Viscoelastic properties of vimentin originate from nonequilibrium conformational changes

Johanna Block1,*, Hannes Witt2†‡, Andrea Candelli3,4, Jordi Cabanas Danes3,4, Erwin J. G. Peterman3, Gijs J. L. Wuite3, Andreas Janshoff2‡, Sarah Köster1‡

Introduction

Vimentin belongs to the protein family of intermediate filaments (IFs), which, together with microfilaments (MFs) and microtubules (MTs), constitute the cytoskeleton of eukaryotic cells. It is widely accepted that this composite network of biopolymers plays an important role in cell mechanics, motility, adhesion, and contraction. Each filament type contributes distinct mechanical features; whereas MFs and MTs are well characterized from a physics point of view, IFs are less well understood. In contrast to actin and MTs, which are evolutionary highly conserved, IFs are encoded by 70 genes in humans (1), subdivided in five categories, and expressed in a cell-type-specific manner. Vimentin not only is the typical IF protein in cells of mesenchymal origin but is also overexpressed in cancer cells of epithelial origin and is associated with metastasis. Moreover, IFs have been proposed to play a major role in mechanical resistance and integrity of cells, especially in response to environmental stress (2–5). All IFs share the secondary structure of the monomer (Fig. 1A and fig. S1), including an α-helical rod domain and intrinsically disordered head and tail domains (6, 7).

The formation of extended filaments from cytoplasmic IF proteins follows a complex hierarchical scheme, distinct from MFs or MTs. First, lateral assembly of monomers via parallel coiled-coil dimers and half-staggered antiparallel tetramer yields unit-length filaments (ULFs) (6), schematically shown in Fig. 1A (middle). Subsequently, longitudinal annealing of these ULFs results in mature, elongated filaments of 10 nm in diameter. This hierarchical filament assembly via dimers, tetramers, octamers, and ULFs and the protodilibrillar organization, which was found, for example, for keratin (8), are in stark contrast to MFs and MTs, which are built from globular subunits, and may be the reasons for the remarkable properties of IFs. It should be noted that IFs, and in particular keratins, also form very stable and resistant natural superstructures such as wool and hair, whose overall mechanical resistance has been extensively studied since the beginning of the last century (9,10). However, the properties of single cytoplasmic IFs, which are the basis for understanding how IFs provide cells with mechanical resistance, cannot be retrieved from these ensemble experiments.

Although IFs display intriguing mechanical characteristics (11–13), little is known about the mechanisms involved at the single filament and molecular levels. Stretching experiments and molecular dynamics simulations of IFs or subunits thereof have shown enormous extensibility with strains up to 3.5 (14–16), different stretching regimes (14, 17), and a loading rate–dependent force response (14). Here, we directly probe the mechanical response of individual vimentin filaments to one-time and repeated stretching-relaxation cycles and to clamping at forces of 50 to 700 pN [force clamp (FC)]. By theoretical modeling and Monte Carlo (MC) simulations based on structure predictions of IF subunits and the hierarchical assembly model, we directly link our experimental results to the molecular architecture of vimentin IFs. With this approach, we find strong evidence for an intricate dissipative behavior and suggest a mechanism for the observed dependence of the mechanical properties on the strain history based on conformational switching between α-helical and β sheet secondary structures. The compiled data reveal how the molecular structure of the vimentin monomer imprints viscoelastic properties on larger length scales and show how extension far from equilibrium permits to dissipate a large amount of energy. We speculate that these concepts of controlled failure due to weak transient bonds and reversible conformational changes are important to protect cells in situations of extreme stresses. From a physics point of view, our observations provide a direct single filament viscoelasticity based on nonequilibrium transformation of the secondary and tertiary protein structure.

Results

Pronounced energy dissipation

To elucidate the energy dissipation mechanisms in vimentin filaments, single filaments were stretched up to forces of 500 to 600 pN and subsequently relaxed (Fig. 1B). Loading and unloading were performed at different velocities varying over two orders of magnitude. The extension curves show three regimes: a linear increase, a plateau (presumably caused by unfolding of α helices in the vimentin monomers, the so-called α-β transition), and a second linear increase with strain (14, 17). In contrast, the relaxation curves do not exhibit a force plateau and are largely...
The effective two-state model is able to quantitatively reproduce the experimental force cycles (Fig. 1, B and C). The main difference between the experimental curves and the corresponding curves obtained from the effective two-state model is the shape of the retraction curves: For the relaxation data, while the two-state model shows a sharp edge at the onset of α helix formation, the experimental curve is more convex, in particular for the slowest loading rate. We may attribute this effect to lowering of the persistence length upon stretching (Fig. S2). The reduction in persistence length might arise from the thinning of the filament (16), from the decoupling of parallel monomers, or from a reduction of the persistence length of β sheets compared to α helices (19).

The apparent energy dissipation comprises more than 70% of the total energy (Fig. 1D) put into the filament, which is comparable to biological high-performance materials such as fibrin fibers, spider silk, or titin (20–24). One might be tempted to attribute the apparent energy dissipation to viscous friction during sliding of IF subunits against each other. However, our analysis indicates that the major contribution to the energy dissipation originates from the unfolding and refolding of the α helices occurring far from equilibrium. Nonequilibrium conformational changes have been identified as a mechanism for energy dissipation early on and can even be considered as an origin of viscous behavior, in contrast to the prevalent “exchange of old neighbors for new ones”—concept of viscosity (10). For the sake of clarity, we will refer to the former as nonequilibrium unfolding and to the latter as viscosity. We compare the contributions of the two different mechanisms to the apparent energy dissipation by switching off the viscous element in the model (light blue curve in Fig. 1E). Surprisingly, we find that the apparent energy dissipation is indeed dominated by nonequilibrium unfolding (red curve), while viscous contributions only become relevant at low loading and relaxation rates. Whether this viscous contribution is due to sliding of subunits or due to the extension of head and tail domains in the filament is not possible to reveal from our current data.
A biopolymer with tensile memory

