Supporting Material

The α-Helix to β-Sheet Transition in Stretched and Compressed Hydrated Fibrin Clots

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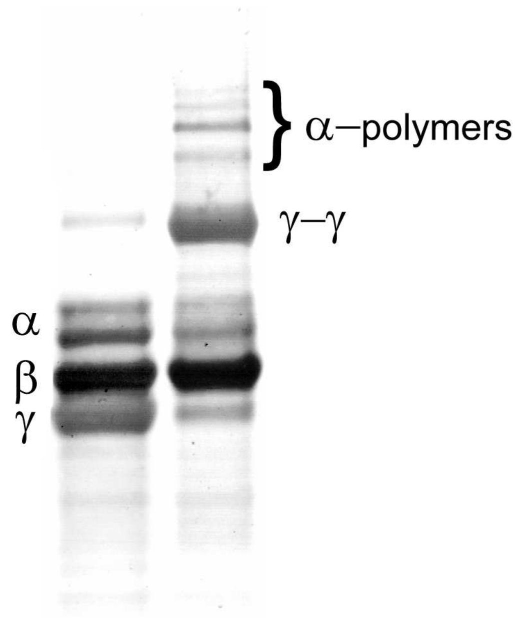

Figure S1. SDS-PAGE in reducing conditions showing the polypeptide composition of mostly uncross-linked human fibrin (left lane) and fibrin heavily cross-linked with the plasma transglutaminase, factor XIIIa (right lane). The cross-linking results in formation of the γ-chain dimers and α-chain polymers which dramatically change mechanical and rheological properties of individual fibrin fibers and an entire clot (1-3).

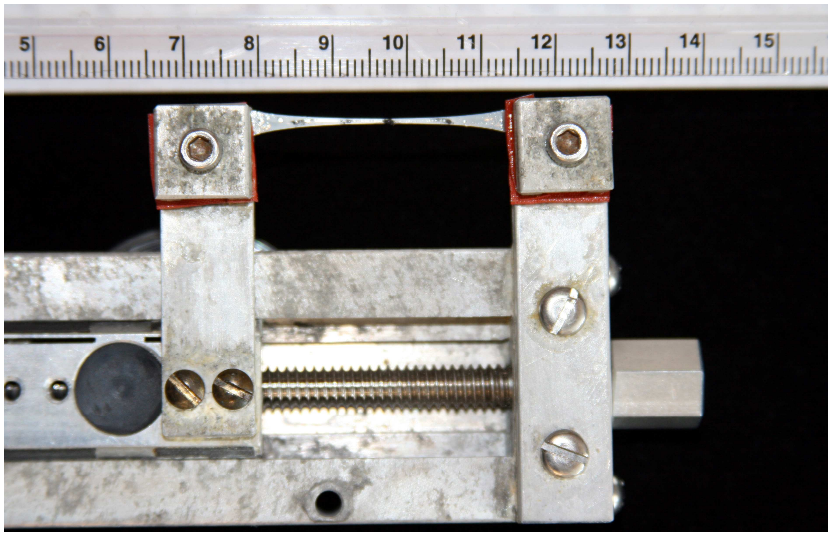

Figure S2. Photograph of a cylindrical plasma clot stretched 3-fold in the custom-built stretching device.

