Strain-controlled criticality governs the nonlinear mechanics of fibre networks

Supplementary Information

Critical strain

We define the critical strain $\gamma_c$ as the inflection point of strain-stiffening curves plotted as the log $K$ vs. log $\gamma$ curves, analogous to the determination of the critical point in a finite size system [1]. Using this definition, we can unambiguously extract the critical strain in a consistent manner for both experiments and simulations. In Fig. S1a, we show the critical strain, obtained from simulations on a 2D phantom triangular network with $\langle z \rangle \approx 3.2$, for different values of the fibre bending rigidity. As expected, one can see, $\gamma_c$ shows a weak dependence on $\tilde{\kappa}$. In the limit of $\tilde{\kappa} \rightarrow 0$, $\gamma_c$ approaches a constant value. Moreover, $\gamma_c$ also marks the strain at which the network exhibits the largest non-affine fluctuations. Given the displacement field $u$ and the affine displacement field $u^A$ of the network, the non-affine fluctuations can be quantified as [2]

$$\delta \Gamma(\gamma) = \frac{\langle |\delta u^{NA}|^2 \rangle}{l^2 d \gamma^2}, \quad (S1)$$

where $\delta \Gamma(\gamma)$ is referred as differential non-affinity, $\delta u^{NA} = u - u^A$ is the differential non-affine displacement of a crosslink to an imposed strain $d \gamma$, $l$ is the typical network mesh size and the angular brackets represent a network average. In fact, in the limit of $\tilde{\kappa} \rightarrow 0$, the fluctuations are expected to diverge. In Fig. S1b, we show $\delta \Gamma(\gamma)$ in the neighbourhood of the critical strain. The fluctuations grow with decreasing $\tilde{\kappa}$, consistent with the idea that the bending rigidity can be considered as an auxiliary field.

Mapping of model onto experimental control parameters

The parameter $\tilde{\kappa}$ in our model is naturally related to the protein concentration $c$ as follows. For an elastic rod of radius $r$ and Young's modulus $E$, $\mu = \pi r^4 E$ and $\kappa = \pi r^4 E/4$, implying that $\tilde{\kappa} \propto (r/l)^2 \propto \phi$, since the volume fraction $\phi = \pi r^2 \rho$, where $\rho \propto 1/l^2$ is the total fibre length per volume [3-5]. Hence, the protein concentration $c$ (or $\rho$) in experiments can be simply related to the reduced bending rigidity $\tilde{\kappa}$ as $\rho \sim \tilde{\kappa}$. We note that the total protein concentration determines both the mesh size $l$ of the network and the bending stiffness $\kappa$ of the fibrils. However, as shown above, it is the reduced bending rigidity $\tilde{\kappa}$ in our model that scales linearly with the protein concentration in experiments. It follows, perhaps surprisingly, that the rheology results, both in magnitude of moduli and stress, as well as functional dependence of the stiffness on strain, should be insensitive to the fibril thickness. The elastic energy involves a summation over all fibres in the network and is a function of the strain $\gamma$ and the reduced bending rigidity $\tilde{\kappa}$. Moreover, since the modulus $K$ involves the energy per unit volume, $K$ is naturally proportional to $\rho$. The modulus can therefore be expressed as

$$K = \mu \rho \kappa(\gamma, \tilde{\kappa}). \quad (S2)$$

In the linear regime, $\gamma \rightarrow 0$, we obtain from the above relation $K \sim \mu \rho \tilde{\kappa} \sim \rho^2$ [6, 7].

Scaling analysis

Finite size scaling

The critical behavior in our model can be tested by performing finite-size scaling, which is sensitive to the divergence of the correlation length. In our system, the order parameter $K$ scales as $|\Delta \gamma|^f$ (Fig. S2), as system size $W \rightarrow \infty$ and $\Delta \gamma \rightarrow 0$. This scaling should be evident when the correlation length, which scales as $|\Delta \gamma|^{-\nu}$, is much smaller than the system size, where $\nu$ is the correlation length exponent. At the critical point, when the correlation length diverges, the modulus should scale with system size as $K \sim W^{-f/\nu}$, such that $K \rightarrow 0$ as $W \rightarrow \infty$. This can be summarized in the following scaling relation.

