Interplay of fibrinogen α_{E}C globular domains and factor XIIIa cross-linking dictates the extensibility and strain stiffening of fibrin networks

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Supplementary Information

Quantification of clottable protein.

The amount of polymerizable protein in the fibrinogen preparations was quantified using a Total Clottable Protein (TCP) assay. Fibrinogen samples were polymerized at 2 mg/ml using 1 U/ml of α-thrombin in Tris Buffered Saline (TBS, pH 7.4) supplemented with 2 mM $CaCl_2$. Samples were left to polymerize for 60 minutes at 37°C. The resulting fibrin polymers were collected by centrifugation and washed three times with TBS. The collected fibrin was dissolved in alkaline urea (6.66 M Urea, 0.2 M NaOH) and the amount of polymerized protein was determined using an OD280 ϵ 1% of 15.87. For rFib340 fibrinogen, the TCP of the start concentration was 99.3% and for rFib420 fibrinogen the TCP was 97.6%.

Detailed description of SEM sample preparation.

In addition to the samples prepared in Petri dishes, we also prepared samples in multi-well imaging chambers (Press-to-Seal, eight wells, 9 mm diameter, 1.0 mm deep, ThermoFisher) in order to obtain duplicates of clots polymerised under the same conditions (Fig. S4A). Sample preparation was similar to the ones prepared in Petri dishes. In both cases, after fixation with glutaraldehyde, samples were dehydrated by a series of buffer solutions containing an increasing volume percentage of ethanol (30, 50 70, 80, 90, 95, 100%) with progressively increasing incubation times (5-20 min). Finally, the samples were dried with 50% HDMS (hexamethyldisilazane, Sigma-Aldrich) in ethanol for 15 min and left overnight with 100% HMDS under a fume hood for full drying. The samples were imaged with a scanning electron microscope (Verios 460, FEI, USA) at an acceleration voltage of 5 or 10 kV.

Detailed description of network branching classification.

The quantification of network branching was performed in three steps. First, regions of interest containing junctions were cropped out of unprocessed SEM images. To prevent bias, we overlaid a grid of 500 x 500 nm onto each image through the ImageJ Grid plugin. Junctions overlapping with the drawn grid lines were selected for further analysis. Second, all cropped images were blindly compiled and manually classified into different categories found across the dataset. Finally, the number of

junctions corresponding to each category were counted and assigned with their corresponding fibrin network.

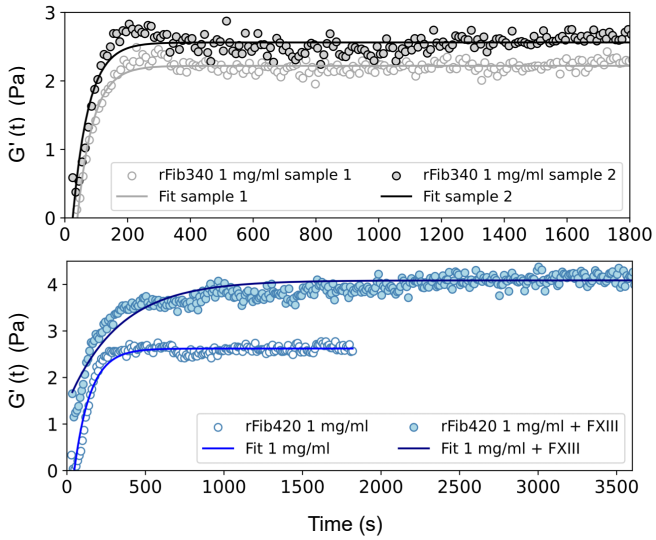

Figure S1: **Polymerisation curves for 1 mg/ml fibrin networks.** Examples of the time dependency of the linear storage modulus G' for rFib340 (top) and rFib420 (bottom) fibrin networks at 1 mg/ml. Time $t = 0$ refers to the moment when the sample was added on the rheometer plate.