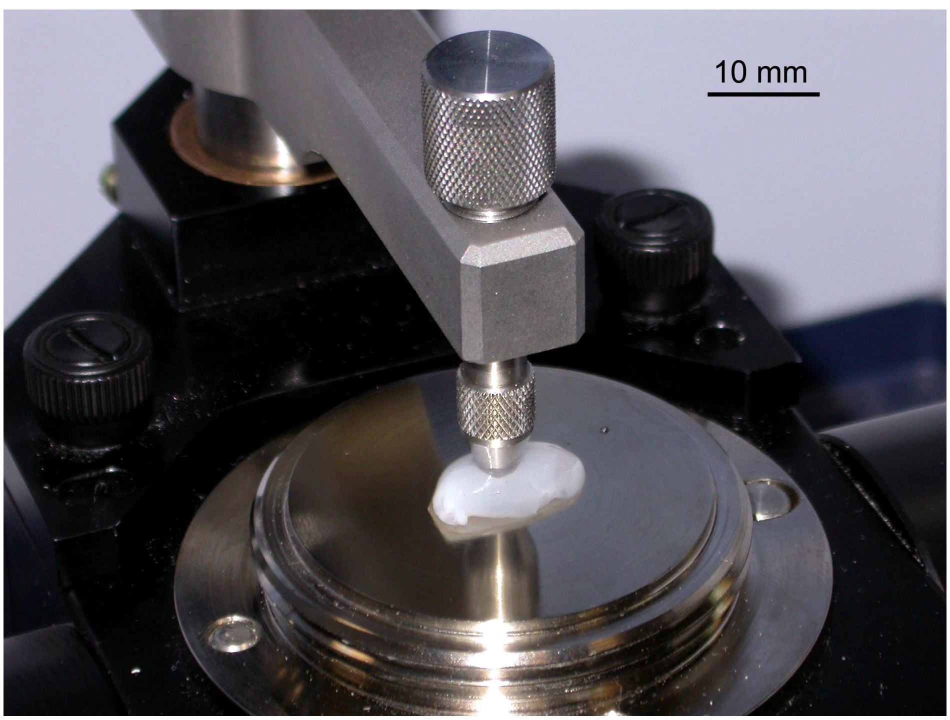

Figure S3. Photograph of a plasma clot compressed directly on the ATR crystal with a pressure calibrated metal knob. The compressed area represents a 2-mm-circle equal to the detecting spot of the ATR crystal.

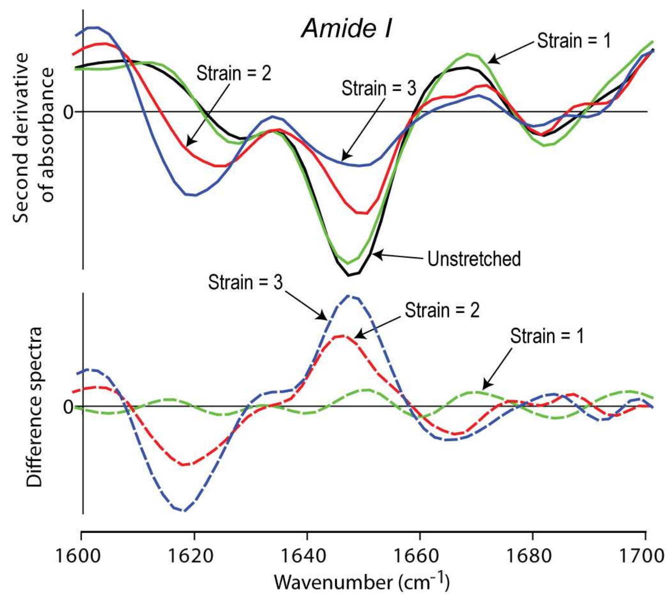

Figure S4. The second derivative spectra of the amide I band of unstretched clot (black line) and the same clot after 2-fold (green solid line), 3-fold (red solid line) and 4-fold (blue solid line) elongation along with their difference spectra (dashed lines of the corresponding color) obtained by subtraction of the second derivative for the initial unstretched clot. The sine-like difference spectrum for strain 1 (green dashed line) reflects minor random fluctuations around zero. Strain is defined as "stretched length/initial length-1". The curves are derived from the raw data shown in Fig. 2.

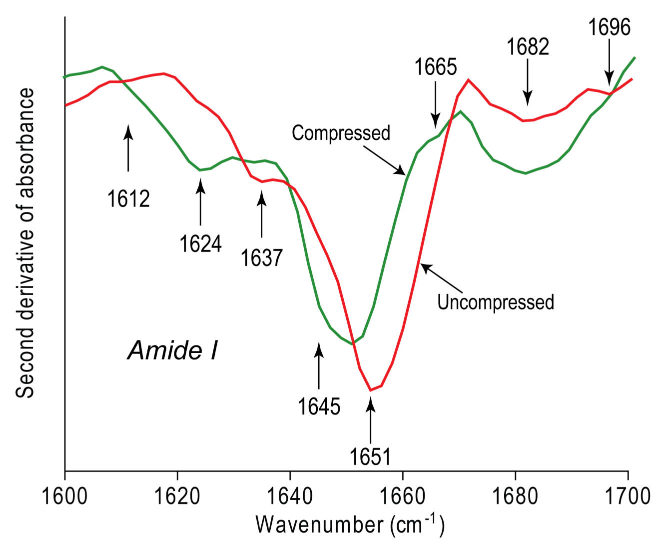

Figure S5. The second derivative spectra of the amide I band of uncompressed (red) and compressed (green) fibrin clots. Peak positions of underlying components are shown by arrows.