To address the question of filament adaptation to mechanical stress, we performed repeated stretching cycles to increasing distances with individual vimentin filaments (Fig. 2A and MC simulations in Fig. 2B) and found a strong dependency on the strain history, which we refer to as tensile memory. The initial slope of the force-strain curve, and therefore the stiffness of the filaments (Fig. 2C), considerably decreases from cycle to cycle. The filaments reach their initial length after each full cycle. This complex tensile behavior cannot be explained by the effective two-state model but requires that each element is able to adopt additional states (10). Therefore, we devise a stochastic model explicitly considering that the filaments consist of multiple [32 on average (6)] monomers per cross section. We assume that, during relaxation of the filament, not all parallel monomers return to the α state but some remain in the β state. The length of the ULF is set by the length of the shortest element, explaining why the filament reaches its initial length after each cycle. For this reason, only the shorter elements experience the applied force upon restretching; therefore, these elements determine the filament’s stiffness, which is approximately proportional to the number of parallel α helices. After each force cycle, an increasing number of helices remain in the β state, therefore lowering the stiffness of the filament with each cycle. A similar mechanism has been proposed for fibrin and collagen networks, where elongation of individual fibers was identified as the origin of the observed tensile memory (25). We performed MC simulations of an idealized vimentin model consisting of 100 ULFs in series (Fig. 2B, inset, and fig. S3), each containing 32 parallel elements. The simulation illustrates that the model qualitatively reproduces the experimentally observed tensile memory.

Figure 2D shows tracking of the numbers of parallel elements in the α state in eight typical MC-modeled ULFs during stretching. In the beginning of the stretching cycle, the number of closed, α-helical elements stays almost constant until all parallel elements in a ULF unfold rapidly in a reaction cascade, justifying the mapping of those parallel monomers on a single element in the two-state model. In contrast, during the relaxation of the filament, the number of elements in the α state in a ULF increases at a steady rate because they do not experience the force acting on the filament, leading to an incomplete refolding of ULFs at high rates, which, in turn, causes the observed softening. In unstressed vimentin monomers, the α helix is the most stable secondary structure with a lower energy compared to the β sheet conformation. Therefore, a part of the energy uptake during stretching, referred to here as apparent dissipation, is stored in the filament as potential energy if monomers remain in the β state after stretching and relaxing. The stored energy is dissipated when the stress is relieved from the filament, since eventually all elements return to the α-helical conformation. Thus, the parallel architecture of vimentin leads to increased apparent dissipation.

Two modes of filament elongation

To gain deeper insight into the different mechanisms of vimentin elongation, we additionally performed constant force experiments at different forces from 50 to 700 pN. Representative FC curves at 500 and 50 pN are shown in Fig. 3 (A and B). The data of all individual curves are presented in a double-logarithmic plot in Fig. 3C. We observe two distinct regimes in filament behavior: a stepwise lengthening at low forces (50 and 100 pN) and a creep-dominated elongation at high forces (500 and 700 pN), with an intermediate regime at 250 pN (Fig. 3C). We still observe steps even at higher forces, but the percentage of lengthening due to steps is reduced compared to lower forces. A histogram of step sizes shows a most likely step size of 10 nm independent of the applied force (Fig 3D and fig. S4). Additional FC experiments with maleimide coupling between filament and beads (fig. S5) and relaxation experiments without force feedback showing improved step height resolution (figs. S6 and S7) confirm that the steps are intrinsic to the filaments.

The observation of discrete elongation steps in vimentin filament agrees with the hypothesis that α-β transitions in the monomers are the main mechanism for vimentin elongation and dissipation at low strains. For full unfolding of one of the three α-helical domains, one would expect a length change between 7.2 nm for the unfolding of domain 1A and 27.8 nm for domain 2 (7, 14, 26). This agrees well with the experimentally observed step sizes. Under FC conditions, the two-state model can be solved analytically. The model predictions for a typical filament length of 10 μm are shown in Fig. 3C for FCs at 50, 100, and 250 pN as orange, green, and magenta lines, respectively, and agree well with the experimental observations. FCs at forces larger than the plateau force show a qualitatively different progression than at lower forces (Fig. 3C). The elongation is dominated by creep (Fig. 3A), and the time-dependent change in length can be well described by a power law (27). Power-law behavior is indicative of viscoelasticity with a broad distribution of relaxation times. The power-law coefficient b is a measure of the system’s viscosity with a

Fig. 2. Response to repeated strain. (A) Top: Distance versus time. Bottom: Force versus strain for a typical filament. (B) Kinetic MC simulations of a simplified vimentin filament that take into account 32 monomers per cross section (inset) reproduce a curve progression resembling the experiment. The force and extension in (B) are given in arbitrary units (a.u.) of force and distance. (C) Fitting the linear regime of the stretching part of every cycle [A]; up to 10% strain] yields the stiffness for each cycle. The progression with cycle number for 77 different filaments is shown. Magenta, average stiffness per cycle. Error bars indicate SEM. (D) Every monomer within the simulated IF is traced by the MC simulation. Transition of the 32 α helices of eight individual ULFs (color-coded) within one filament.
DISCUSSION

Vimentin has remarkable mechanical properties, such as tensile memory, an enormous degree of energy dissipation upon stretching, mostly due to nonequilibrium α-β transitions, and high durability sustaining stresses of several megapascals, properties otherwise only observed in high-performance biomaterials such as fibrin/fibrinogen or spider silk (20–24). The mechanical features of vimentin filaments are most distinct at high strains and large loads of several hundreds of piconewtons. This finding raises the following questions: To what extent are these properties of physiological relevance? In particular, are IFs in the cytoskeleton under tensile load and do cells experience and survive strains of that magnitude? Fudge et al. (32) found not only that keratinocytes survived strains of 138% but also that large strains cause straightening of keratin IFs, confirming tensile load, and lead to elongation of IFs. Although there are no direct observations of IFs within cells stretched to large strains in vivo, the strain resilience of cells observed in vitro supports a physiological relevance of the strains applied here.

