Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Insights into the Biophysical Forces Between Proteins Involved in Elastic Fiber Assembly

Sean O'Neill Moore, Tyler Jacob Grubb, Chandrasekhar R. Kothapalli*

Supplementary Information

Table S1. The coating conditions for various proteins on cantilever tip or glass slide, prior to testing with AFM.

Protein	Coating	Concentration (µg/mL)	Amount (µL)	Incubation time, h	Details
Tropoelastin	AFM tip	20	5	3	Washed with PBS
LOXL2	AFM tip	1	5	2	Washed with PBS
Fibulin-5	AFM tip	20	5	3	Washed with PBS
Tropoelastin	Cover glass	20	5	3	Submerged in 4 mL PBS
GLB-1	Cover glass	50	5	overnight	Submerged in 4 mL PBS
Fibulin-5	Cover Glass	20	5	3	Submerged in 4 mL PBS
LOXL2	Cover glass	1	5	2	Submerged in 4 mL PBS
Fibrillin-1	Cover glass	50	5	overnight	Submerged in 4 mL PBS
Elastin	Cover glass	20	5	overnight	Submerged in 4 mL PBS
Laminin-1	Cover glass	1000	5	3	Submerged in 4 mL PBS
Rat Collagen-1	Cover glass	50	5	3	Submerged in 4 mL PBS
Human Collagen-1	Cover glass	50	5	3	Submerged in 4 mL PBS

Table S2. Number of data points collected for n each combination with AFM testing.

Tip coating	Glass slide coating	Number of data points
Tropoelastin	Uncoated	33
	Laminin	32
	Rat-tail derived type I collagen	30
	Human tissue derived type I collagen	31
	Tropoelastin	53
	GLB-1	29
	Fibulin-5	31
	LOXL2	32
	Fibrillin-1	34
	Mature elastin	36
	Tropoelastin + LOXL2	38
	Fibrillin-1 + LOXL2	34
Blank tip	Uncoated	34
	Laminin	32
	Rat-tail derived type I collagen	32
	Human tissue derived type I collagen	30
	Tropoelastin	56
	GLB-1	37
	LOXL2	32
	Fibrillin-1	34
	Tropoelastin + LOXL2	34
	Mature elastin	29
	Fibulin-5	32
LOXL2	Fibrillin-1	39
	Fibulin-5	32
Fibulin-5	Fibrillin-1	31

Table S3. Hydrodynamic radii (R_h) of proteins tested in this study. The R_h values of proteins not involved in elastogenesis were also computed. The molecular weight of Nidogen-1 is ~139 kDa. The values were computed using approaches described by Wilkins et al[1].

[1] Wilkins DK, Grimshaw SB, Receveur V, Dobson CM, Jones JA, Smith LJ. Hydrodynamic radii of native and

Protein	R_h, nm
Tropoelastin	3.06
GLB-1	3.14
Fibulin-5	3.01
LOXL2	3.28
Fibrillin-1	2.57
Mature elastin	3.1
Laminin-1	6.37
Rat Collagen	3.77
Human Collagen	3.69
Nidogen-1	3.77

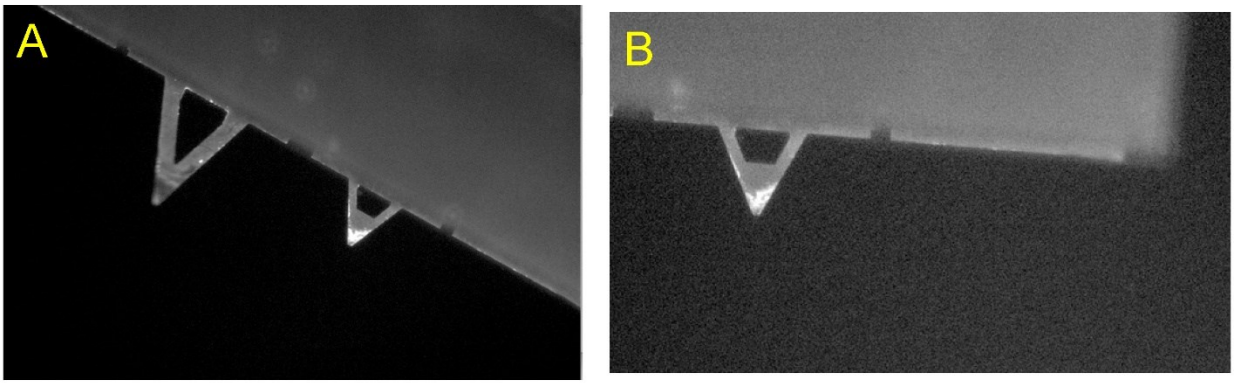

Figure S1. Representative immunofluorescence images of protein-coated silicon nitride cantilever tips obtained using an Axio Vert.A1 light microscope. Here, tropoelastin coating was detected on the cantilever tip using respective primary and secondary antibodies.