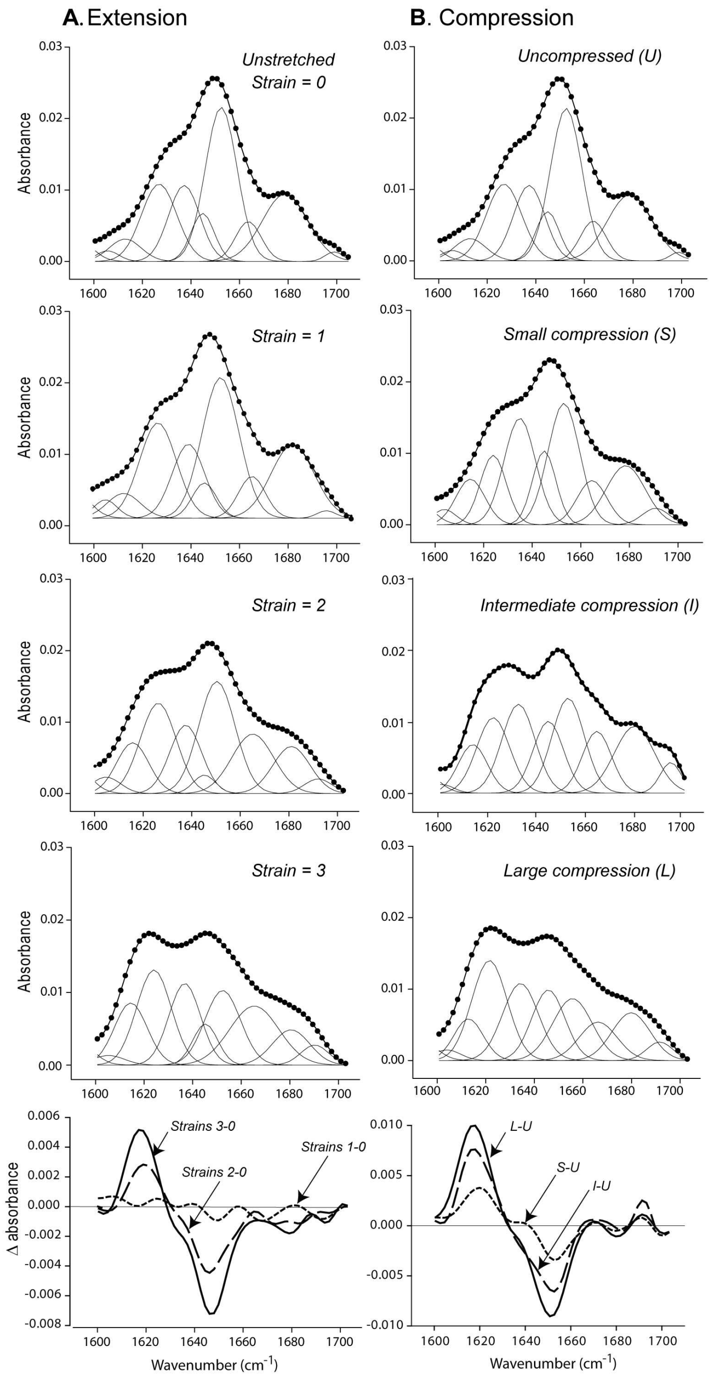

Figure S6. Deconvolution of resolution-enhanced FTIR ATR spectra (amide I band) of fibrin clots: (**A**) unstretched (strain 0) and stretched at three different strains (strains 1, 2, and 3) along with the difference spectra (bottom) for each strain (1-0, 2-0, and 3-0, respectively) obtained by subtraction of the initial spectrum for the unstretched clot. (**B**) Uncompressed (U) and compressed at a relatively small (S, 10-50 bar), intermediate (I, 100-150 bar), and large (L, 200-250 bar) pressure along with the difference spectra (bottom) for each compression (S-U, I-U, and L-U, respectively) obtained by subtraction of the initial spectrum (U) for the uncompressed clot.