The use of two different levels of mechanical modeling, a stochastic model explicitly taking the parallel monomers into account, and a coarser numerical effective two-state model foregoing parallel elements, allowed us to relate our experimental observations to molecular processes. Both models are limited in their application. The numerical two-state model fails to reproduce all phenomena related to parallel elements in different folding states, such as the tensile memory, and can therefore only be applied to initially relaxed filaments. However, in this case, the model is suitable to quantitatively reproduce the nonlinear force response and the energy dissipation during vimentin elongation. By contrast, the stochastic parallel element model is computationally much more demanding, rendering any attempt of direct data fitting unfeasible, and is difficult to combine with additional elements such as viscous contributions. Nevertheless, both models are consistent with each other and reflect well how the hierarchical organization of the vimentin filament affects its overall mechanical behavior. Both the tensile memory and the pronounced energy dissipation can be related to vimentin’s architecture of parallel monomers. In our analysis, ULFs partially in α-helical and β sheet configuration play a crucial role by allowing a change in the filament’s stiffness without altering its length. We were able to demonstrate that the observed energy dissipation can be mostly explained by nonequilibrium α-β transitions in the vimentin monomer. This reaction has been observed early on in strained wool fibers (9) and, more recently, was also directly measured in an engineered vimentin superstructure using wide-angle x-ray scattering (33). Simulations of strained vimentin dimers and tetramers illustrate how this transition occurs on the molecular level (17). While it is so
far not possible to directly observe this conformational change at the level of single filaments, the good agreement between the experimentally recorded force-distance curves and the modeled curves based on this known conformational transition supports the computational prediction (14). The strategy to use a nonequilibrium transition providing additional length to the vimentin filament to maximize energy dissipation is fundamentally the same mechanism as observed for spider silk, titin, or fibrin (20–24), albeit using the more subtle α-β transition compared to the unfolding of globular protein domains in titin and fibrin or β sheet crystals in silk.

Because of their low persistence length compared to MFs and MTs, IFs were often assumed to be of minor relevance for cell mechanics. However, the pronounced energy dissipation observed here might indicate that IFs act as intracellular shock absorbers against tensile stresses, as they are able to efficiently dissipate large amounts of mechanical energy, thereby preserving the cellular integrity. This agrees with the observation that the effect of a keratin knockout in keratinocytes on the mechanical properties is much more pronounced when the cells are subjected to tensile stresses compared to compressive loading (2, 4).

We found that individual vimentin filaments get softer upon repeated stretching, whereas the filaments’ length is not influenced by the strain history. While similar behavior was observed in networks composed of fibrin or collagen (25), vimentin behavior is notably distinct from the observations for single fibrin (25) or titin (34) fibers, which show a change in length upon repeated stretching cycles while maintaining their mechanical properties. This behavior is consistent with our proposed mechanism based on the supramolecular architecture of vimentin filaments using numerous highly ordered parallel monomers in contrast to both titin and fibrin.

Vimentin is found in cells of mesenchymal origin that are exposed to relevant stresses, and the ability to dissipate large amounts of mechanical energy may help prevent mechanical damage. For example, the endothelium and smooth muscles form the intima and the media of blood vessels. The tensile memory could potentially help these cells to be compliant with the beating of the heart and the repeated pressure shift between systolic and diastolic blood pressure. This hypothesis is supported by the observation that vimentin expression is elevated in cardiovascular segments subjected to high blood pressure such as the pulmonary trunk, the aorta, and the left ventricle (35).

To conclude, our data reveal how the molecular structure of the vimentin monomer imparts viscoelastic properties at larger length scales and show how extension far from equilibrium permits the dissipation of a large amount of energy. We were able to directly observe stepwise elongation of vimentin filaments attributed to α-β transitions in the protein monomers. The ULF architecture of parallel extensible monomers permits the establishment of tensile memory at repeated load, while sliding of the filaments gives rise to power-law viscoelasticity at large strains. Thus, our results explain the intriguing mechanical properties of vimentin IFs, such as extreme extensibility, tensile memory, and a nonequilibrium mechanism for energy dissipation by the particular molecular architecture of IF reliance on weak bonds that display controlled and reversible failure in the filaments.

MATERIALS AND METHODS

Vimentin expression and purification protocol

Escherichia coli T61 cells (#3017, Zymo Research) were used to recombinantly express human vimentin C328A with additional amino acids GGC at the C terminus (plasmid DNA from H. Herrmann, German Cancer Research Center). The protocol was adopted from Herrmann et al. (36). One hundred microliters of thawed bacteria solution and 1 μl of plasmid solution (1 μg/μl; diluted 1:100) were mixed and incubated on ice for 5 min. Fifty microliters of this mixture was plated on an ampicillin-containing lysogeny broth agar plate (L5667, Sigma-Aldrich) and incubated at 37°C overnight.

Single bacteria colonies were picked, transferred into 50 ml of terrific broth (TB; 47.6 g/liter; #70918, Sigma-Aldrich) medium containing glycerol (10 to 11 g/liter) and ampicillin (0.1 mg/ml; Sigma-Aldrich), and incubated at 37°C and 150 rpm. After 6 hours, the bacteria solution was transferred to 1 liter of fresh TB medium containing ampicillin (0.1 mg/ml) and was allowed to grow at 37°C and 150 rpm overnight.