denatured proteins measured by pulse field gradient NMR techniques. Biochemistry, 1999; 38(50): 16424-31.

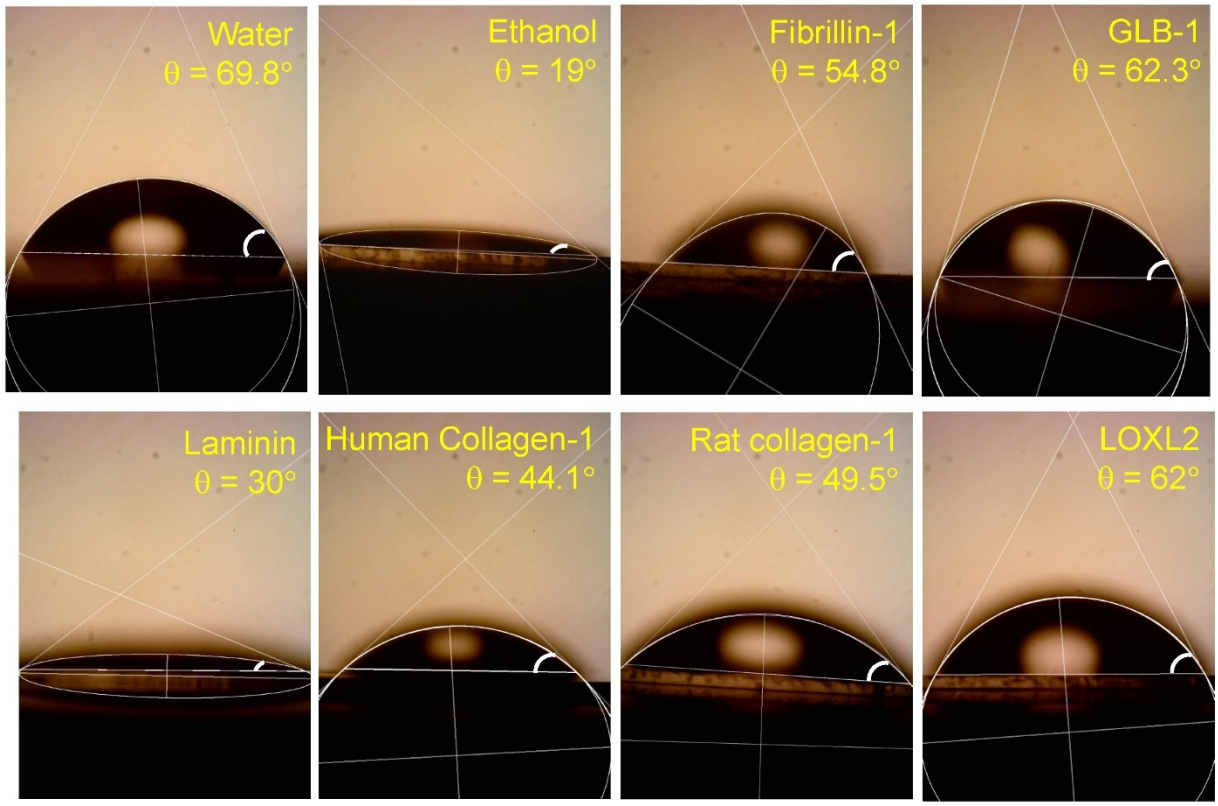

Figure S2. Representative contact angle measurements using a Rame-Hart model 200 standard contact angle goniometer and ImageJ built in contact angle software. Contact angle measurement for water on a non-protein coated surface and for ethanol on a non-protein coated surface. Contact angle measurements for water on selected protein-coated surfaces were shown for comparison. The values shown here are averages from multiple measurements for each case. ImageJ's built in software measures the contact angle using the sphere and ellipse approximations. Images were analyzed using ImageJ's contact angle plug-in which uses the sphere approximation ($\theta = 2arctan(\frac{2h}{l})$), where h and l are the height and length of the tangent lines from the surface, air, droplet interface, and ellipse approximation as previously described[2].

