004 |
| NHS type2 | 3.6±0.6 | $0.120{\pm}0.040$ |