Slack-taut transition and emergent stiffness in bioinspired entangled filament networks

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Inspired by massive intermediate filament (IF) reorganization in superstretched epithelia, we examine computationally the principles controlling the mechanics of a set of entangled filaments whose ends slide on the cell boundary. We identify an entanglement metric and percolation threshold beyond which random loose networks self-organize into structurally optimal star-shaped configurations. A simple model connecting cellular and filament strains links emergent mechanics to cell geometry, network topology, and filament mechanics. We identify a safety net mechanism in IF networks and provide a framework to harness entanglement in soft materials.

Epithelial tissues are cohesive cellular sheets lining free surfaces within animals, which perform crucial physiological functions including morphogenesis, protection, secretion, and absorption. They must preserve integrity within active and challenging mechanical environments. Tissue mechanics depends on intracellular cytoskeletal networks, which are mechanically integrated at the tissue scale through cell-cell junctions. The cell cytoskeleton is a composite system combining widely diverse biopolymers, which interact chemically, physically and through biological regulation. Cytoskeletal actin filaments, which are short (≤ 1 µm) and stiff both to stretch and bending (persistence length ≈ 10 µm), form heavily crosslinked networks undergoing turnover within minutes. In contrast, intermediate filaments (IFs) are bendable (persistence length ≈ 1 µm), can reach several µm in length, and form cell-wide physically entangled networks with turnover times of hours. IF coupling across cell pairs is mediated by adhesion complexes known as desmosomes. A hierarchical architecture, relying on subunits that can slide and uncoil, enables extreme extension of IFs (up to 3 – 4.5×), with a highly nonlinear force-stretch behavior featuring a plateau and subsequent re-stiffening at large deformations. Together, these features support the widespread view that IFs provide a “safety belt” against fast and large deformations, although load transfer from the epithelial scale to individual IFs remains elusive.

Recent stretching experiments on epithelial monolayers suggest synergistic interactions between the actin cytoskeleton — controlling epithelial mechanics at moderate stretches — and IFs, providing load bearing under severe cell deformations. Laser ablation confirms that the thick radial bundles formed by IFs in such conditions, white arrowheads in Fig. 1(a), provide structural integrity to superstretched cells, as the actin network becomes progressively diluted. Since IFs appear wavy and arranged cortically in unloaded cells, we wondered about the physical mechanisms underlying this slack-taut transition and the corresponding effects on the network-scale mechanical behavior.

To address these questions, we idealized the IF cytoskeleton within a cell as a loose and entangled network of non-crosslinked, bendable, and extensible filaments, whose ends are attached to the lateral boundaries of a prismatic domain, Fig. 1(b). These attachments simulate desmosomes and prevent unraveling of the network by filament reptation. We view this cell as a representative volume element of a tissue, cf. yellow hexagons in Fig. 1(a). We developed computational simulations of cell stretching using the cytoskeletal simulation suite cytopsim, which describes the Brownian dynamics of inextensible and bendable filaments and which we customized to model extensible filaments with general...
We prepared computational models comprising $N_f$ cylindrical filaments of reference length $l_0$, diameter $\phi \ll l_0$, and cross-sectional area $A_f = \pi \phi^2/4$. Initially, we considered linearly elastic filaments with modulus $E$. Steric interactions prevent filament crossing and all filament points are confined inside the cell volume. The cell is modeled as a right regular prism, with $N_c$ lateral junctions. As we change $A_c$, we quantify the forces acting on the side walls. If $F^+_i$ and $F^-_i$ are the forces on the $i$-th filament ends, and $\hat{n}^+_i$ and $\hat{n}^-_i$ are the normals to the walls that constrain those ends, the total force is

$$F_c = \sum_{i=1}^{N_f} (F^+_i \cdot \hat{n}^+_i + F^-_i \cdot \hat{n}^-_i),$$

from which we define the nominal tissue surface tension, $T_c = F_c/N_c s_0$, and its dimensionless form, $T_c^* = F_c a_0/EA_f N_c s_0$. We performed stretch athermally and quasi-statically by ensuring that the applied strain rate is much smaller than the inverse intrinsic time constant of the system ($\dot{\epsilon}_c \ll E/\nu)$, as further discussed later.

In the absence of crosslinkers, entanglement is the only mechanism for the network to develop mechanical resistance. We thus established a system preparation protocol that allows controlling entanglement by modifying the fraction of time during which filaments grow unconstrained or attached to cell walls, §1, 30 and Movie S1. In agreement with our rationale, a loose and randomly organized network with default parameters and strong entanglement undergoes a dramatic spontaneous reorganization when stretched. This yields a central tight tangle from which filament bundles radiate perpendicularly to the lateral cell boundaries. The formation of such ‘star-shaped’ configuration, reminiscent of IFs in superstretched epithelial cells, involves significant lateral motion of the attachment points and results in all filaments carrying load, Movie S2. Conversely, an equivalent system with low entanglement develops less predictable and directed network reorganizations, where only a small fraction of filaments become taut under stretch, Movie S3.