[2] Lamour G, Hamraoui A, Buvailo A, Xing Y, Keuleyan S, Prakash V, Eftekhari-Bafrooei A, Borguet E. Contact angle measurements using a simplified experimental setup. *J Chem Educ*. 2010, 87, 12, 1403-1407.

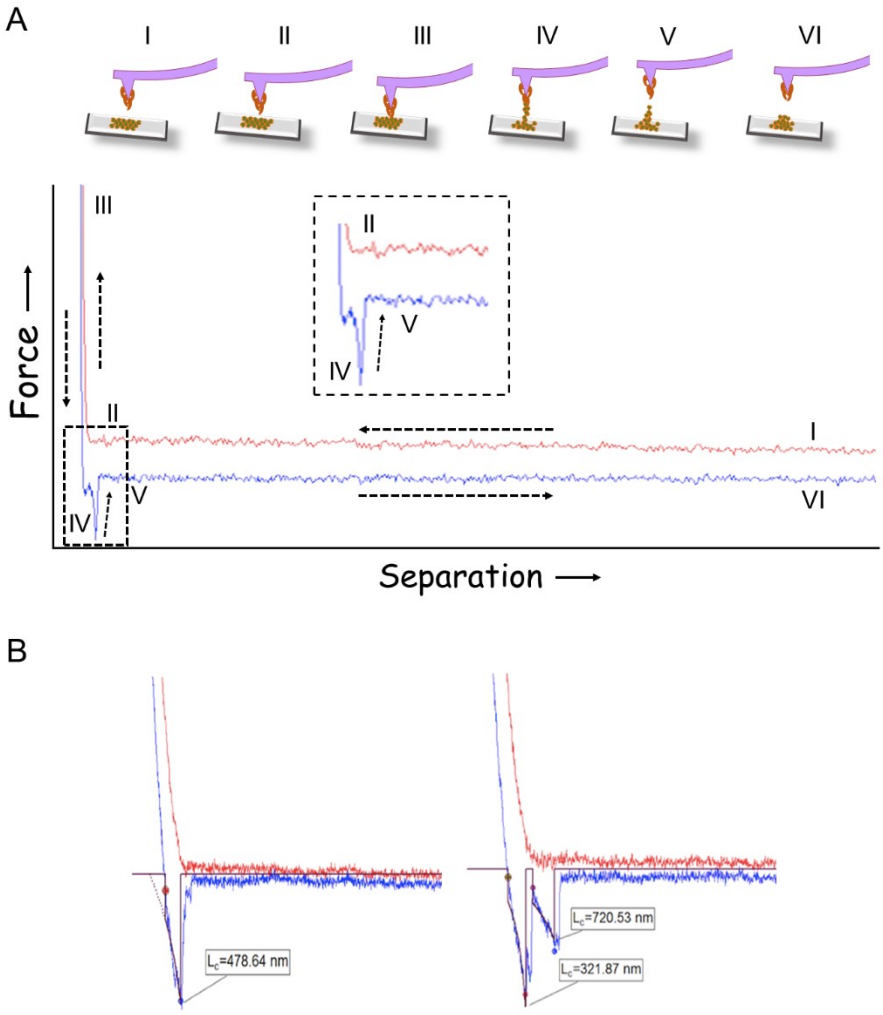

Figure S3. (**A**) A representative force deflection curve produced by AFM and schematics of corresponding cantilever placement along the z-axis for clarity. **(I-II)** Probe approaches and contacts sample. **(II-III)** Repulsive forces exhibited by probe indentation on sample. **(III-IV)** Probe retracts and forces switch from repulsive to attractive **(IV-V)** until it ultimately traces back to its starting position **(VI)**. Adhesion force measured as difference between maximum attractive deflection and zero probe deflection. Note. The difference in positioning between **I** and **VI** can be contributed to hysteresis within the cantilever deflection. (**B**) A Worm-Like Chain (WLC) model fitted to force-separation curve of non-coated AFM tip and GLB-1**. (Left)** WLC model fitted for single molecule stretching of GLB-1. **(Right)** WLC model fitted for multiple molecule stretching of GLB-1. **Note**. L_C corresponds to the contour length determined for each individual molecule stretching obtained by WLC model.