Protein preparation started by pelleting bacteria with a Beckman centrifuge (Beckmann Coulter Avanti J-26 XP, rotor JS5.3) at 5000g for 15 min at 4°C. During the subsequent steps, the protein was always kept on ice. The pellet was transferred to a cooled douncer, homogenized with 16 ml of tris buffer (50 mM; pH 8.0; Carl Roth GmbH) containing saccharose (25%, w/v), EDTA (1 mM; Carl Roth GmbH), and lysozyme (10 mg/ml; Roche Diagnostics), and incubated on ice for 30 min. Eight hundred microliters of MgCl₂ solution (1 M; Sigma-Aldrich), 80 μl of deoxyribonuclease I (DNase I; 50 mg/ml; Sigma-Aldrich) in tris buffer (10 mM; pH 7.5) containing NaCl (100 mM; Carl Roth GmbH), 80 μl of ribonuclease A (RNase A; 10 mg/ml; Roche Diagnostics) in tris buffer (100 mM; pH 7.5), 800 μl of saturated phenylmethylsulfonyl fluoride (PMSF; Serva) in ethanol, and 1.6 ml of 10% NP-40 (Roche Diagnostics) were added and mixed several times by homogenization. Forty milliliters of tris buffer (20 mM; pH 7.5), containing NaCl (200 mM), NP-40 (1%), EDTA (2 mM), and 3,5-dihydroxy-5-β-cholanic acid sodium salt (1%, Sigma-Aldrich), was complemented by 800 μl of a 1 M, 1,4-dithiothreitol solution (DTT; Carl Roth GmbH), 1.2 ml of PMSF, and 400 μl of a 50 mM Pefabloc SC solution (Carl Roth GmbH) and added to the douncer/bacteria solution. The mixture was incubated for 10 min, transferred to Nalgene centrifuge bottles (250-ml PPCO-centrifuge bottles with sealing closure, Thermo Fisher Scientific), and centrifuged for 25 min at 10,000g and 4°C using a Beckman centrifuge (Beckman Coulter Avanti J-26 XP, rotor JLA 16.250). The supernatant was discarded, and the pellet was washed by homogenization in the douncer using washing buffer [80 ml of tris buffer (20 mM; pH 8.0) containing Triton X-100 (0.5%, v/v; Carl Roth GmbH) and EDTA (5 mM)] complemented by 80 μl of a 1 M DTT solution, 400 μl of PMSF, and 200 μl of a 50 mM Pefabloc SC solution. The mixture was incubated 10 min on ice and centrifuged using the same conditions as for the previous step. Supernatant was again discarded, and the pellet was washed using 80 ml of washing buffer containing KCl (1.5 M) complemented by 80 μl of a 1 M DTT solution, 400 μl of PMSF, and 200 μl of a 50 mM Pefabloc SC solution. After homogenization, the mixture was incubated for 30 min before centrifugation, which was performed as before. The third washing step was the exact repeat of the first washing step. For the last washing step, 80 ml of tris buffer (10 mM; pH 8.0), containing EDTA (0.1 mM) complemented by 80 μl of a 1 M DTT solution, 400 μl of PMSF, and 200 μl of a 50 mM Pefabloc SC solution, was used. As before, the pellet was washed and incubated for 10 min, and the mixture was centrifuged. The supernatant was discarded, and the pellet was transferred to a douncer, warmed to room temperature, and solubilized in urea solution (9.5 M). The recipe for the urea solution was as follows: 7.125 ml of urea (9.5 M; Carl Roth GmbH), 75 μl of 1 M tris (pH 7.5), 75 μl of 0.5 M EDTA, 75 μl of PMSF, and 75 μl of 1 M DTT. To yield a high protein concentration, as little of the urea solution as possible was used to dissolve the pellet. The solution was homogenized and finally centrifuged using a Beckman ultracentrifuge (Beckman Coulter Optima L90K, rotor Ti70) at 50000g and 4°C for 70 min.
20°C and 100,000g for 60 min. The vimentin-containing supernatant was transferred to a Falcon tube, and methylamine hydrochloride solution (MAC; Sigma-Aldrich) was added to a final concentration of 10 mM. For purification from the supernatant, an anion exchange chromatography step was followed by a cation exchange chromatography step. For anion exchange chromatography, a chromatography column (inner diameter, 15 mm; length, 200 mm; volume, 35 ml; #7317-1522, Bio-Rad) was prepared according to the user’s manual. As column material, 10 ml of DEAE Sepharose (GE Healthcare) was mixed with 10 ml of column buffer [400 ml of urea (10 M) and 50 ml of 50 mM tris (pH 8.0), containing EDTA (10 mM) and EGTA (1 mM)], filled to 500 ml with MilliQ water and complemented by 500 μl of 1 M DTT] [a final pH of 7.5 was adjusted by hydrochloric acid (Carl Roth GmbH)]. A KCl gradient was used to elute the protein. To do so, a gradient mixer (2 × 50 ml; Schütt 24) was filled with 40 ml of column buffer in the front chamber and 40 ml of column buffer containing 0.896 g of KCl (Carl Roth GmbH) in the rear chamber. When the sample entered the column, it was washed with two bed volumes of column buffer using a slow flow velocity. When the collected flow-through was equal to the dead volume of the column, the KCl gradient was started, and sample collection began (fractioning in 1.5-ml tubes). The protein concentration was monitored by measuring the absorption at 280 nm (NanoDrop, ND-1000, Thermo Fisher Scientific). As an additional quality control, an 8% SDS gel was performed using samples collected after each purification step and from fractions of interest after chromatography. Fractions with high protein concentration after the DEAE column were pooled for a cation exchange chromatography, which was performed in analogy to the anion exchange chromatography but with CM Sepharose (GE Healthcare) as column material. In addition, the rear chamber was filled with 40 ml of column buffer containing 1.792 g of KCl. The protein concentration of collected liquid was again monitored by measuring the absorption at 280 nm, and fractions with high protein concentration were pooled. Last, MAC was added to the vimentin solution to a final concentration of 10 mM. Aliquots of the protein solution were stored at −80°C.