We sought a quantitative measure of entanglement capable of discerning between the two behaviors. In topology, the entanglement of knots (embeddings of the unit circle in $\mathbb{R}^3$) and links (collections of knots) can be classified using invariants 31, 32. To characterize the entanglement of a link comprising two oriented spatial curves, $\delta_i$ and $\delta_j$, we focus on the pairwise Gaussian linking number, $L_{k_{i,j}}$, used previously to study topological properties of proteins and DNA 33, 35. This is an integer measuring the number of times that $\delta_i$ winds around $\delta_j$, which is invariant with respect to deformations that respect mutual filament avoidance. Parameterizing $\delta_i$ and $\delta_j$ as $\mathbf{r}_i(t)$ and $\mathbf{r}_j(t)$ with $t \in [0, 2\pi)$, $L_{k_{i,j}}$ can be computed as 31, 30:

$$L_{k_{i,j}} = \frac{1}{4\pi} \int_0^{2\pi} \int_0^{2\pi} \frac{\mathbf{r}_i(t_i) - \mathbf{r}_j(t_j)}{|\mathbf{r}_i(t_i) - \mathbf{r}_j(t_j)|^2} \cdot \left[ \mathbf{r}'_i(t_i) \times \mathbf{r}'_j(t_j) \right] dt_i dt_j,$$

where the prime denotes differentiation with respect to $t$.

An embedding that includes closed and open curves with their ends fixed in space is termed a tangle, a topological concept appropriate to describe our system and generalizing knots, links, and braids 31, 32. For tangles, $L_{k_{i,j}}$ is no longer an integer nor a strict invariant 37, but is still useful to characterize pairwise linking 31, 32, 33, 34. Measuring $L_{k_{i,j}}$ for each pair of filaments in an ensemble, Fig. 1(c), allows us to evaluate the total pairwise Gaussian linking number 33, $L_{k s} = \sum_{j>i} |L_{k_{i,j}}| \approx N_w$, which approximates the total number of windings in the network, neglecting self-winding, $N_w$.

To test whether $L_{k s}$ predicts the system behavior, we considered networks with equal $L_{k s}$ but different $N_f$. We found that neither $L_{k s}$ nor the average pairwise Gaussian linking number per filament, $L_{k s}/N_f$, provide measures of entanglement capable of predicting the network ability to develop the tight tangle/radial bundles configuration, §3, 30. Instead, the average pairwise Gaussian linking number

$$\mathcal{E} = \frac{L_{k s}}{N_p} = \frac{2}{N_f(N_f-1)} \sum_{j>i} |L_{k_{i,j}}| \approx \frac{2N_w}{N_f(N_f-1)},$$

where the average is obtained dividing $L_{k s}$ by the number of filament pairs, $N_p$, accurately predicts this transition, §3, 30. This quantity is not a strict topological invariant but we checked that it is essentially independent of cell deformation, §3, 30.

To systematically examine the role of entanglement, we considered filament ensembles whose parameters are set to their default values and varied $\mathcal{E}$. For insufficiently entangled systems ($\mathcal{E} \lesssim 0.3$), the networks do not exhibit coherent reorganization and, concomitantly, the buildup of tension is insignificant (cyan and green curves in Fig. 2 and Movies S3, S4), in line with previous
findings on non-woven textiles. Since modest levels of $E$ correspond to limited mutual winding, IFs not directly bridging opposite sides can accommodate cell-scale deformations without elongating (green filament strain distribution in Fig. 2 inset). By remaining slack (green arrowhead in Fig. 2 and inset), these filaments do not contribute to the emergent mechanical response. Note that, while cell-scale load bearing could be in principle supported using low $E$ if all IFs were to bridge opposite cell walls, this arrangement is unlikely when network generation is stochastic, as presumably occurring in cells.

For $E \approx 0.4$, the networks develop several tight tangles connecting taut filaments. This topological reorganization enables sustained cell-scale stiffening (blue curve in Fig. 2 and Movie S5). However, the distribution of filament strains is extremely broad, indicating that some are strongly elongated while others are essentially unloaded, blue arrowhead in Fig. 2 and inset.

For $E \gtrsim 0.5$, the networks robustly reorganizes into star-shaped configurations, which mobilize all filaments with similar elongation (Fig. 2 inset) and yield stiffening beyond an activation strain $\varepsilon_c^A$, Fig. 2 and Movies S2, S6. We infer that entanglement enables network self-organization into a structurally optimal filament arrangement, akin to IF reorganization in super stretched epithelial cells. The transition of system behavior beyond a critical degree of entanglement can be interpreted as a percolation threshold.

To understand the parameters controlling $\varepsilon_c^A$ and the subsequent tension-strain relation, we developed an analytical model assuming percolation, S5 S60. From the star-shaped geometry of the stretched network, and accounting for the filament length stored in the central tight tangle, this model establishes a connection between cell- and filament-scale deformation to estimate the cellular activation strain:

$$\varepsilon_c^A \approx \frac{1}{4a_0^2} \left[ \ell_0 - \frac{\pi}{4} \phi(E) (N_f - 1) \right]^2 - 1,$$

where $\langle a \rangle = 0$ if $a < 0$ and $\langle a \rangle = a$ otherwise. Thus, the only fitting parameter is $\gamma(E)$, which should be close to 1 for barely percolated networks and increase with $E$.

We found a nearly quantitative match between the analytical model with $\gamma = 1$ and simulations at the onset of percolation, $E \approx 0.5$. For higher degrees of entanglement, we found very good agreement increasing $\gamma$ to 2.2 and 2.9 for $E \approx 0.7$ and $E \approx 1.0$, Fig. 2 consistent with the idea that networks with larger $E$ involve windings of increasing complexity. In agreement with the analytical model, the mechanical response of the system beyond percolation ($E \gtrsim 0.5$) — and particularly the emergent stiffness $\partial T_c^*/\partial \varepsilon_c^A$ — is essentially independent of entanglement except for the shift in $\varepsilon_c^A$. Accordingly, we considered a default entanglement $E \approx 0.5$ in subsequent simulations and set $\gamma = 1$ for the theoretical fits. We note that the small sensitivity of mechanical behavior on entanglement is specific to our system exhibiting corralled entanglement, since mechanical properties of entangled polymer networks generally depend on this topological parameter S9 S40.