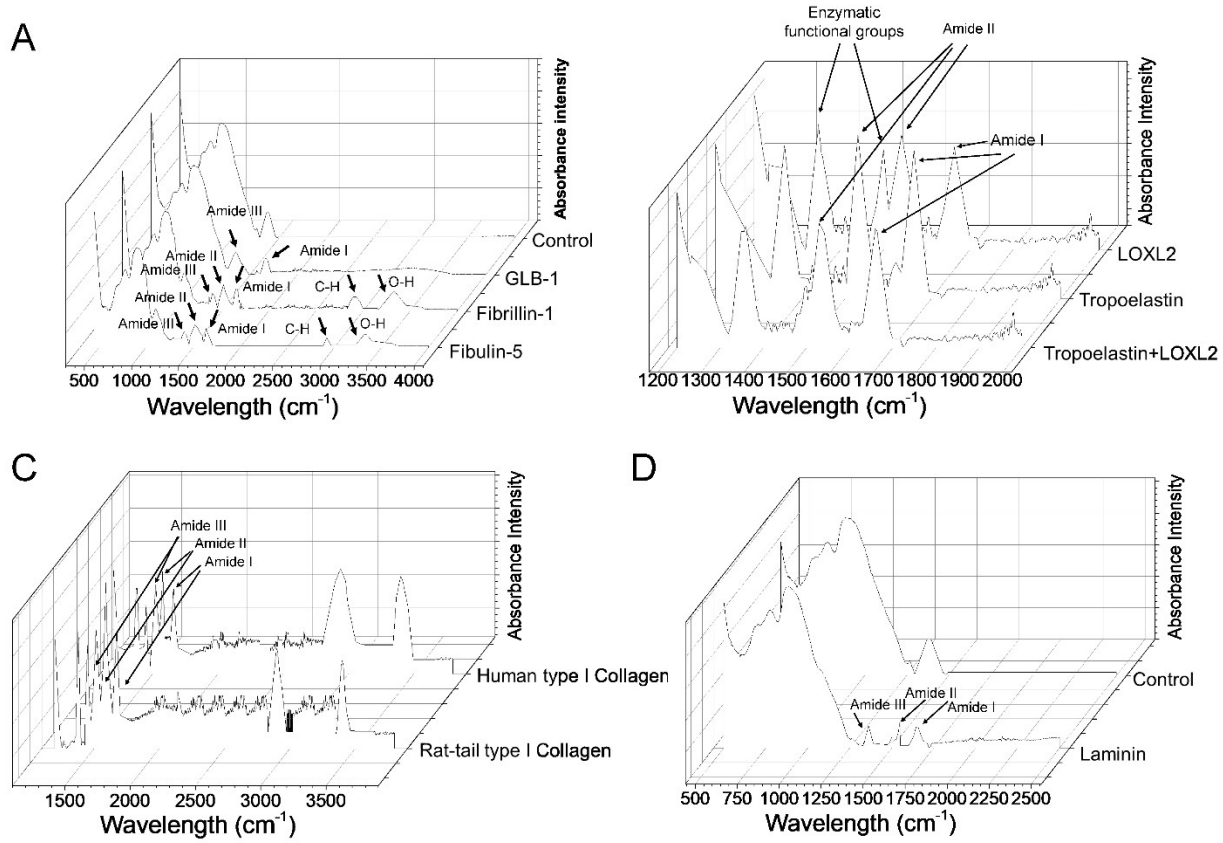

Figure S4. (**A**) FTIR absorbance intensity for proteins fibrillin-1, GLB-1, and fibulin-5. Fibrillin-1 and fibulin-5 show specific protein associated chemical groups at ~3333 cm^{-1}, ~2917 cm^{-1}, ~1700 cm^{-1}, ~1550 cm^{-1}, ~1427 cm^{-1}, and ~1375 cm^{-1}. The peaks at ~3333 cm^{-1} and ~2917 cm^{-1} show the O-H and the stretching of carbon-hydrogen bonds. GLB-1 shows similar peaks at the amide I wavelength (~1700 cm^{-1}) and amide III wavelength (~1427 cm^{-1}). (**B**) FTIR absorbance intensity over wavelengths 2000 to 1200 cm^{-1} for LOXL2, tropoelastin, and the cover glass coated with the combination of LOXL2 and tropoelastin. Individual amide I, II, and III peaks can be seen for both LOXL2 and tropoelastin separately, as well as the absorbance bands for when both proteins were coated together. LOXL2 has dominating peaks at wavelengths of ~1346 cm^{-1} and ~1529 cm^{-1} while tropoelastin has peaks at corresponding ~1650 cm^{-1} and ~1530 cm^{-1} wavelengths. (**C**) FTIR absorbance intensity for human collagen-1 and rat tail collagen-1. Individual peaks associated with both sets of collagen-1 can be seen at ~3400 cm^{-1}, ~2900 cm^{-1}, ~1630 cm^{-1}, ~1600 cm^{-1}, ~1550 cm^{-1}, and ~1400 cm^{-1}. The amide I, II, and III groups can be seen at the corresponding wavelengths of ~1630 cm^{-1}, ~1600 cm^{-1}, ~1550 cm^{-1}, and ~1400 cm^{-1}. (**D**) FTIR absorbance intensity for laminin-1. Laminin-1 has absorption peaks at the amide I, II, and III regions.