**Vimentin labeling**

For fluorescence imaging of vimentin filaments and binding to streptavidin-coated beads, the protein was labeled with ATTO 647N maleimide (ATTO-Tech GmbH) and biotin-maleimide (Jena Bioscience GmbH), respectively. Labeling was performed as described by Block et al. (14) and Winheim et al. (37).

**Vimentin reconstruction and assembly**

Labeled and unlabeled vimentin was stored in 8 M urea at −80°C. By stepwise dialysis (8, 6, 4, 2, 1, 0 M urea) into phosphate buffer [2 mM; pH 7.5; disodium hydrogen phosphate (Sigma-Aldrich) and sodium dihydrogen phosphate (Sigma-Aldrich)], vimentin renaturation and dimer and tetramer formation were initiated. For dialysis, unlabeled and labeled vimentin was mixed in the desired ratio and transferred to 50-kDa cutoff dialysis tubing (SpectraPor, Carl Roth GmbH). Each dialysis step had a duration of 30 min. Last, the dialysis tubing containing the vimentin was placed in 2 mM phosphate buffer for 2 hours at room temperature or at 10°C overnight.

Filament formation was initiated by KCl. To do so, the vimentin concentration was adjusted to 0.2 g/liter, monitored by ultraviolet-visible spectrometry, by adding phosphate buffer. Afterward, vimentin was placed in a fresh piece of 50-kDa cutoff dialysis tubing, placed in phosphate buffer (2 mM; pH 7.5), containing KCl (100 mM), and kept at 37°C overnight. This dialysis procedure ensured filaments that were homogeneous in diameter (6). Assembled filaments were diluted 1:10 with KCl-containing phosphate buffer and kept at 4°C or on ice until further use. The morphology of the assembled filaments was monitored by epifluorescence microscopy, atomic force microscopy, and electron microscopy and found to be typical of IFs (see fig. S9).

**Maleimide functionalization of beads**

Functionalization of carboxylated polystyrene beads (4.0 to 4.4 μm; 5%, w/v; PPS-4.2COOH, Kisker Biotech GmbH & Co. KG) was performed according to Janissen et al. (38). Beads were coated using maleimide-PEG-NH₂ (molecular weight, 5000 Da; PG2-AMM2-5k, Scholz Labor- und Klinikversorgungs GmbH), NH₂-PEG-ÖH (Iris BioTech), and 1-ethyl-3-(3-dimethylaminopropyl)-carboxiimide (E1769-1G, Sigma-Aldrich). Functionalized beads were stored in 1× phosphate-buffered saline (lot no. 73604799A, Invitrogen AG) containing 2% bovine serum albumin (A7906-10G, lot no. SLBB476V, Sigma-Aldrich).

**Experimental setup and procedure**

Vimentin force-extension experiments were performed using instruments combining dual optical tweezers (for manipulation) and fluorescence microscopy (for visualization). Most experiments were performed on a LUMICKS C-Trap equipped with confocal fluorescence microscopy. Some experiments were performed on a laboratory-built instrument equipped with wide-field fluorescence microscopy (39). Both instruments use comparable, dual optical traps and an identical microfluidic flow cell containing at least three parallel laminar-flow channels (Fig. 1A, top).

All solutions (beads in KCl-containing phosphate buffer, vimentin in KCl-containing phosphate buffer, and pure KCl-containing phosphate buffer) were flushed into the microfluidic chamber using air pressure. Because of laminar flow, they do not mix but flow parallel to each other. For every data set, a fresh pair of beads (4.5 μm streptavidin-coated polystyrene beads, Spherotech Inc.) was captured in the bead channel (channel 1) and, by moving the microfluidic chamber with respect to the tweezers, passed through the vimentin channel (channel 2) into the buffer channel (channel 3), where the measurement was performed after the flow had been stopped. While passing through channel 2, vimentin filaments bound to the beads. By moving the second bead closer to the first bead, one filament eventually bound to the second bead. To be sure that there was only one filament between the two beads, confocal imaging was used. Experiments were performed by moving the second bead, while the first stable bead was used for the force measurement.

**Analysis of optical tweezers data**

Data sets were exported in ASCII format from the measuring software (Twom, LUMICKS). The bead diameter was subtracted from the distance measurements, and time and force values were directly used as measured. Only data sets with filaments that were stable for at least three cycles were taken into account. As a threshold to determine the initial filament length, the measured distance at the highest force still smaller than 5 pN at the beginning of the stretching process was used. Using the initial filament length, the strain was calculated for all data sets. The data point density was very high, and missing bead tracking occurred only rarely. Thus, data points with falsely zero distance due to lost tracking were neglected for further analysis. We also neglected data points recorded before the initial filament length was reached and after the filaments broke.

The slopes of repeated force cycles were determined by fitting the force-distance curves for strains between 0 and 0.1 by a straight line. The error bars in Fig. 2C show the SEM.
To determine the power-law coefficient $b$, a power law, $\frac{dN}{dt} = A \left( \frac{x}{x_0} \right)^b$, with the power-law prefactor $A$, the initial length $L_0$, and the time scale set arbitrarily to $t_0 = 1$ s, was fitted to the time-dependent change in length $\Delta L$ during the FC experiment.