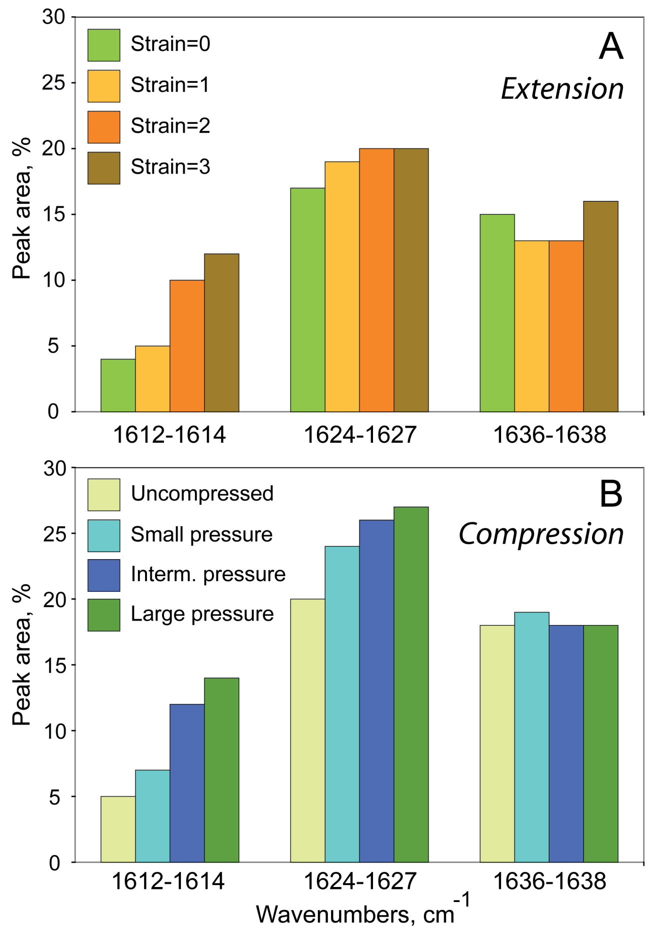

Figure S7. Changes in the proportion of major types of the β-structures peaked at 1612-1614 cm^{-1}, 1624-1627 cm^{-1}, and 1636-1638 cm^{-1} in FTIR spectra of fibrin clots at different degrees of extension (**A**) and compression (**B**). The figure is based on the data in Tables S1-S8.

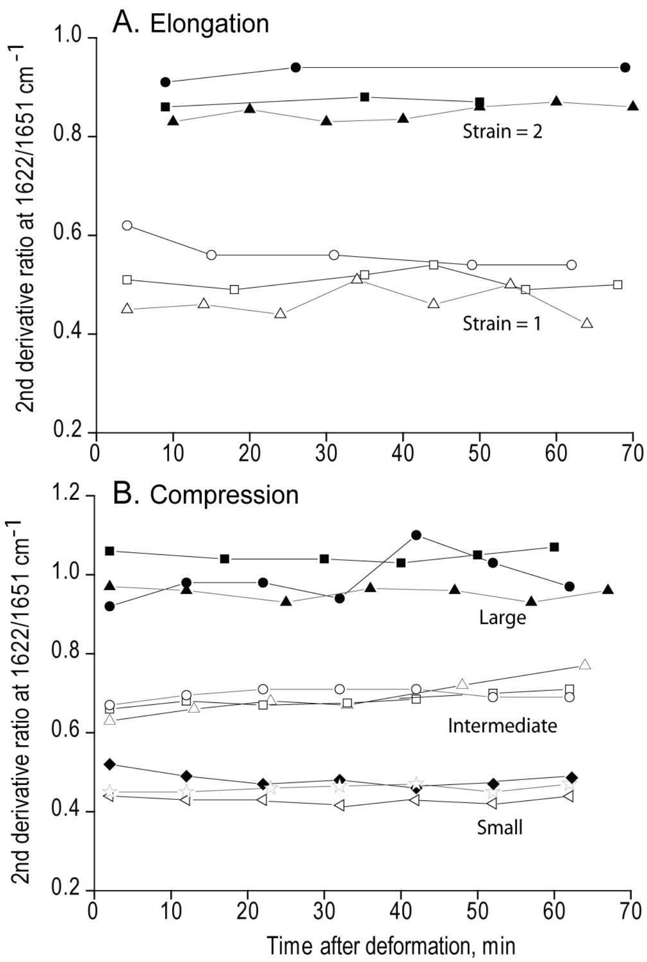

Figure S8. Time course of the deformation-induced changes in FTIR ATR spectra of fibrin clots. The ratio of second derivative absorbance intensities at 1622/1651 cm^{-1} in (**A**) stretched fibrin clots at different strains (1 and 2) and (**B**) in compressed fibrin clots at different pressures (small, intermediate, and large) as a function of time after the deformations. Three individual clots were followed under each experimental condition of deformation.