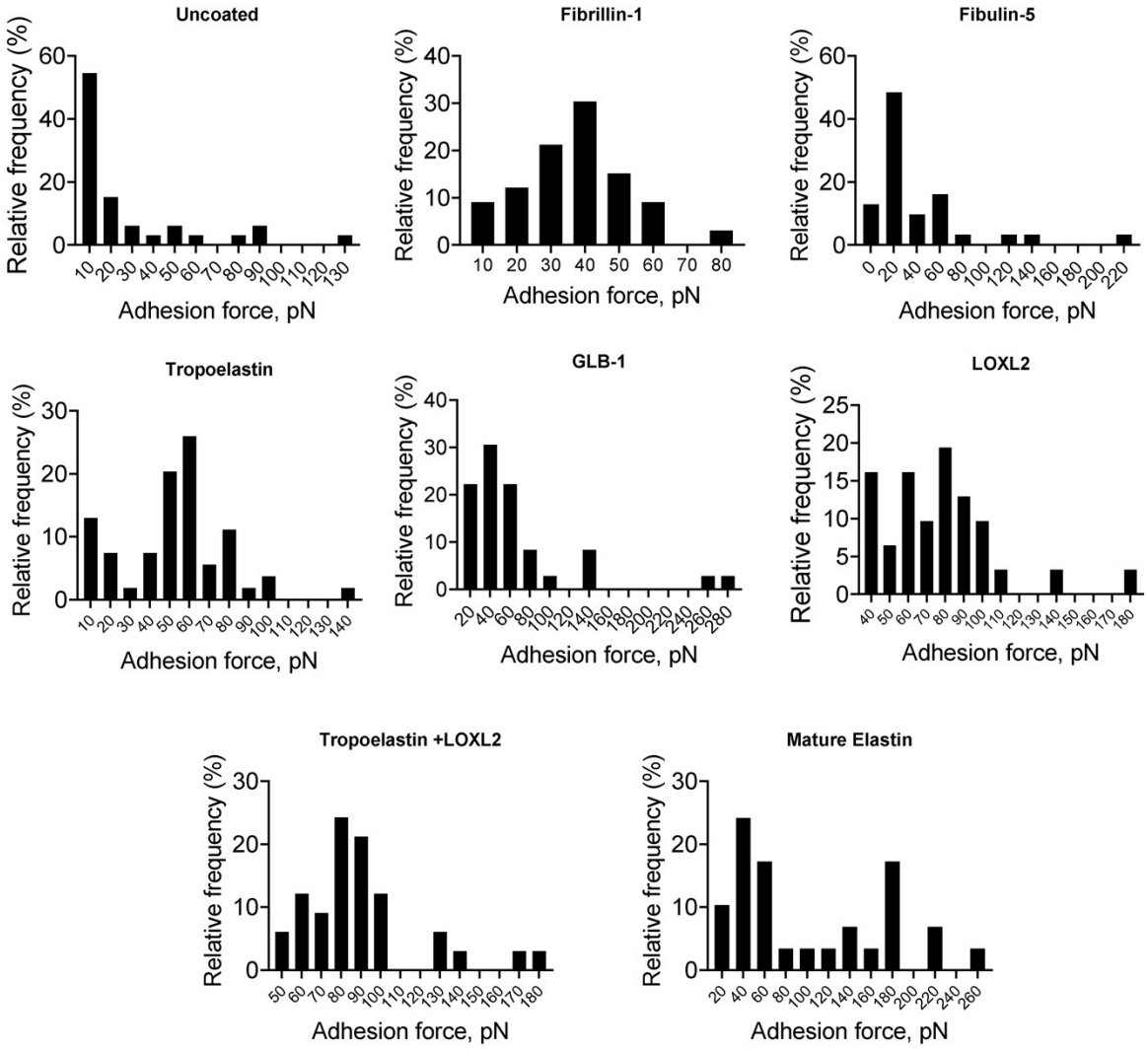

Figure S5. Frequency distributions of adhesion force measurements of proteins involved in elastic fiber synthesis using a non-coated cantilever tip (data shown in Fig. 3B).

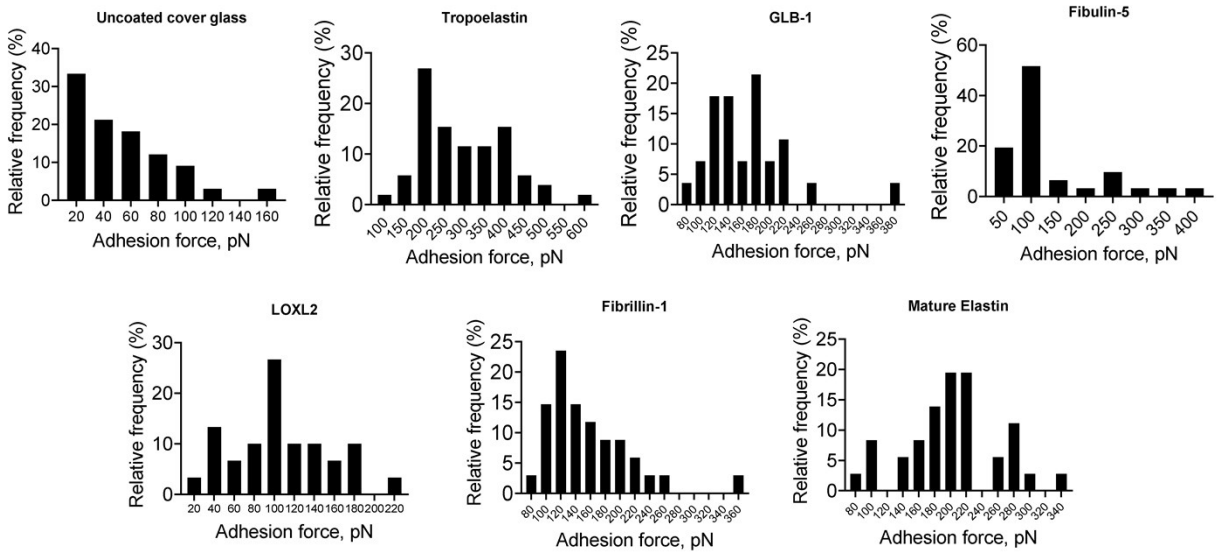

Figure S6. Frequency distributions of adhesion force measurements of proteins involved in elastic fiber synthesis using a tropoelastin-coated cantilever tip (data shown in Fig. 3C).