$$K = W^{-f/\nu} F_{\pm} \left( |\Delta \gamma| W^{1/\nu} \right). \quad (S3)$$

In order to perform finite-size scaling, we chose a finite but small bending rigidity. We chose a finite $\tilde{\kappa}$ to avoid the numerical problems associated with a rope-like network ($\tilde{\kappa} = 0$). In a rope-like network, due to finite size effects, the energy density shows a jump at the critical strain. Since one needs to take derivatives of energy density, it is numerically problematic to unambiguously extract the modulus at the critical strain. In Fig. S3a we show the stiffening curves for $\tilde{\kappa} = 10^{-7}$ for system sizes from $W = 40$ to a maximum of $W = 200$ in 2D. In Fig. S3b we present the collapse (Eq. (S3)) of stiffening curves obtained for two other bending rigidities, $\tilde{\kappa} = 10^{-6}$ and $10^{-8}$. For the three values of $\tilde{\kappa}$, the data collapse is indistinguishable except for the lower branch. The lower branch converges to a value which scales with $\tilde{\kappa}$. It follows that the lower branch extends continuously to zero as $\tilde{\kappa} \rightarrow 0$, consistent with the fact that a central-force sub-isostatic network is unstable for $\gamma \leq \gamma_c$.

Widom-like scaling of the stiffness for finite $\tilde{\kappa}$

In the absence of bending, i.e., for $\tilde{\kappa} = 0$, the network stiffness scales as $K \sim |\Delta \gamma|^f$ in the regime where $\Delta \gamma = \gamma - \gamma_c > 0$. For $\gamma < \gamma_c$, the effect of stabilization by bending leads to $K \sim \tilde{\kappa}$. These regimes can be summarized by the scaling form

$$K \propto |\Delta \gamma|^f G_{\pm} \left( \tilde{\kappa}/|\Delta \gamma|^s \right), \quad (S4)$$
Supplementary Figure S1: (a) Critical strain $\gamma_c$, obtained from simulations on a 2D phantom triangular network with $\langle z \rangle \approx 3.2$, as the inflection point of the log $K$ vs. log $\gamma$ curves (circles). Squares show the strain at which the non-affine fluctuations show a maximum. It is clear that the maximum in the non-affine fluctuations occurs at $\gamma \approx \gamma_c$. (b) Differential non-affinity $\delta\Gamma(\gamma)$ obtained from the same simulations in (a) as a function of the applied strain. $\delta\Gamma(\gamma)$ peaks at $\gamma \approx \gamma_c$. As expected, the height of the peak increases with decreasing $\tilde{\kappa}$ since the displacement field of these networks becomes highly non-affine as $\gamma \rightarrow \gamma_c$.

where $G_{\pm}$ is a scaling function with the positive and negative branches corresponding to $\Delta \gamma > 0$ and $\Delta \gamma < 0$, respectively. Mapping protein concentration to $\tilde{\kappa}$ allows us to obtain an analogous scaling relation applicable to experimental data:

$$K/c \propto |\Delta \gamma|^f G_{\pm} \left( c/|\Delta \gamma|^\phi \right). \quad (S5)$$

In the main text, we applied this scaling relation to experimentally measured stiffness data of collagen networks prepared at $T = 37^\circ$. As shown in Fig. S4, the same analysis successfully captures the nonlinear stiffness of a network prepared at $T = 30^\circ$ with rest of the experimental parameters kept the same. In The stiffness curves in Fig. S4(a) are collapsed according to Eq. (S5) in Fig. S4(b). An excellent collapse is obtained with critical exponents $f = 0.8$ and $\phi = 2.1$. For networks prepared at $T = 30^\circ$, the critical exponent $f/\phi \approx 0.38$ is slightly larger than that for networks prepared at $T = 37^\circ$. Although the difference between the exponents is not substantial and is within error margin, it is an interesting possibility that the higher value at $T = 30^\circ$ has its origin in a slightly larger average connectivity at $T = 30^\circ$ than $T = 37^\circ$. A slightly larger connectivity would result in slightly smaller critical strains, which is indeed what we observe in experiments. However, at present this proposition remains speculative and would require more experimental study to establish.