According to our theory, as more filament length is stored in the central tight tangle, less length is available for the bundles to bridge cell boundaries. As a result, not only does the strain at which bundles become taut decrease, but the filament strain for a given cellular strain also increases. Individual filament strain distribution confirmed this rationale, black and red distributions in Fig. 2 inset. Additional simulations show that the mechanical response and network mechanisms described here are not modified by thermal vibrations, S7 S8 S9, strain rate, S8 S9, or changes in filament bending rigidity, S9 S30, and that the emergent stiffness scales proportionally to the filament elastic modulus S10 S9.

To further test our theory, we examined the role of filament length, which according to Eqs. S4 modifies the activation strain, $\varepsilon_c^A$, and the emergent tension, $T_c^*$. In agreement with model predictions, shorter/longer filaments lead to smaller/larger activation strains and
stiffer/softer networks, Fig. 3(a), with a marked downward shift in filament strain distributions for longer filaments as more filament length is available to accommodate cellular strain. Per Eq. (4), the tension-strain curves of networks with different filament lengths collapse when representing $\ell_0 T^*_c$, Fig. 3(a)-inset, reflecting the increased compliance of longer filaments. Varying the number of filaments, $N_f$, directly affects the slope of the cell-scale response, Fig. 3(b), in agreement with our theory that predicts a linear increase of tension and stiffness with $N_f$, which we further confirmed by representing $T^*_c/N_f$ in Fig. 3(b)-inset. Instead, the number of filaments mildly impacts the activation strain and hence the transfer of cellular strain into filament strain, Fig. 3(b)-inset, although $N_f$ appears explicitly on an equal footing as $E$ in Eq. (6). We attribute this difference to the fact that increasing $E$ beyond the percolation threshold, as in Fig. 2, complexifies the windings, storing more filament length in the central tight tangle and leading to a very strong effect on $\varepsilon^0_a$, which our model captures by increasing $E(c)$. Conversely, when fixing $E \approx 0.5$ and modifying $N_f$, the winding complexity is not significantly affected, as confirmed by the accurate fit of the emergent mechanics obtained with $\gamma = 1$. It follows that filament loading in our entangled networks is determined primarily by $E$ and $\ell_0$ and only mildly by $N_f$, whereas emergent tension and stiffness are directly controlled by $N_f$ and to a lesser extent by $\ell_0$.

Since IFs exhibit a highly nonlinear force-stretch relation, we then wondered whether the filament constitutive behavior affected the slack-taut transition and the emergent mechanics. We considered filaments that soften to $E/5$ for $\varepsilon_f \in [10\% - 40\%]$ and eventually re-stiffen to reach $10E$, hence exhibiting the typical supereastic response [17][22]. The emergent stiffness of the taut network mirrors the individual IF constitutive relations, Fig. 3(c), consistent with the narrow filament strain distribution beyond percolation. To examine the influence of filament length variability, we sampled $\ell_0$ from a normal distribution with mean $5a_0$ and standard deviation $0.5a_0$. For linearly elastic filaments, this reduces the activation strains, as shorter filaments are mobilized earlier. For nonlinear filaments, the plateau in the filament response is lost at the cellular scale, as the emergent behavior results from convolving the nonlinear constitutive laws of unequally strained filaments. Cell-scale stiffening is also reached earlier when including shorter filaments. However, the slack-taut transition corresponding to the formation of radial IF bundles remains unchanged. [ST] [30]. Thus, while the shape of the emergent mechanical response past the activation strain depends on the constitutive relation, the number, and the length distribution of the filaments, their self-organization into a star-shaped configuration is solely determined by network entanglement.

Following this rationale, the slack-taut transition should also be preserved when varying the cell shape. We thus prepared networks with default parameters and enclosed them in cells with the same $a_0$ but different

**FIG. 3.** Cell-scale mechanical response (solid lines and shadings: mean ± standard deviation of 8 model realizations; dashed lines: 1D analytical model with $\gamma = 1$) and filament strain distribution at $\varepsilon_c = 1000\%$ for varying $\ell_0$ (a), $N_f$ (b), $\ell_0$ distribution and linear vs. nonlinear filament extensibility (c).

**FIG. 4.** Network reorganization for alternative shapes of the enclosing cell.
According to the three regular tessellations of the plane \([11]\), we compared triangular, square, and hexagonal cells \([N_c=3, 4, 6]\). Remarkably, the percolation threshold and network reorganization are independent of cell shape, yielding \(N_c\) filament bundles that bridge the lateral walls to an approximately central tight tangle, Fig. 7 and S12 \([30]\).

In summary, inspired by the phenomenology of IFs during epithelial stretching \([3]\), we have studied the physical principles supporting the emergent mechanical behavior of an ensemble of entangled extensible filaments confined to a cell, with laterally moving boundary attachments. We identify a metric of entanglement, \(\mathcal{E}\), which robustly predicts a percolation threshold, \(\mathcal{E} \gtrsim 0.5\), leading to self-organization of random filament networks into structurally optimal configurations beyond an activation strain. The occurrence of the transition is purely topological, whereas the emergent mechanics depend on filament length, number, and constitutive response, enabling independent control of activation strain and stiffness.

Our work suggests that, through entanglement and self-organization within cells, IF networks provide a “safety net” against extreme strains, complementary to their role as “safety belt” against fast strain rates hinging on the IF rate-dependent behavior \([21]\). Beyond the biological context, network entanglement has been exploited to enhance mechanical properties of hydrogels \([12, 13]\) and is at the core of textile materials \([14, 15]\). Here, we identify corralled entanglement as a scale-free principle for extremely deformable bioinspired materials, whose organization lies between random networks and woven materials. By relying on self-organization, this principle is devoid of the synthesis challenges of weaving or knitting at a molecular scale \([17]\).