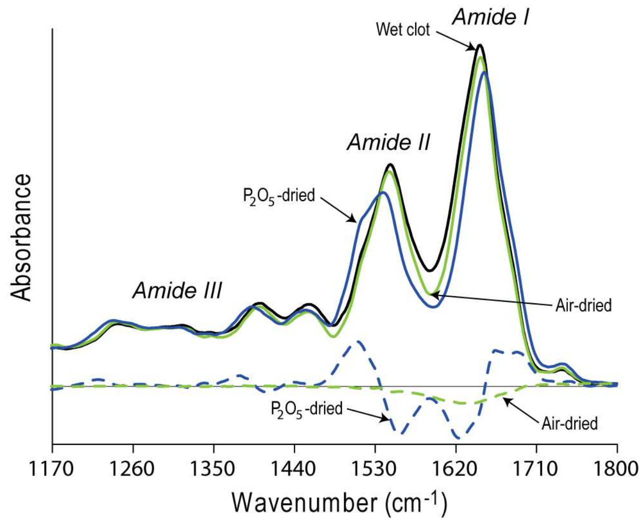

Figure S9. FTIR ATR spectral changes during stepwise dehydration of a plasma clot. Black spectrum – initial wet clot, green solid spectrum – the air-dried clot (dashed green line – the corresponding difference spectrum), blue solid spectrum – the clot thoroughly dried over P_2O_5 (dashed blue line – the corresponding difference spectrum).

Quantification of the secondary structure elements

Table S1. Unstretched fibrin clot

Centroid, cm^{-1}	*Height*	*Area*	*FWHM, cm^{-1}*	*Assignment*
1698.92	0.00133	0.01054	8.64395	β-structures
1678.73	0.00943	0.1891	21.9296	turns
1663.61	0.00557	0.06375	12.5082	turns/loops
1652.45	0.02158	0.30371	15.3883	α-helix
1644.8	0.00672	0.06909	11.2354	random coils
1637.22	0.01064	0.14696	15.0989	β-structures
1626.89	0.01083	0.16937	17.1033	β-structures
1612.9	0.00314	0.04428	15.4101	β-structures

Summarized content

	Mean value	Standard deviation	COV*
α-helix	0.30371	0.0355	0.117
β-sheets	0.37115	0.03934	0.106
turns/random coils/loops	0.32195	0.0314	0.097

*Coefficient of variation (COV) = (Standard deviation) / (Mean value)

Table S2. 2-fold stretched fibrin clot (strain=1)

Centroid, cm^{-1}	*Height*	*Area*	*FWHM, cm^{-1}*	*Assignment*
1693.07	8.36519E-4	0.0077	8.64394	β-structures
1679.7	0.00822	0.17648	20.1596	turns
1663.61	0.00468	0.06231	12.5082	turns/loops
1651.2	0.01577	0.28886	17.2041	α-helix
1644.8	0.00395	0.04729	11.2354	random coils
1638.5	0.00831	0.1335	15.0989	β-structures
1626.44	0.01069	0.19465	17.1033	β-structures
1612.9	0.00278	0.0456	15.4101	β-structures

Summarized content

α-helix	0.30203
β-sheets	0.39884
turns/random coils/loops	0.29912

Table S3. 3-fold stretched fibrin clot (strain=2)

Centroid, cm^{-1}	*Height*	*Area*	*FWHM, cm^{-1}*	*Assignment*
1692.11	0.00205	0.025	13.349	β-structures
1681.06	0.0066	0.10772	17.9008	turns
1665.12	0.00833	0.15409	20.2803	turns/loops
1650.37	0.0157	0.25178	17.5748	α-helix
1645.2	0.00259	0.02537	10.7309	random coils
1637.51	0.00959	0.1346	15.3909	β-structures
1626.2	0.01267	0.20053	17.3529	β-structures
1615.68	0.00712	0.10093	15.5384	β-structures

Summarized content

α-helix	0.25178
β-sheets	0.46106
turns/random coils/loops	0.28718

Table S4. 4-fold stretched fibrin clot (strain=3)