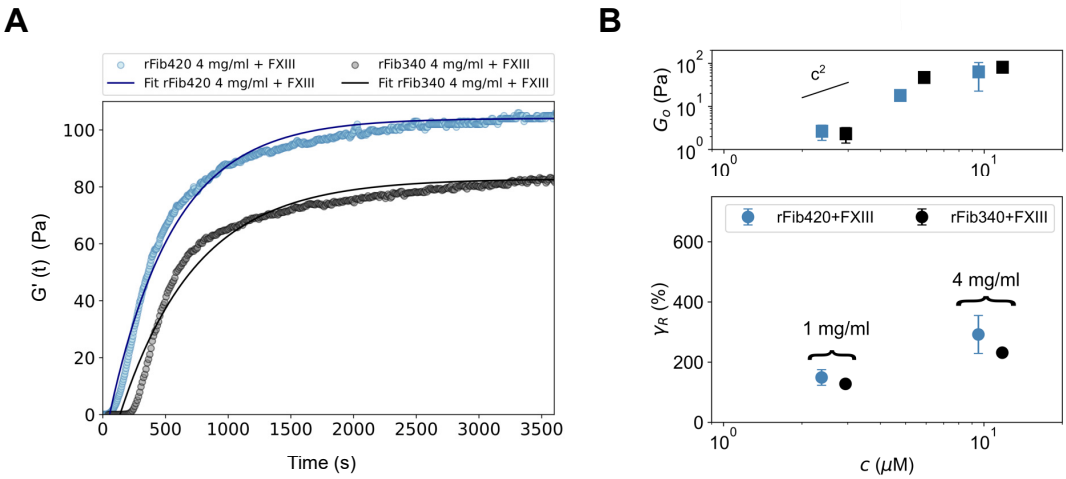

Figure S2: **Rheology of crosslinked fibrin networks.** (A) Examples of polymerisation curves for crosslinked rFib340 (blue) and rFib420 (gray) networks at 4 mg/ml. (B) Mean linear storage modulus (top) and mean rupture strain (bottom) as a function of fibrinogen concentration, c. The solid line shows the c^2-scaling expected for semiflexible polymer networks.

Table S1: **Mechanical properties of rFib340 and rFib420 fibrin networks under shear rheology.** The values reported are the mean values and the standard deviation for n samples. Unless explicitly stated, $n = 2$.

[Fg]	FXIII	G_o (Pa)		γ_R (%)	
		rFib340	rFib420	rFib340	rFib420
1 mg/ml	-	2.86 ± 0.89 (n=8)	3.15 ± 0.69 (n=6)	124.2 ± 4.1 (n=3)	209.7 ± 25.9 (n=3)
	+	2.32 ± 0.91 (n=4)	2.65 ± 1.02 (n=3)	127.9 ± 7.4 (n=3)	149.2 ± 25.9 (n=3)
2 mg/ml	-	21.74 ± 2.84 (n=7)	16.36 ± 5.58 (n=4)	222.8 ± 14.3	378.8 ± 28.1
	+	46.92 ± 7.02	17.93 ± 0.83	-	-
4 mg/ml	-	95.56 ± 45.80	87.22 ± 6.83	229.2 ± 49.3	551.6 ± 42.1
	+	81.00 ± 1.86	63.35 ± 40.74	231.5 ± 12.2	292.1 ± 63.1

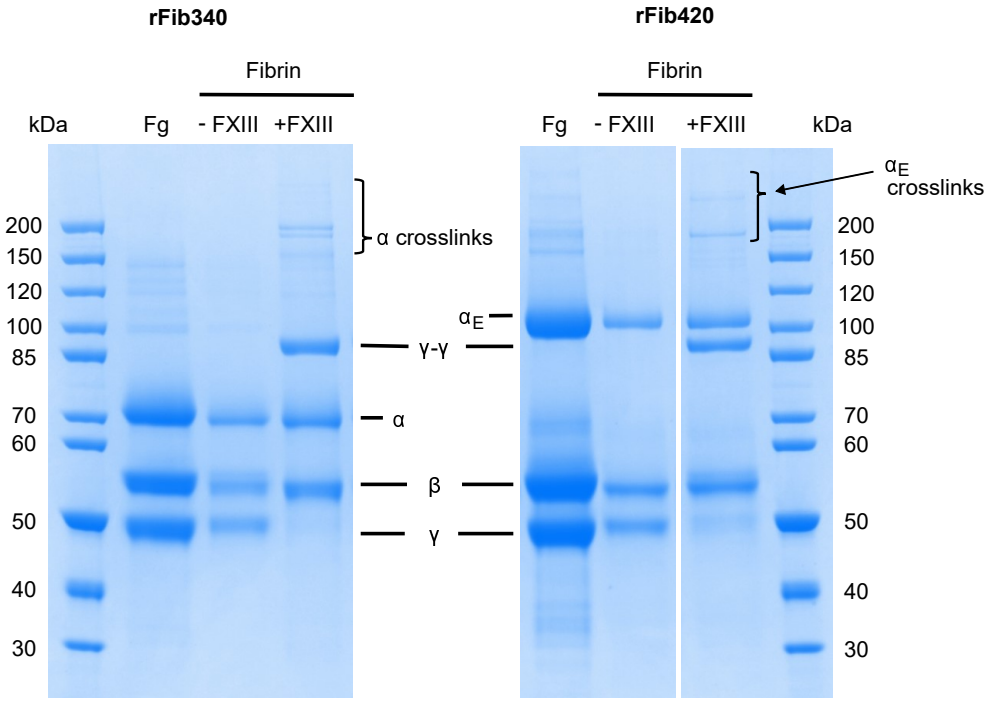

Figure S3: **SDS-PAGE of fibrin(ogen) samples from rFib340 and rFib420, with or without FXIII.** The bands corresponding to the polypeptide chains α, α_{E}, β, and γ are indicated, as well as those corresponding to the cross-links α-multimers and γ-dimers, formed with the addition of FXIII.