Acknowledgements. We thankfully acknowledge the computer resources at Calèndula (SCAYLE) and the technical support provided by Barcelona Supercomputing Center (RES-IM-2021-2-0017 & RES-IM-2021-2-0028). This work was supported through funding from the Spanish Ministry of Science and Innovation & NextGenerationEU/PRTR (PCI2021-122049-2B), the EU Research Council (CoG-681434), the EU Commission & NextGenerationEU/PRTR (PCI2021-122049-2B), the technical support provided by Barcelona Supercomputing Center (RES-IM-2021-2-0017 & RES-IM-2021-2-0028). This work was supported through funding from the Technical University of Catalonia (SCAI, RES-IM-2021-2-0017 & RES-IM-2021-2-0028). This work was supported through funding from the Technical University of Catalonia (SCAI, RES-IM-2021-2-0017 & RES-IM-2021-2-0028).

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[30] See Supplemental Material for further details on: model generation (S1), quantification of the pairwise linking number in discrete systems (S2), comparison of alternative definitions of system-wide entanglement (S3), influence of cell deformation on $E$ (S4), analytical model development (S5), cell stiffening in the absence of filament entanglement (S6), influence of system temperature (S7), cell deformation rate (S8), filament bending rigidity (S9), elastic modulus (S10), length distribution and constitutive behavior (S11), as well as enclosing cell shape (S12) on cell-scale mechanics.
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Supplemental Material: Slack-taut transition and emergent stiffness in bioinspired entangled filament networks

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S1. GENERATION OF ENTANGLED FILAMENT NETWORK MODELS IN CYTOSIM

To establish computational models of entangled filament networks, we leverage cytosim’s ability to simulate the growth of inextensible filaments in a confined cellular space, which we model as a right regular prism. The cell base has $N_c$ edges; its apothem length and height in the reference configuration are $a_0$ and $h_0 = a_0/4$. We seed $N_f$ filaments of initial length $\ell_0 = 0.03a_0$ and bending rigidity $\kappa \approx 3EA_f^2 \times 10^{-9}$, i.e. a persistence length $\ell_p = a_0$ at room temperature, on the surface of a cylinder of radius $R = 0.9a_0$, coaxial with the prism, Fig. S1(a). The filament diameter, $\phi = a_0/50$, is represented by enforcing repulsive steric interactions through a harmonic potential of stiffness $k_s = 500EA_f/a_0$, resulting in frictionless contacts among filaments. All filament points are confined inside the cell volume by a harmonic potential whose stiffness is set to $k_c = 50EA_f/a_0 \gg EA_f/R_0$ for points outside the cell volume, and to 0 for points located inside. To form entanglements, we let filaments grow at room temperature ($k_BT = 0.0042$ pN µm) in an environment of effective viscosity $\nu = 1$ pNs/µm$^2$, Fig. S1(b), resulting in a random-walk-like stochastic process. Importantly, filament ends are not constrained to lie on the side walls of the cell at this stage.

During this process, we track $E$. Slightly before reaching the prescribed value of $E$, we activate a confining potential, also of stiffness $k_c$, which brings each filament end on the closest side face of the enclosing prism, Fig. S1(c). At this point, the length of the filaments is adjusted while holding their ends with very stiff springs (red dots in Fig. S1(d)), such that the network entanglement cannot change significantly. The entire system is then equilibrated at room temperature to eliminate any pretension that might arise during model generation, Fig. S1(e). Since the described steps involve stochastic events, we consider 8 model realizations for each parameter set. We treat the filaments as inextensible ($E = \infty$) during model generation, and only set $k_BT \approx 0$ pNµm when simulating cell stretching.

(a) Seed $N_f$ filaments  (b) Form entanglements  (c) Ends on membrane  (d) Growth at fixed $E$  (e) Equilibration

FIG. S1. Model generation, performed at room temperature ($k_BT > 0$). $N_f$ filaments are seeded within the confining space (a) and let grow to form entanglements (b). Upon reaching the prescribed $E$, a confining potential brings the filament ends on the cell side walls (c). Then, $\ell_0$ is adjusted to reach the desired value by letting filaments grow while their ends are fixed, so that $E$ cannot change significantly (d). A long equilibration phase (e) ensures that any pretension possibly associated with model generation is removed prior to stretching for mechanical characterization.
S2. QUANTIFICATION OF THE PAIRWISE LINKING NUMBER FOR CLOSED AND OPEN CURVES

To evaluate entanglement in our computational models, where filaments are represented by sequences of segments, we approximate Eq. (1) in the main text by

$$L_{ki,j} \approx \frac{1}{4\pi} \sum_{A=1}^{N_i-1} \sum_{B=1}^{N_j-1} \frac{\mathbf{R}_A^{(i)} - \mathbf{R}_B^{(j)}}{|\mathbf{R}_A^{(i)} - \mathbf{R}_B^{(j)}|^3} \cdot [d\mathbf{R}_A^{(i)} \times d\mathbf{R}_B^{(j)}],$$

(S1)

where $\delta_i$ and $\delta_j$ have been discretized using $N_i$ and $N_j$ points that define the piecewise straight segments $d\mathbf{R}_A^{(i)}$ and $d\mathbf{R}_B^{(j)}$ with midpoint locations $\mathbf{R}_A^{(i)}$ and $\mathbf{R}_B^{(j)}$.