Equation of state

In ferromagnetism, the magnetization, $m$, in presence of an applied field $h$ and reduced temperature $t = (T - T_c)/T_c$ can be captured in the following scaling relation [8]:

$$h/|t|^\Delta \sim m/|t|^\beta \left( \pm 1 + m^{1/\beta} |t|^\Delta \right)^{(\Delta - \beta)} \frac{1}{|t|^\beta}. \quad (S6)$$

Here, the $(\pm)$ branch corresponds to $t \gtrless 0$ and $\Delta$ and $\beta$ are critical exponents. The analogous quantities for a fibre network are the following:

$$\Delta \leftrightarrow \phi \quad (S7)$$

$$\beta \leftrightarrow f \quad (S8)$$

$$h/|t|^\Delta \leftrightarrow \tilde{\kappa}/|\Delta \gamma|^\phi \quad (S9)$$

$$m/|t|^\beta \leftrightarrow K/|\Delta \gamma|^f \quad (S10)$$

Based on the above analogy, we obtain the following scaling law.

$$\tilde{\kappa}/|\Delta \gamma|^\phi \sim K/|\Delta \gamma|^f \left( \pm 1 + K^{1/f}/|\Delta \gamma|^\phi \right)^{(\phi-f)}. \quad (S11)$$

For $\Delta \gamma = 0$, this scaling relation correctly reproduces $K \sim \tilde{\kappa}^{f/\phi}$ at the critical point.

In the main text, we use Eq. (S11) to obtain fit to the experimental $K$ vs. $\gamma$ data. We replot an enlarged version of this figure for clarity in Fig. S5 showing nonlinear
stiffness versus strain measured up to the point of rupture. Consistent with previous reports [9, 10], network rupture occurs at 30-50% strain. The fitting is done in the following way. We first focus on the linear regime. In the linear regime, we know from simulations that the modulus (in units of $\rho \mu$) scales linearly with $\tilde{\kappa}$ which itself scales as $\tilde{\kappa} \sim \rho$ giving rise to the $c^2$ (or $\rho^2$) dependence of the linear modulus where $c$ is the protein concentration. It follows that in order to compare experimental $K$ with the corresponding data obtained from simulations we should first rescale the experimental $K$ by $c^{1.2}$ so that the rescaled modulus scales as $K/c^{1.2} \sim c \sim \tilde{\kappa}$. As the next step, we obtain the individual critical strains, $\gamma_c$, for each of the concentrations as the inflection point of the log $K$ vs. log $\gamma$ curve. We then consider the experimental data (rescaled by $c^{1.2}$) for each concentration along with its $\gamma_c$ and fit the entire curve to Eq. (S11) with $\tilde{\kappa}$ as the only free parameter. Different samples for a given concentration are fitted independently. As can be seen in the inset of Fig. S5, for each concentration, we obtain three values of $\tilde{\kappa}$ corresponding to three different samples. The average value of $\tilde{\kappa}$ scales linearly with the concentration consistent with the predictions of our model.

**Average connectivity of collagen networks**

In Fig. S6, we show typical Scanning Electron Microscope (SEM) images of collagen networks prepared at two different temperatures, $T = 30^\circ C$ (panel (c)) and $T = 37^\circ C$ (panel (d)), and concentration of 4 mg/ml. For SEM, collagen gels (50-100 µl) were polymerized overnight in 5 ml eppendorf tubes in humid conditions. After polymerization, samples were washed three times with sodium cacodylate buffer (50 mM cacodylate, 150 mM NaCl, pH 7.4) for 30-60 min each, at their polymerization temperature. Samples were fixed with 2.5% glutaraldehyde in the same buffer for at least 2 hours. Next, samples were washed three times with sodium cacodylate buffer (room temperature) and dehydrated with increasing percentage of ethanol. After complete dehydration (100% ethanol), 50% hexamethyldisilazane (HMDS) in

**Supplementary Figure S2**: Stiffening curves for $\gamma > \gamma_c$ in a 2D network (phantom triangular, $\langle z \rangle \simeq 3.2$) show that in the limit $\tilde{\kappa} \to 0$, $K \sim |\Delta \gamma|^{0.75}$ where for this network $f \simeq 0.75$. 

![Stiffening curves for $\gamma > \gamma_c$](image)
**Supplementary Figure S3:** (a) Stiffening curves for $\bar{\kappa} = 10^{-7}$ from a 2D triangular lattice based network with $\langle z \rangle = 3.2$ for different system sizes. (b) Collapse of the stiffening curves according to Eq. (S3) for different system sizes $W$. The critical exponent $f$, obtained as the slope of the upper branch (thick black line), is $f = 0.75 \pm 0.05$. The exponent associated with divergence of correlation length is $\nu = 2.0 \pm 0.1$. Stiffening curves are obtained for three different bending rigidities (see legend). The lower branch converges to a value that scales with the bending rigidity $\bar{\kappa}$. In the limit of $\bar{\kappa} \to 0$, the lower branch will extend all the way down to zero.