To access the size of the jumps in filament length during FC experiments, we analyzed the distribution of the measured filament lengths. The distribution is shown as histograms on the $y$ axes in Fig. 3 (A and B) and fig. S7 (A and B). The plateaus between the jumps correspond to peaks in the filament length distribution. Therefore, the step size can be obtained as the distance between two adjacent peaks. To avoid artifacts due to binning, we used kernel density estimates with a Gaussian kernel with width $\sigma = 1.7$ nm (the experimental uncertainty) instead of histograms. Only peaks with a minimal height of 5% of the highest peak were analyzed.

**Models**

**Effective two-state model**

The filament with length $x$ was modeled as an elastic spring with extension $x_1$ coupled to an equivalent freely jointed chain (eFJC) with end-to-end distance $x_2$. Since each monomer, and hence the whole filament, is able to change its contour length $L_c$ in response to applied forces via the transition of the $\alpha$ helices into an elongated $\beta$ sheet, the contour length $L_c(F, t)$ becomes a function of time $t$ and force $F(t)$ (for the sake of readability, we will state neither explicit nor implicit dependencies going forward).

Assuming a Bell-Evans kinetic for the $\alpha$-$\beta$ transition for each of the three $\alpha$ helices in the monomer (the three $\alpha$ helices, 1A, 1B, and 2, are indicated by subscripts $i = 1, 2, 3$), the number $N_{\alpha,i}$ of monomers with the $i$th subunit in the $\beta$ state is described by the following rate equation

$$\frac{dN_{\alpha,i}}{dt} = (N_0 - N_{\beta,i}) k_i^{\alpha\rightarrow\beta} \cdot \exp \left( \frac{F \cdot x_{i\alpha}}{k_B T} \right) - N_{\beta,i} k_i^{\beta\rightarrow\alpha} \cdot \exp \left( -\frac{F \cdot x_{i\beta}}{k_B T} \right)$$

(1)

with zero-force reaction rate $k_i^{\alpha\rightarrow\beta}$ and potential width $x_{i\alpha}$ for the $\alpha$-$\beta$ transition, zero-force reaction rate $k_i^{\beta\rightarrow\alpha}$ and potential width $x_{i\beta}$ of the reverse $\beta$-$\alpha$ transition, and the total number of monomers in a filament $N_0 = N_{\alpha,i} + N_{\beta,i}$. Here, we assumed that parallel monomers can be described by a single kinetic, as described by Friddle et al. (40). From $N_{\beta,i}$ and the lengths of $\alpha$ helices $l_{i\alpha,i}$, $\beta$ sheets $l_{i\beta,i}$, and all combined linker regions $l_{i0}$, we can calculate the contour length $L_c$ of the filament

$$L_c = N_0 l_0 + \sum_i (N_{\beta,i} l_{i\beta,i} + N_{\alpha,i} l_{i\alpha,i})$$

(2)

Using the large-force approximation for an eFJC characterized by the Kuhn length $L_K$, we can calculate the extension of the entropic spring $x_2$ as

$$x_2 \approx L_c \left( 1 - \frac{F_K}{F} \right)$$

(3)

with the force scale $F_K = \frac{h k_B T}{x_0}$. For a semiflexible chain, the Kuhn length is equivalent to twice the persistence length $L_K = 2L_p$ (41). The serial Hookian spring, which combines elastic contributions from both the filament and the pulling apparatus, is characterized by its spring constant $k_{\text{eff}}$ and its extension $x_1$. The force acting on the spring is $F = k_{\text{eff}} x_1 = k_{\text{eff}} (x - x_2)$. With Eq. 3, we obtain

$$F = k_{\text{eff}} \left[ x - L_c \left( 1 - \frac{F_K}{F} \right) \right]$$

(4)

When the filament is stretched up to the time $t_{\text{max}}$ and subsequently relaxed with a constant velocity $v$, the extension of the filament changes to

$$x = \begin{cases} vt & \text{for } t \leq t_{\text{max}} \\ vt_{\text{max}} - v(t - t_{\text{max}}) & \text{for } t \geq t_{\text{max}} \end{cases}$$

(5)

The refolding of $\alpha$ helices from $\beta$ sheets was neglected previously ($x_i^{\beta\rightarrow\alpha} = 0$) because refolding was expected to be suppressed by the high forces during stretching of the filaments but plays an important role during relaxation, which occurs at lower forces. Equation 1 includes a nonobvious assumption: We described all parallel elements by a single effective element, which is possible since unfolding of all parallel elements occurs in a rapid reaction cascade, essentially all or nothing process (see Fig. 2D). Describing the formation of $\alpha$ helices as the back reaction of the $\alpha$-$\beta$ transition implies that both reactions have the same specific characteristics, that is, that all parallel elements react rapidly from $\beta$ sheets to $\alpha$ helices. However, we actually know from repeated pulling and relaxation experiments that this assumption is not valid, since we observed a tensile memory attributed to a fraction of parallel elements remaining in the $\beta$ state (see also Fig. 2D). Equation 1 still provides a useful effective description of both the stretching and the relaxation curve of vimentin filaments, since the partially folded array of parallel elements is similar in length to the all-$\alpha$ configuration. However, $x_i^{\beta\rightarrow\alpha}$ and $x_i^{\alpha\rightarrow\beta}$ should be strictly considered apparent or effective values and not be overinterpreted numerically. In the effective two-state model, a ULF will still appear as the shorter $\alpha$ helix, even if some of the parallel elements in that ULF are in the extended $\beta$ state. This means that, like the experimental curves, the energy dissipation in the effective two-state model is an apparent value, since a certain amount of energy is stored as potential energy in the $\beta$ sheets.