To address the influence of such discretization on the quantity $L_{ki,j}$, we consider a link with known linking number and apply Eq. (S1) with varying $N_i$ and $N_j$, assuming for simplicity that $N_i = N_j = N$. For a Hopf link, i.e. a set of two closed curves interlinked in the simplest possible way, the linking number is 1. As shown in Fig. S2(a), it is sufficient to take $N \geq 10$ to keep the error induced by discretization below 10%. This confirms that a discrete version of Eq. (1) in the main text can be used to provide a quite accurate estimate of $L_{ki,j}$ for closed curves. However, the filaments that we model are open sequences of segments. Applying Eq. (S1) to pairs of filaments akin to those in our network models shows that $L_{ki,j}$ provides a good estimate of the degree of mutual winding between filaments, Fig. S2(b).

FIG. S2. Quantification of the pairwise linking number for closed and open curves. For a pair of closed curves forming a Hopf link and discretized by a finite number of points, $L_{ki,j}$ converges to its exact value as the discretization is refined (a). For pairs of open curves, $L_{ki,j}$ can be used to identify mutual winding despite not being a strict topological invariant (b).
S3. ALTERNATIVE NETWORK ENTANGLEMENT METRICS AND THEIR LINK TO MECHANICS

To substantiate the use of the system-wide entanglement metric adopted in the main text, Eq. 2, we compare alternative definitions and discuss their suitability to capture the characteristic self-organization of our filament ensembles. Specifically, we aim to confirm that $E$ can discern between the percolated and non-percolated network behaviors, whereas $Lks$ and $Lks/N_f$ cannot. We recall next the definitions of these entanglement measures:

- $Lks$ is the total pairwise Gaussian linking number, approximating the total number of windings in the network if self-winding is neglected:

$$Lks = \sum_{j>i} |Lk_{i,j}| \approx N_w,$$

(S2)

- $Lks/N_f$ is the average pairwise Gaussian linking number per filament, which approximates the average number of windings per filament and, in principle, could provide an approach to compare systems with different $N_f$

$$\frac{Lks}{N_f} = \frac{1}{N_f} \sum_{j>i} |Lk_{i,j}| \approx \frac{N_w}{N_f},$$

(S3)

- $E$ is the average pairwise Gaussian linking number per filament pair, which has been adopted in the main text and approximates the average number of windings per filament pair

$$E = \frac{Lks}{N_p} = \frac{2}{N_f(N_f-1)} \sum_{j>i} |Lk_{i,j}| \approx \frac{N_w}{N_p},$$

(S4)

Neither $Lks$, Fig. S3(a), nor $Lks/N_f$, Fig. S3(b), are useful predictors of network percolation leading to self-organization, as shown by comparing ensembles with different $N_f$ and otherwise identical parameters and values of the considered entanglement metric, Fig. S3. Quantifying the emergent mechanical behavior of these networks also shows that, fixing $Lks$, Fig. S3(c), or $Lks/N_f$, Fig. S3(f), and increasing $N_f$ leads to a softer response. Thus, we discard these measures of entanglement. In contrast, $E$ predicts percolation independently of $N_f$, Fig. S3(c, d, g, h).

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**FIG. S3.** Alternative definitions of system-wide entanglement and their link to network self-organization for ensembles with comparable level of entanglement according to each definition (a-d; dot and braces: mean ± standard deviation of 8 model realizations). Neither $Lks$ (a) nor $Lks/N_f$ (b) predict percolation when varying the number of filaments $N_f$. They also lead to a counterintuitive network softening when increasing $N_f$ (e, f). Conversely, $E$ is a robust predictor of percolation irrespective of $N_f$ (c, d, g, h). Solid lines and shadings: mean ± standard deviation of 8 model realizations.
S4. INFLUENCE OF CELL STRAIN ON THE MEASURE OF NETWORK ENTANGLEMENT $\mathcal{E}$

To further test the robustness the proposed entanglement metric, we report its variation throughout the performed cell stretching simulations. As shown in Fig. S4 for the systems compared in Fig. 2, $\mathcal{E}$ is only mildly affected by the applied cell deformation. Although $\mathcal{E}$ is not constant, its variations during stretching do not modify the ranking of the networks according to their entanglement, Fig. S4(a). Likewise, systems with different $\ell_0$, Fig. S4(b), or $N_f$, Fig. S4(c), but a comparable level of entanglement maintain similar values of $\mathcal{E}$ throughout the simulations. Taken together, these data corroborate the choice of $\mathcal{E}$ as a suitable system-wide entanglement metric for the filament networks considered here.

![Diagram of network entanglement evolution](https://via.placeholder.com/150)

FIG. S4. Evolution of network entanglement during stretching simulations for systems with clearly different $\mathcal{E}$ (a) and for systems with comparable entanglement but different $\ell_0$ (b) or $N_f$ (c). In all cases, $\mathcal{E}$ is very robust to deformations of the cell enclosing the filaments.
S5. DERIVATION OF AN ANALYTICAL MODEL OF EQUIBIAXIAL CELL STRETCHING

To establish an analytical model of cell stretching for percolated filament networks, we focus on a 2D projection of the system on a plane that is parallel to the base of the prism enclosing the $N_f$ filaments, Fig. S5(a). Assuming that all filaments have the same circular cross-sectional area, $A_f = \pi \phi^2 / 4$, $\phi$ being their diameter, constant elastic modulus, $E$, and undergo the same strain, $\varepsilon_f$, when the cell is subjected to an equibiaxial areal strain, $\varepsilon_c$, it is immediate to derive the total cell force as:

$$F_c = \sum_{i=1}^{2N_f} F_i = 2N_f EA_f \varepsilon_f.$$  \hfill (S5)

The corresponding dimensionless nominal cell tension is thus:

$$T_c^* = \frac{F_c a_0}{EA_f N_c s_0} = \frac{N_f \varepsilon_f}{N_c \tan(\pi/N_e)},$$  \hfill (S6)

where we have used the geometrical relation between the side and apothem length of a regular polygon, $s_0 = 2a_0 \tan(\pi/N_e)$.