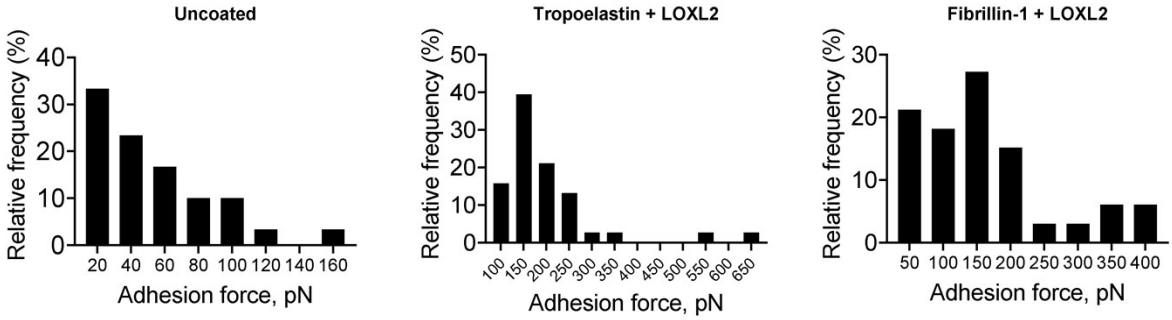

Figure S7. Frequency distributions of adhesion force measurements of proteins involved in elastic fiber synthesis: Tropoelastin-coated tip was used to obtain adhesion force measurements when more than one protein was coated on the cover glass, primarily to assess the effect of cross-linker LOXL2. Data was shown in Fig. 3D.