Equation 1 can be solved numerically for each of the three $\alpha$ helices together with Eqs. 2 and 4 to obtain $F$. It was shown previously for simple stretching experiments without subsequent relaxation that this simple model was able to reproduce experimental curves over a wide range of loading rates, even if real structural data to determine $l_{i\alpha,i}$ (5.4, 13.8, and 20.9 nm), $l_{i\beta,i}$ (12.6, 32.2, and 48.7 nm), and $l_0$ (29.7 nm) and literature data for $k_i^{\alpha\rightarrow\beta} = 3.3 \times 10^5$ s$^{-1}$ were used (14). The most likely parameters in the fits of 244 experimental curves were found to be $k_{\text{eff}} = 0.23$ pN/nm, $x_{i\alpha} = 0.11$ nm, $x_{i\beta} = 0.13$ nm, and $x_{i\alpha,i} = 0.15$ nm. Since the extension curve was only slightly affected by the inclusion of the backwards reaction, we used the parameters determined previously for $k_{\text{eff}}$ and $x_{i\alpha,i}$. $x_{i\beta,i}$ was chosen such that there was a fixed ratio between the two potential widths $r_p = \frac{x_{i\alpha,i}}{x_{i\beta,i}} = 2.3$ for all the helices. The back-reaction rate was set to $k_i^{\beta\rightarrow\alpha} = 5.4$ s$^{-1}$. 

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To account for creep behavior, we introduced a dashpot with length $x_3$ as a third element in series to the entropic and the linear spring such that the total length is $x = x_1 + x_2 + x_3$. This element shows idealized viscous behavior that can be described by

$$\frac{dx_3}{dt} = x_3 \eta$$

with the strain $\epsilon$, the viscosity $\eta$, and the stress $\sigma = F/A$ with the area of the cross section $A$. The cross-section area was estimated from the filament diameter of $d \approx 10$ nm. The time derivative of the spring can be related to the time derivative of $x_3$ via $\frac{dx_3}{dt} = x_3 \eta$ with a typical value for the initial filament length of $L_0 = 10 \mu$m. Equation 6 is solved numerically together with Eq. 1 using $\eta = 3.2$ GPa.s.

The shape of the experimental retraction curve indicates a strong decrease of the persistence length of vimentin filaments during stretching and $\alpha$-$\beta$ transition. The persistence length of the unstretched filament with all monomers in $\alpha$-helical configuration was known from literature to be $L_p = 1000$ nm, leading to the force scale $F_{ka} \approx 0.002$ pN (42). However, the shape of the relaxation curve suggested a substantially smaller persistence length (see fig. S2). Therefore, we introduced a second persistence length for the stretched filament $L_{p,\beta}$ with a corresponding force scale $F_{ka,\beta}$. We interpolated linearly between the two different force scales, $F_{ka,\beta}$ for $N_{\beta,j} = 0$ and $F_{ka,\beta}$ for $N_{\beta,j} = N_0$, leading to a strain dependent of the persistence length.

**Analytical solution for FC conditions**

Under FC conditions, that is, at constant force, the analytical solution of Eq. 1 becomes straightforward. Since we rarely observed any shortening of the filament during FC experiments, even at the lowest force of 50 pN, we can neglect the restoration of $\alpha$ helices from $\beta$ sheets. When the force $F$ is constant, the force-dependent reaction rate $k_F^{\alpha,\beta} = k_0^{\alpha,\beta} \cdot \exp(\frac{F_{ka,\beta}}{kT})$ also becomes a constant. Therefore, the solution of Eq. 1 reads

$$N_{\alpha,i,F} = N_0 \exp\left(-k_0^{\alpha,\beta} \cdot \frac{F_{ka,\beta}}{kT} t\right)$$

Equation 2 is then used to calculate $l_c$, and Eq. 3 allows us to calculate $x_2, x = x_1 + x_2$ is used to calculate the filament length. The strain is calculated using $\epsilon = \frac{x - L_0}{L_0}$ with a typical value for the initial filament length $L_0 = 10 \mu$m. The value used for $L_0$ only affects the contribution of the elastic extension $x_1$ to the strain $\epsilon$ and has therefore only a minor impact on the resulting curves.

**MC simulation of unfolding and refolding of parallel elements**

More complex behavior of vimentin IFs, such as the tensile memory, cannot be described without explicitly considering the architecture of ULFs consisting of parallel elements, as sketched in fig. S3. It is convenient to introduce dimensionless force $\phi = F/L_0$ by normalization with the force scale of the $\alpha$-$\beta$ transition $F_{ka}$, dimensionless length scale $\lambda = L/\Delta L$ using the length difference $\Delta L$ between $\alpha$ state and $\beta$ state, and dimensionless time $\tau = t \cdot F_{ka,\beta}$ with the zero-force reaction rate $k_0^{\alpha,\beta}$ of the $\alpha$-$\beta$ transition of a single monomer.

The dimensionless stiffness of the filament $\kappa_{TOT} = \frac{\Delta \kappa}{\Delta \lambda}$ depends on the stiffness of the ULF $\kappa_{ULF}$ and the number of ULFs in the filament $N_S$

$$\kappa_{TOT} = \left( \frac{1}{N_S \cdot \kappa_{ULF,j}} \right)^{-1}$$

The simplest model to describe the elasticity of the ULF is a parallel arrangement of $N_T$ springs with stiffness $\kappa_p$. Each of these springs is arranged in series to an element that can change its length in a force dependent manner, representing the $\alpha$ helices that can switch into an extended $\beta$ sheet, with $N_p$ elements in $\alpha$-helical configuration. Together with an additional spring with stiffness $\kappa_V$ serial to this element representing linkers and the connection between ULFs, the stiffness of one ULF is

$$\kappa_{ULF,j} = \left( \frac{1}{\kappa_V} + \frac{1}{\kappa_p N_{p,j}} \right)^{-1}$$

To illustrate that this mechanism was suitable to generate tensile memory, we performed MC simulations. For simplicity, we neglected entropic contributions to the elasticity and viscous contributions and used only one extendable element for each ULF, as opposed to the three helices used in the numerical description above.