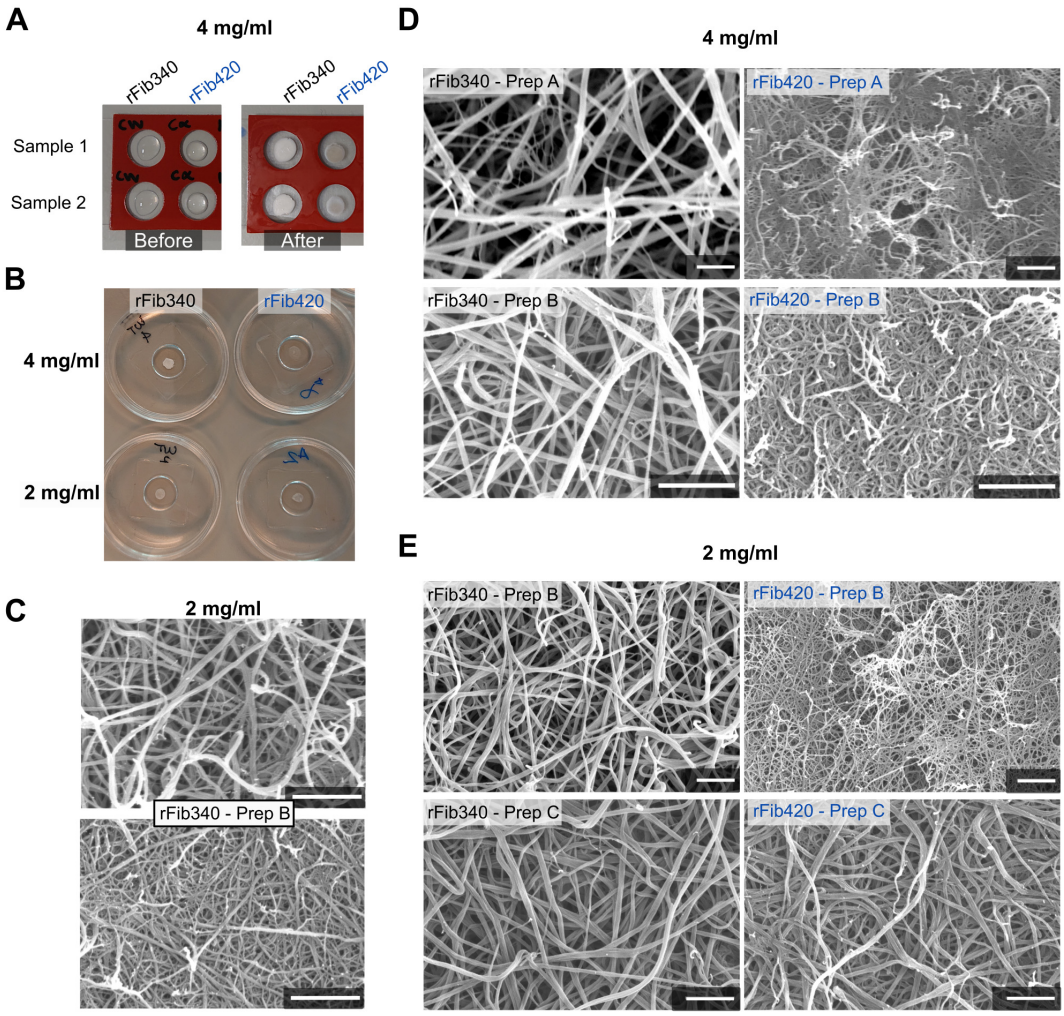

Figure S4: **Sample heterogeneity in SEM imaging.** (A) Pictures showing examples of droplets of 4 mg/ml fibrin networks before and after treatment for SEM imaging, as prepared in multi-well chambers. The "before" picture was taken after 45 minutes of polymerisation, and the "after" picture was taken after full dehydration. The length of the orange stripe containing the wells is 25 mm. (B) Picture of fibrin networks droplets after dehydration for SEM, as prepared in Petri dishes. All four samples were prepared simultaneously. Top row: 4 mg/ml rFib340 (left) and 4 mg/ml rFib420 (right) fibrin networks. Bottom row: 2 mg/ml rFib340 (left) and 2 mg/ml rFib420 (right) networks. Samples in A and in B were prepared on different days, from different single-use aliquots of protein. (C) SEM images of 2 mg/ml rFib340 fibrin networks. Both images were taken at the same magnification from two different regions in the same sample. "Prep B" refers to the sample prepared in (B)(bottom left). Most of the sample showed the network morphology seen at the top image, and only a couple of small regions showed the morphology seen in the bottom image, likely due to artefacts of sample mounting on SEM stubs. (D) SEM images of 4 mg/ml rFib340 (left) and rFib420 (right) fibrin networks. "Prep A" refers to the samples prepared in (A). (E) SEM images for 2 mg/ml rFib340 (left) and rFib420 (right) fibrin networks. "Prep C" refers to a different sample set not shown in this figure, prepared in petri dishes, similar to "prep B". Scale bar in (C-E) is 1 μm.