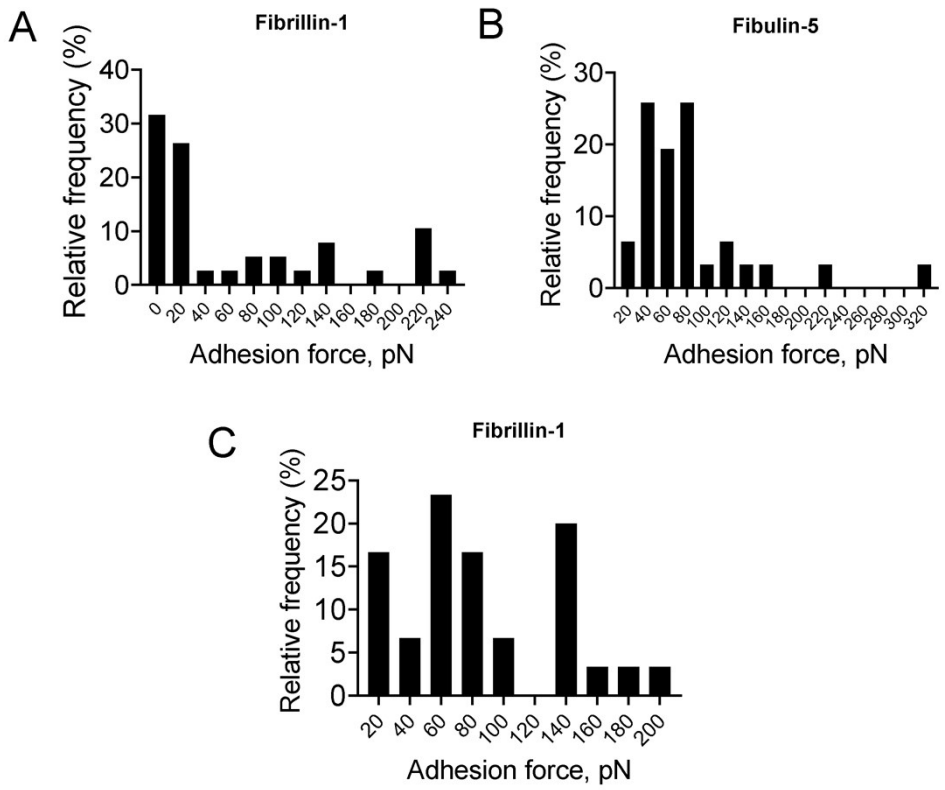

Figure S8. Frequency distributions of adhesion force measurements of proteins involved in elastic fiber synthesis: Interaction of LOXL2-coated cantilever tip with (A) fibrillin-1 and (B) fibulin-5, or (C) fibulin-5-coated cantilever tip with fibrillin-1. Data was shown in Fig. 3E.