To determine the filament strain, we need to link the cell-scale deformation to the current filament length. From simple kinematical arguments, we can express the current apothem length, $a$, in terms of its reference value, $a_0$, and the cell areal strain, $\varepsilon_c$:

$$a = a_0 \sqrt{\varepsilon_c + 1}.$$  \hfill (S7)

Assuming that the central tight tangle that forms at large cell deformations is located at the center of the enclosing regular polygon, we can also approximate the current apothem length in terms of the current filament length, $\ell$, by deducting the length stored in the clump corresponding to the tight tangle, $\ell_{clump}$, as sketched in Fig. S5(a):

$$a \approx \frac{1}{2} (\ell - \ell_{clump}).$$  \hfill (S8)

To determine $\ell_{clump}$, we first consider the case of a filament (i) being wound around another one (j) in the simplest possible way, Fig. S5(b). The length used to form such a winding is simply:

$$\ell_{wn}^{(i)} = \frac{\pi \phi}{2} = \ell_w.$$  \hfill (S9)

For a filament (i) that forms a total of $N_w^{(i)}$ windings of this kind with other filaments in the ensemble, the length available to bridge the cell walls will thus be reduced by the quantity:

$$\ell_{clump} = N_w^{(i)} \ell_w = \frac{N_w^{(i)} \pi \phi}{2}.$$  \hfill (S10)

FIG. S5. Derivation of a 1D analytical model of cell stretching. (a) The current filament length, $\ell$, and apothem length, $a$, are related via the length stored in the central tight tangle, $\ell_{clump}$. (b) The simplest type of winding is that formed by a filament (i) looping around another filament (j) for half a circle. (c) Windings that involve more filaments correspond to a larger filament length stored in the tight tangle, as captured by the scalar parameter $\gamma(\mathcal{E}) \geq 1$. 

However, filaments might form more complex windings than the one sketched in Fig. S5(b). For instance, a filament could wind around more than one other filament, Fig. S5(c), such that the length $\ell_{wn}^{(i)}$ will be larger than $\ell_w$ and possibly not be the same for all windings in the system, leading to the following general expression:

$$\ell_{clump} = \sum_{n=1}^{N_w^{(i)}} \ell_{wn}^{(i)}.$$  \hspace{1cm} (S11)

To account for the additional length used by filaments when they form more complex windings as compared to that in Fig. S5(b), we introduce a scalar, $\gamma$, defined as:

$$\gamma = \frac{\ell_{clump}}{N_w^{(i)}} \frac{\ell_w}{\bar{\ell}_w}.$$  \hspace{1cm} (S12)

The parameter $\gamma$ can be interpreted as a phenomenological measure of the typical complexity of the windings in a given system. Its value will be 1 when all windings correspond to the sketch in Fig. S5(b), whereas $\gamma$ will increase for systems that feature more complex windings, Fig. S5(c), as we expect to occur when $E$ increases past the percolation threshold. Thus, using the definition of $\gamma$ given in Eq. (S12) and the definition of $\bar{\ell}_w$ given in Eq. (S9), we can write:

$$\ell_{clump} = \gamma N_w^{(i)} \ell_w = \frac{\gamma}{2} N_w^{(i)} \pi \phi.$$  \hspace{1cm} (S13)

Since more strongly entangled systems will have a tendency to form more complex windings, we infer that the value of $\ell_{clump}$ will be larger for such systems and hence that $\gamma(E)$ should be an increasing function.

Recalling the definition of $E$ provided in Eq. (S4), we can further approximate the number of windings formed by the $i$-th filament in a system that has a total of $N_w$ windings as:

$$N_w^{(i)} \approx \frac{N_w}{N_f} \approx \frac{E}{2} (N_f - 1),$$  \hspace{1cm} (S14)

so that Eq. (S13) becomes

$$\ell_{clump} \approx \frac{\gamma}{4} E (N_f - 1) \pi \phi,$$  \hspace{1cm} (S15)

and Eq. (S8)

$$a \approx \frac{1}{2} \left[ \ell - \frac{\pi}{4} \gamma E \phi (N_f - 1) \right].$$  \hspace{1cm} (S16)

Finally, we can relate the cell areal strain, $\varepsilon_c$, and the current filament length, $\ell$, by combining Eq. (S7) and Eq. (S18):

$$a_0 \sqrt{\varepsilon_c + 1} \approx \frac{1}{2} \left[ \ell - \frac{\pi}{4} \gamma E \phi (N_f - 1) \right],$$  \hspace{1cm} (S17)

so that

$$\ell \approx 2a_0 \sqrt{\varepsilon_c + 1} + \frac{\pi}{4} \gamma E \phi (N_f - 1).$$  \hspace{1cm} (S18)

The cell activation strain, $\varepsilon_c^A$, i.e. the areal strain at which filaments become taut and begin contributing to the cell-scale response, corresponds to a filament length $\ell = \ell_0$:

$$\ell_0 \approx 2a_0 \sqrt{\varepsilon_c^A + 1} + \frac{\pi}{4} \gamma E \phi (N_f - 1).$$  \hspace{1cm} (S19)