**Supplementary Figure S4:** (a) Nonlinear stiffness vs. strain measured for collagen networks prepared at $T = 30^\circ$ and different concentrations $c$, namely 2 mg/ml ($\triangle$), 3 mg/ml ($\bigcirc$), 4 mg/ml ($\bullet$), and 5 mg/ml ($\square$). For each concentration, at least three different samples were measured. Red filled symbols mark the critical strain $\gamma_c$. The collapse of the experimental data according to Eq. (S5) is shown in (b) with $f = 0.8$ and $\phi = 2.1$. The inset of (b) shows the scaling of the critical strain with concentration for the two samples. The line is the model prediction.

Ethanol was added (under the hood) and afterwards replaced after 30 min by 100% HMDS. The HMDS was left to evaporate overnight. The samples were transported to a stub with carbon tape and sputter coated using a K575X sputter coater (Quorum Technologies, Gouda, The Netherlands). A layer of 15.4 nm of Au/Pd was sputtered using a current of 80 mA. The SEM samples were visualized using a Scanning Transmission Electron Microscope (STEM) setup (Verios 460, FEI Company, Eindhoven, the Netherlands) using 50 pA, 5 kV and 4 mm working distance, in immersion mode. The connectivity was determined manually on SEM images with 20,000-50,000 times magnification. The images were divided by a grid of 5x5 squares and in every other square all junctions were taken into account. Per sample, a total of at least 100 junctions were counted. The average connectivity of a collagen network at both $37^\circ$ and $30^\circ$ was determined to be $3.3 \pm 0.1$. 
Supplementary Figure S5: Nonlinear stiffness vs. strain measured for collagen networks prepared at different concentrations (see legend). The inflection point ($\gamma_c$) is in each case marked in red enlarged symbols. The dashed lines are the prediction of Eq. (S11). For each concentration, three different samples were prepared. The fit values of $\tilde{\kappa}$ based on Eq. (S11) are shown in the inset on a linear scale. The average value of $\tilde{\kappa}$ for each concentration is shown as filled red circles. The linear scaling of $\tilde{\kappa}$ with the concentration $c$ is consistent with the predictions of our model.

To verify that the SEM preparation does not influence the network architecture, we imaged 4 mg/ml collagen networks in the hydrated stated using confocal reflectance microscopy (See Fig. S6(a) and (b)). The images were collected using an inverted Eclipse Ti microscope (Nikon) using an 488 Ar laser (Melles Griot, Albuquerque, NM) for illumination. We obtained z-stacks by recording confocal slices over a total distance of 20 $\mu$m with 0.2 $\mu$m z-spacing using a 100x (N.A. 1.49) oil objective, starting at least 10 $\mu$m away from the coverslip surface. For display purposes, the confocal stacks were summed to give an impression of the 3D network structure and the typical mesh size.

The confocal reflectance images show that networks polymerized at 30°C and 37°C are homogeneous (See Fig. S6(a) and (b)). The observation is consistent with SEM (See Fig. S6(c) and (d)). The images obtained by SEM are consistent with the qualitative observation that the fibres are thinner and more numerous at 37°C than at 30°C. Quantitative measurements of turbidity using spectrophotometry confirmed that the fibres are somewhat thinner at 37°C than at 30°C. These findings are in agreement with previous studies on collagen, where a decrease in mesh size as determined by confocal imaging was correlated with a decrease in mesh size and an increase in fibre diameter as determined by SEM [11–13]. We do note, however, that the diameter one obtains from SEM is likely to be different from the diameter in the hydrated state due to loss of water [14]. We therefore think that SEM conserves the network structure, even though the absolute value of the pore size and fibre thickness are likely different from the hydrated state. Furthermore, we note that the average $z$ determined with SEM is in excellent agreement with earlier measurements in collagen [15].
and fibrin networks [16].