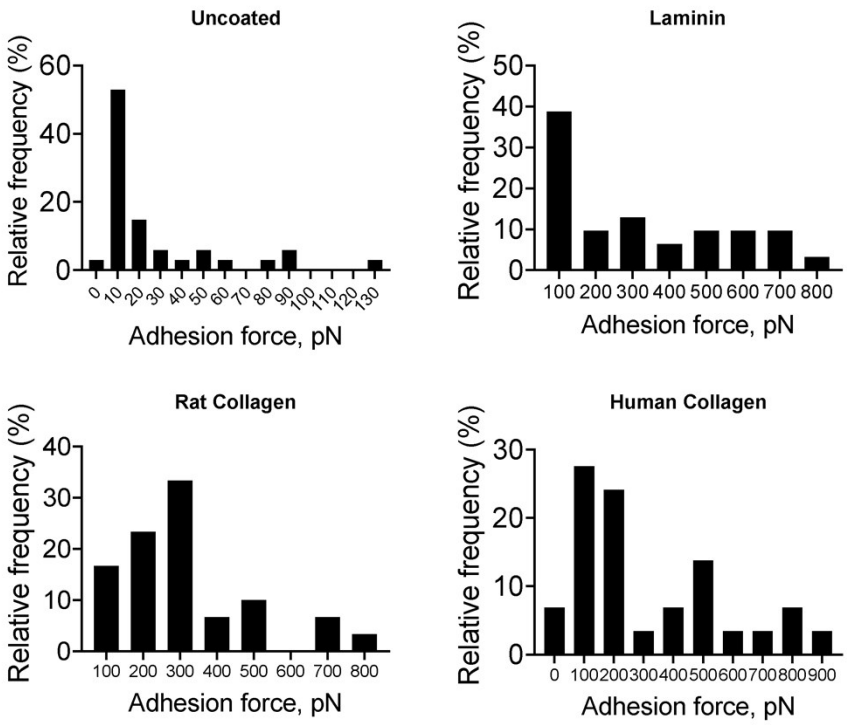

Figure S9. Frequency distributions of adhesion force measurements of proteins involved in elastic fiber synthesis: Non-coated cantilever tip was used to measure adhesive forces of ECM proteins not involved in elastic fiber synthesis, i.e., laminin and type I collagen (rat or human tissues derived). Data was shown in Fig. 3F.

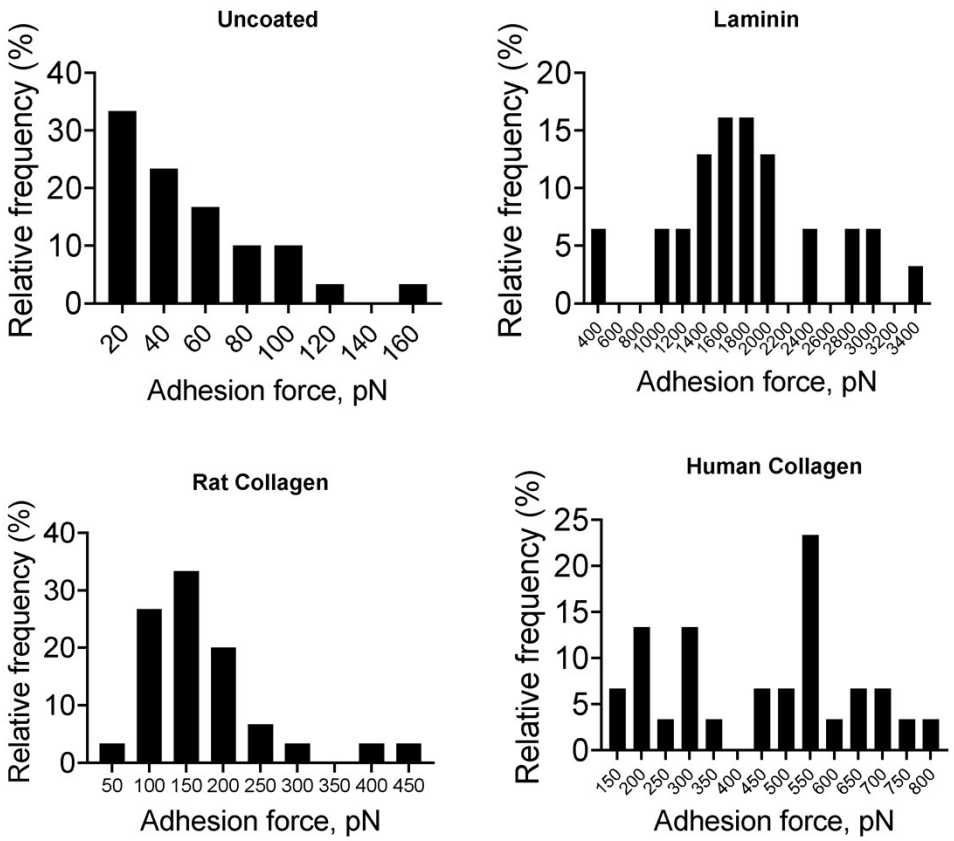

Figure S10. Frequency distributions of adhesion force measurements of proteins involved in elastic fiber synthesis: Tropoelastin-coated cantilever tip was used to measure adhesive forces of ECM proteins not involved in elastic fiber synthesis, i.e., laminin and type I collagen (rat or human tissues derived). Data was shown in Fig. 3G.