Solving Eq. (S19) for $\varepsilon_c^A$, we obtain the approximation provided in Eq. (3) in the main text:

$$\varepsilon_c^A \approx \frac{1}{4a_0^2} \left[ \varepsilon_c - \frac{\pi}{4} \gamma \phi (E) (N_f - 1) \right]^2 - 1.$$  \hspace{1cm} (S20)

Per its definition, we also obtain the current filament strain for $\varepsilon_c \geq \varepsilon_c^A$ as:

$$\varepsilon_f = \frac{\ell - \ell_0}{\ell_0} \approx \frac{2a_0}{\ell_0} \left( \sqrt{\varepsilon_c + 1} - \sqrt{\varepsilon_c^A + 1} \right).$$  \hspace{1cm} (S21)
Since the filament strain for $\varepsilon_c \leq \varepsilon_c^A$ is zero, we can introduce Macaulay brackets and obtain an general expression valid for any $\varepsilon_c$:

$$\varepsilon_f \approx \frac{2a_0}{l_0} \left( \sqrt{\varepsilon_c + 1} - \sqrt{\varepsilon_c^A + 1} \right).$$  \hspace{1cm} (S22)

Plugging Eq. (S22) into Eq. (S6), we can finally write

$$T^* = \frac{2N_f a_0}{l_0 N_e \tan(\pi/N_e)} \left( \sqrt{\varepsilon_c + 1} - \sqrt{\varepsilon_c^A + 1} \right),$$  \hspace{1cm} (S23)

which coincides with Eq. (4) in the main text and provides an analytical expression to estimate the dimensionless nominal tissue tension for an entangled filament ensemble of known characteristics.
S6. LOAD BEARING IN UNENTANGLED FILAMENT ENSEMBLES

As mentioned in the main text, cell-scale load bearing using ensembles of unentangled filaments is possible, though quite unlikely in stochastically-generated systems. Here we use our analytical model to examine such situation in detail by focusing on a system that shares all properties with the one considered in S5 but its filaments always bridge opposite cell sides without forming entanglements ($E = 0$). We can immediately notice that the filaments will only be extended when the apothem length, $a$, corresponding to the applied areal strain, $\varepsilon_c$, satisfies the condition $2a \geq \ell_0$. Thus, the filament-scale strain can be expressed as follows:

$$\varepsilon_f = \left( \frac{2a - \ell_0}{\ell_0} \right). \quad (S24)$$

We note that this expression is equivalent to the one that results from setting $E = 0$ in Eq. (S22) and recalling Eq. (S7):

$$\varepsilon_f = \frac{2a_0}{\ell_0} \left( \sqrt{\varepsilon_c + 1} - \sqrt{\varepsilon_c^A + 1} \right) = \left( \frac{2a}{\ell_0} - 1 \right), \quad (S25)$$

leading to the following dimensionless nominal tissue tension

$$T^*_c = \frac{N_f}{N_c \tan(\pi/N_f)} \left( \frac{2a}{\ell_0} - 1 \right). \quad (S26)$$

We can also explicitly write the activation strain for the unentangled filament network as:

$$\varepsilon^A_c = \frac{\ell^2_0}{4a^2_0} - 1, \quad (S27)$$

which coincides with the expression obtained from Eq. (S20) by setting $E = 0$.

Using Eq. (S26), we can investigate the influence of $\ell_0$ and $N_f$ on the cell-scale response. The results in Fig. S6b, c) show that an unentangled network where all filaments connect opposite cell walls can provide control over the cell-scale activation strain and stiffness but, unlike an entangled ensemble, it lacks the additional degree of freedom provided by $E$ (cf. Fig. 2). More importantly, these unentangled network require a precise assembly whereas entangled networks past the percolation threshold self-organize to provide load-bearing capacity.

![Figure S6](image)

**FIG. S6.** Equibiaxial stretching of an unentangled filament ensemble. (a) Idealized network rearrangement under stretching. Influence of $\ell_0$ (b) and $N_f$ (c) on the emergent cell-scale mechanical response.
To validate the assumption that cell deformation can be modeled as an athermal process, we simulate stretching at room temperature for 8 realizations of the model with default parameters. As shown in Fig. S7, the difference between thermal and athermal simulations is minor, confirming that neglecting the role of thermal fluctuations in the context of cell stretching is a reasonable assumption.

**FIG. S7.** Influence of $k_B T$ on the mechanical response of the entangled network, confirming the negligible role of thermal fluctuations in the present context. Solid lines and shadings: mean ± standard deviation of 8 model realizations.
S8. INFLUENCE OF STRAIN RATE

To confirm that cell deformation is applied quasi-statically and that the results reported in the main text have negligible dependence on the ambient viscosity, $\nu$, we subject 8 realizations of the model with default parameters to varying strain rates, $\dot{\varepsilon}_c$. As shown in Fig. S8, neither the cell-scale response in the stiffening regime nor the activation strain are significantly affected by $\dot{\varepsilon}_c^* = \dot{\varepsilon}_c \nu / E$. This indicates that all rates in the explored range correspond to very slow stretching with respect to the intrinsic time scale of the system, $\tau_{load} \gg \tau = \nu / E$, confirming the negligible influence of $\nu$ on the reported cell-scale mechanical behaviors. This is further confirmed by the extremely small value of the dimensionless cell strain rate: $\dot{\varepsilon}_c^* = \dot{\varepsilon}_c \nu / E = \varepsilon_c^{max} \tau / \tau_{load} \approx 2 \times 10^{-6}$.