Each ULF $j$ is characterized by the number of elements $N_{p,j}$ in the shorter $\alpha$-helical state. Since the elements can switch between $\alpha$ state and $\beta$ state, the number of $\alpha$-helical elements in each individual ULF is described by the master equation

$$\frac{dP_N}{dt} = r_0^{-\alpha,\beta} P_{N-1} + r_0^{\alpha,\beta} P_{N+1} - \left[ r_0^{-\alpha,\beta} + r_0^{\alpha,\beta} \right] P_N$$

where $P_N$ represents the probability that $N$ parallel elements are in the $\alpha$-helical state, $r_0^{\alpha,\beta}$ is the rate of the $\alpha$-$\beta$ transition, and $r_0^{-\alpha,\beta}$ is the rate of the reverse reaction.

When all parallel elements are in the elongated state, that is, the $\beta$ state, the ULF is extended by $\Delta L$. Since the length scale is normalized by $\lambda$, the dimensionless extension of the $j$th ULF $\lambda_j$ can be described as

$$\lambda_j = \begin{cases} 0 & \text{for } N_{p,j} > 0 \\ 1 & \text{for } N_{p,j} = 0 \end{cases}$$

The total extension of the filament is $\lambda_{TOT} = \sum_j \lambda_j$. The total force acting on a filament with end-to-end distance $x$ is given by $\phi = \kappa_{TOT} \cdot (x - \lambda_{TOT})$.

The time-dependent end-to-end distance of the filament $x(t)$ is calculated according to

$$x = \begin{cases} vt & \text{for } \tau \leq \tau_{max} \\ x_{max} - v(\tau - \tau_{max}) & \text{for } \tau \geq \tau_{max} \end{cases}$$

using the dimensionless velocity $v = d\lambda/d\tau$ and the maximal extension $x_{max} = ut_{max}$ at the time point $\tau_{max}$. The reaction rates were chosen similar to the ones for the effective two-state model described above. The rates are a function of the force acting on each of the parallel elements $\phi_{p,j}$. If there is at least one element in $\alpha$ state, then each of these elements experiences $\phi_{p,j} = \frac{\phi}{N_{p,j}}$, while the extended elements are force-free. The reaction rate $r_0^{\alpha,\beta} P_{N,j}$ can therefore be expressed as

$$r_0^{\alpha,\beta} = N_{p,j} \exp\left( \frac{\phi}{N_{p,j}} \right)$$

The simplest model to describe the elasticity of the ULF is a parallel arrangement of $N_T$ springs with stiffness $\kappa_p$. Each of these springs is arranged in series to an element that can change its length in a force dependent manner, representing the $\alpha$ helices that can switch into an extended $\beta$ sheet, with $N_p$ elements in $\alpha$-helical configuration. Together with an additional spring with stiffness $\kappa_V$ serial to this element representing linkers and the connection between ULFs, the stiffness of one ULF is

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To illustrate that this mechanism was suitable to generate tensile memory, we performed MC simulations. For simplicity, we neglected entropic contributions to the elasticity and viscous contributions and used only one extendable element for each ULF, as opposed to the three helices used in the numerical description above.

Each ULF $j$ is characterized by the number of elements $N_{p,j}$ in the shorter $\alpha$-helical state. Since the elements can switch between $\alpha$ state and $\beta$ state, the number of $\alpha$-helical elements in each individual ULF is described by the master equation

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where $P_N$ represents the probability that $N$ parallel elements are in the $\alpha$-helical state, $r_0^{\alpha,\beta}$ is the rate of the $\alpha$-$\beta$ transition, and $r_0^{-\alpha,\beta}$ is the rate of the reverse reaction.

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$$r_0^{\alpha,\beta} = N_{p,j} \exp\left( \frac{\phi}{N_{p,j}} \right)$$
If all elements of an ULF are in the extended β sheet conformation, then the force will be shared between them such that \( \frac{\partial f}{\partial x} = \frac{N_f}{N_T} \). Therefore, the reaction rate is

\[
 \frac{dN_f}{dt} = \begin{cases} 
 (N_T - N_{P_j}) \gamma & \text{for } N_{P_j} > 0 \\
 N_T \gamma \exp \left( \frac{-\theta}{N_T} \right) & \text{for } N_{P_j} = 0
 \end{cases}
\]  

(14)

using the normalized rebinding constant \( \gamma = \frac{k_{on}}{k_{off}} \) with the zero-force reaction rate \( k_{on} \) of refolding. The free-energy difference between the α helix and the β sheet is \( \Delta G/k_B T = \ln(\gamma) \).

This set of equations was solved by MC methods using the Gillespie algorithm with initial condition \( N_f(t=0) = N_T(1 - \exp(-\Delta G/k_B T)) \), that is, the system being in thermal equilibrium. When multiple consecutive pulling cycles are simulated, \( N_{P_j} \) at the end of each cycle was used as the initial condition for the next cycle. The parameters used to produce Fig. 2 (B and D) were \( N_f = 100, N_T = 32, k_\theta = 10, k_\gamma = 100, \nu = 2000, \) and \( \Delta G/k_B T = 2 \). The Jupyter Notebook (43, 44) used to produce the data shown in Fig. 2 (B and D) is included as supplementary information.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/4/6/eaat1161/DC1

**REFERENCES AND NOTES**


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Viscoelastic properties of vimentin originate from nonequilibrium conformational changes

Johanna Block, Hannes Witt, Andrea Candelli, Jordi Cabanas Danes, Erwin J. G. Peterman, Gijs J. L. Wuite, Andreas Janshoff and Sarah Köster

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