Centroid, cm^{-1}	*Height*	*Area*	*FWHM, cm^{-1}*	*Assignment*
1690.22	0.00276	0.03385	13.3978	β-structures
1680.5	0.00486	0.07988	17.9538	turns
1665.36	0.00814	0.17782	23.8528	turns/loops
1652.33	0.01029	0.16247	17.2536	α-helix
1645.2	0.00566	0.06253	12.0724	random coils
1636.72	0.01125	0.15777	15.3147	β-structures
1624.04	0.01311	0.20112	16.7528	β-structures
1614.5	0.00855	0.12456	15.9041	β-structures

Summarized content

α-helix	0.16247
β-sheets	0.5173
turns/random coils/loops	0.32023

Table S5. Uncompressed fibrin clot

Centroid, cm^{-1}	*Height*	*Area*	*FWHM, cm^{-1}*	*Assignment*
1698.38	0.00114	0.00858	7.08076	β-structures
1678.72	0.00961	0.21898	21.3971	turns
1663.62	0.00592	0.07897	12.5246	turns/loops
1652.49	0.02257	0.36746	15.2964	α-helix
1644.83	0.00734	0.08808	11.2717	random coils
1637.18	0.01107	0.17784	15.0989	β-structures
1626.96	0.01113	0.20266	17.1033	β-structures
1613.57	0.00286	0.04685	15.4101	β-structures

Summarized content

α-helix	0.31038
β-sheets	0.37192
turns/random coils/loops	0.32357

Table S6. Fibrin clot under small compression

Centroid, cm^{-1}	*Height*	*Area*	*FWHM, cm^{-1}*	*Assignment*
1692.44	0.00146	0.01815	11.6953	β-structures
1678.13	0.00894	0.20366	21.397	turns
1663.85	0.00564	0.07525	12.5246	turns/loops
1652.49	0.01785	0.2906	15.2964	α-helix
1644.83	0.00741	0.08889	11.2717	random coils
1637.18	0.01185	0.19045	15.0989	β-structures
1624.89	0.01305	0.2375	17.1033	β-structures
1611.77	0.00426	0.06993	15.4101	β-structures

Summarized content

α-helix	0.25026
β-sheets	0.44135
turns/random coils/loops	0.31214

Table S7. Fibrin clot under intermediate compression

Centroid, cm^{-1}	*Height*	*Area*	*FWHM, cm^{-1}*	*Assignment*
1691.42	6.3671E-4	0.00536	7.90788	β-structures
1678.57	0.00843	0.22518	25.099	turns
1664.96	0.00459	0.06519	13.3519	turns/loops
1653.24	0.0139	0.2263	15.2964	α-helix
1644.83	0.00698	0.08369	11.2717	random coils
1637.18	0.01118	0.17962	15.0989	β-structures
1624.27	0.01451	0.26414	17.1033	β-structures
1613.57	0.0071	0.11644	15.4101	β-structures

Summarized content

α-helix	0.19026
β-sheets	0.48123
turns/random coils/loops	0.33112

Table S8. Fibrin clot under large compression

Centroid, cm^{-1}	*Height*	*Area*	*FWHM, cm^{-1}*	*Assignment*
1691.71	7.65398E-4	0.00595	7.30041	β-structures
1678.77	0.00802	0.22057	25.823	turns
1663.74	0.00571	0.09217	15.1674	turns/loops
1652.49	0.01173	0.19106	15.2964	α-helix
1644.83	0.00623	0.07469	11.2717	random coils
1636.58	0.01151	0.18499	15.0989	β-structures
1623.49	0.01461	0.26598	17.1033	β-structures
1613.28	0.00826	0.13543	15.4101	β-structures

Summarized content

α-helix	0.16467
β-sheets	0.51243
turns/random coils/loops	0.33214

Supporting references

1. Collet, J. P., H. Shuman, R. E. Ledger, S. Lee, and J. W. Weisel. 2005. The elasticity of an individual fibrin fiber in a clot. Proc Natl Acad Sci U S A 102:9133-9137.
2. Liu, W., L. M. Jawerth, E. A. Sparks, M. R. Falvo, R. R. Hantgan, R. Superfine, S. T. Lord, and M. Guthold. 2006. Fibrin fibers have extraordinary extensibility and elasticity. Science 313:634.
3. Brown, A. E. X., R. I. Litvinov, D. E. Discher, P. K. Purohit, and J. W. Weisel. 2009. Multiscale mechanics of fibrin polymer: gel stretching with protein unfolding and loss of water. Science 325:741-744.