Reversibility of collagen strain-stiffening and Gap Size Dependence

To test whether network creep influences the determination of network stiffness $K$ [17], we checked whether the strain-stiffening behavior of the collagen networks is reversible. We first ramped the prestress up in a stepwise manner then ramped it back down to the linear regime, as shown Fig. S7. The collagen concentration was kept constant at 4 mg/ml whereas the polymerization temperature was varied from 22 (a) to 30 (b) and 37°C (c). For the 22°C case, we can see hysteresis between the upward and downward curves and significant softening. At the two higher temperatures, however, we see no difference between the upward and downward curves and the stiffness returns to its original value.

We note that we did not see an effect of gap size in our collagen systems. In preliminary experiments, we have done tests using a 40 mm cone-plate with slightly larger cone angle (2° instead of the 1° used throughout this paper). This did not have any significant influence on our strain-stiffening curves (not shown). Our observations of critical phenomena in collagen networks may provide an explanation for the size-dependent rheology reported in Ref. [18]. In collagen networks, where the natural small length scale for the network is its mesh size (typically of order ~ 10 microns), the correlation length associated with criticality such as we observe here can easily be as large as ~ 100 – 150 microns. This is the scale over which sample size effects were observed in Ref. [18]. It is an intriguing possibility that the prior sample-size dependence can be understood in terms of the size dependence well known to occur in critical systems exemplified in Fig. 4 in the main text.

Critical strain as a function of connectivity

In Fig. S8, we show the critical strain as a function of average connectivity in a 2D phantom triangular network. In the main text, a schematic version of such a figure is shown as Fig. 1. As can be seen in Fig. S8, $\gamma^c$ approaches zero as the connectivity approaches the isostatic threshold of $\langle z \rangle = 4$ in 2D. In Fig. S8, we mark as shaded rectangle the connectivity-range relevant for collagen networks. In the main text, the overall decrease in the critical strain in the experiments (Fig. 3b inset in the main text) with increasing concentration of collagen is about 30%. Increasing concentration of collagen is expected to result in an increase in the average connectivity of the network. Consistent with this, one can see in Fig. S8 that the critical strain decreases with increasing connectivity. In fact, $\gamma^c$ decreases significantly from 0.2 to 0.1, as the connectivity increases from 3.2 to 3.6.

Near isostatic 3D FCC network

Although the theoretical results shown in the main text correspond to average connectivity value $\langle z \rangle$ close to the experimentally relevant values for collagen networks, we also studied a disordered FCC lattice with $\langle z \rangle$ close to 5. The predicted scaling is shown in Fig. S9. Such a network is close to the isostatic threshold of $\langle z \rangle = 6$ in 3D. The near-isostatic case of the FCC lattice at $\langle z \rangle \approx 5$ exhibits a value $f/\phi \approx 1/2$, consistent with the $K \sim n^{0.5}$ scaling reported in Ref. [19] for an isostatic network. The individual exponents are $f = 1.45$ and $\phi = 2.9$. These critical exponents are only defined for sub-isostatic networks, and thus are not expected to coincide with studies of isostatic systems [19, 20].

References


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Supplementary Figure S6: Confocal reflectance microscopy images of collagen networks prepared at a concentration of 4 mg/ml and $T = 30^\circ$ in (a) and $T = 37^\circ$ in (b). SEM images of the same networks are shown in (c) and (d) revealing the zoomed-in (50,000 magnification) structure of the networks in (a) and (b), respectively. The branched structure of the collagen fibres is visible. The white scale bar in (b) represents 20 µm for (a) and (b), while the scale bar denotes 1 µm for (c) and (d).
Supplementary Figure S7: Nonlinear stiffness vs. shear stress measured for collagen networks prepared at three different polymerization temperatures, namely 22°C (a), 30°C (b) and 37°C (c). The collagen concentration was fixed at 4 mg/ml. The vertical dashed lines indicate the shear stress at which a reverse measurement is made. The red dashed curves show the measured stiffness under stress reversal.
Supplementary Figure S8: Critical strain as a function of the average connectivity in a 2D network (phantom triangular). In the shaded rectangular region, $\langle z \rangle$ changes from 3.2 to 3.6, a range relevant for collagen.
Supplementary Figure S9: Collapse of data from a 3D FCC lattice network with \( \langle z \rangle \approx 5 \). The critical exponents are \( f = 1.45 \) and \( \phi = 2.9 \). The ratio \( f/\phi \approx 1/2 \) is consistent with the \( K \sim \kappa^{0.5} \) scaling reported in Ref. [19] for an isostatic network in 3D.