We also observe that the initial region of the cell-scale response exhibits some dependence on $\dot{\varepsilon}_c^*$, with slightly higher tensions for fast strain rates, indicating that the tension for $\varepsilon_c < \varepsilon_c^A$ is mainly determined by the drag of filaments past the frictional medium.

![Graph showing influence of strain rate on mechanical response](image)

**FIG. S8.** Influence of strain rate on the mechanical response of the entangled network, confirming the negligible effect of $\dot{\varepsilon}_c^*$. Solid lines and shadings: mean ± standard deviation of 8 model realizations.
The role of filament bending rigidity, $\kappa$, has been neglected in developing the 1D analytical model, cf. §S5. To validate such assumption, we simulate the stretching of 8 realizations of the model where all parameters but $\kappa$ are set to their default values. We introduce the dimensionless bending rigidity, $\kappa^* = \kappa/Ea^4_0$, and vary it in a range spanning two orders of magnitude, confirming its negligible influence on the emergent cell-scale response, Fig. S9. We also observe that sufficiently large values of $\kappa^*$ (red curve and shading, corresponding to a $100\times$ increase) have a noticeable effect and make the slack-taut transition less abrupt. However, the effect at very large strains remains negligible. We expect that system response might be affected by choosing even larger values of $\kappa^*$, since the straightening of initially curved filaments is clearly related to their bending rigidity. However, we note that an Euler-Bernoulli beam with circular cross-section of diameter $\phi = a_0/50$ has $\kappa_{\text{beam}}^* \approx 7.9 \times 10^{-9}$, which is about $3\times$ our default value for $\kappa^*$. This suggests that values of $\kappa^*$ having a significant influence on the cell-scale response are of little practical interest. Moreover, the persistence length of IFs is $(0.2 - 1) \mu$m [15]. At room temperature, and using the above normalization, this corresponds to a dimensionless bending rigidity $\kappa_{\text{IF}}^* \approx (1 - 5) \times 10^{-10}$. This value is about one order of magnitude below the smallest $\kappa^*$ considered in our simulations, indicating that the contribution of IF bending rigidity to cell deformability is most certainly negligible.

FIG. S9. Influence of filament bending rigidity on the mechanical response of the entangled network, confirming the negligible role of $\kappa^*$ within the examined range. Solid lines and shadings: mean ± standard deviation of 8 model realizations.
S10. INFLUENCE OF FILAMENT ELASTIC MODULUS

When addressing the influence of the filament elastic modulus, $E$, on the emergent response of a network of linearly elastic filaments, we recall that $T_c^* = T_c a_0/E A_f$ is normalized with respect to $E$. Therefore, we expect a negligible influence of $E$ on $T_c^*$, particularly at large cell strains where the emergent response is dominated by filament stretching. Both the simulations and the analytical model, Fig. S10, support this rationale, suggesting that the dimensional cell tension, $T_c$, scales linearly with $E$.

![Graph](image)

FIG. S10. Influence of filament elastic modulus on the mechanical response of the entangled network. Owing to the normalization included in its definition, $T_c^*$ is only marginally affected by $E$, implying that $T_c$ depends linearly on $E$. Solid lines and shadings: mean ± standard deviation of 8 model realizations. Dashed lines: 1D analytical model with $\gamma = 1$. 
S11. INFLUENCE OF FILAMENT LENGTH DISTRIBUTION AND CONSTITUTIVE BEHAVIOR

As mentioned in the main text, network self-organization into a star-shaped configuration is solely determined by its entanglement. Here we further confirm that this is the case by comparing models with default parameters except for the filament constitutive behavior, Fig. S11(b, d), or their initial length distribution, Fig. S11(c, d). Under equibiaxial loading, all models self-organize into the characteristics star-shape configuration observed for the default model, Fig. S11(a).

FIG. S11. Slack-taut transition in cell-scale models that differ in terms of length distribution or constitutive behavior of the filaments, showing that these parameters do not affect network self-organization. The models are colored according to the curves and shadings in Fig. 3(c). (a) Default model, where all filaments have equal initial length and are mechanically linear. (b) Model featuring filaments with homogeneous initial length but a nonlinear constitutive behavior. (c) Model comprising mechanically-linear filaments with inhomogeneous initial length. (d) Model featuring nonlinear filaments with inhomogeneous initial length.
S12. INFLUENCE OF ENCLOSING CELL SHAPE

In the main text, we have shown that the occurrence of the slack-taut transition does not depend on the enclosing cell shape for triangular, square, or hexagonal prisms. Here, we focus on the emergent mechanical responses of 8 model realizations featuring default parameters but enclosed in cells with \( N_e = 3, 4, \) or 6. While both our simulations and analytical predictions indicate larger \( T^*_c \) for larger \( N_e \), this effect is purely associated with the definition of \( T^*_c \), which involves normalization of the cell-scale force by the reference cell perimeter, \( 2p_0 \propto N_e \tan(\pi/N_e) \), a quantity decreasing monotonically with \( N_e \) for \( N_e \geq 3 \). Indeed, the cell-scale force is independent of \( N_e \), as shown in the inset and also predicted analytically: \( F_c/E_A f = 4N_f a_0 (\sqrt{\varepsilon_c + 1} - \sqrt{\varepsilon_A^A + 1})/\ell_0 \).

FIG. S12. Influence of enclosing cell shape on the mechanical response of entangled networks. Since all networks have default parameters, the emergent response is only affected by the cell perimeter involved in the definition of \( T^*_c \), which changes with \( N_e \). The inset confirms that cell-scale forces are consistent across the considered models. Solid lines and shadings: mean ± standard deviation of 8 model realizations. Dashed lines: 1D analytical model with \( \gamma = 1 \).