Mechanical Behavior of Axonal Actin, Spectrin, and Their Periodic Structure: A Brief Review

Md Ishak Khan1 · Sheikh Fahad Ferdous2 · Ashfaq Adnan1

Received: 21 May 2021 / Revised: 14 July 2021 / Accepted: 23 July 2021 / Published online: 4 August 2021
© Korea Multi-Scale Mechanics (KMSM) 2021

Abstract

Actin and spectrin are important constituents of axonal cytoskeleton. Periodic actin-spectrin structures are found in dendrites, initial segment of axon, and main axon. Actin-spectrin periodicity has been hypothesized to be manipulating the axon stability and mechanical behavior. Several experimental and computational studies have been performed focusing on the mechanical behavior of actin, spectrin, and actin-spectrin network. However, most of the actin studies focus on typical long F-actin and do not provide quantitative comparison between the mechanical behavior of short and long actin filaments. Also, most of the spectrin studies focus on erythrocytic spectrin and do not shed light on the behavior of structurally different axonal spectrin. Only a few studies have highlighted forced unfolding of axonal spectrin which are relevant to brain injury scenario. A comprehensive, strain rate dependent mechanical study is still absent in the literature. Moreover, the current opinions regarding periodic actin-spectrin network structure in axon are disputed due to conflicting results on actin ring organization—as argued by recent super-resolution microscopy studies. This review summarizes the ongoing limitations in this regard and provides insights on possible approaches to address them. This study will invoke further investigation into relevant high strain rate response of actin, spectrin, and actin-spectrin network—shedding light into brain pathology scenario such as traumatic brain injury (TBI).

Ashfaq Adnan
aadnan@uta.edu

1 Department of Mechanical and Aerospace Engineering, University of Texas at Arlington, Arlington, TX 76019, USA

2 Department of Applied Engineering and Technology Management, Indiana State University, Terre Haute, IN 47809, USA
Keywords Actin · Spectrin · Axon · Actin-spectrin periodic lattice · Cytoskeletal components · Mechanical behavior · Brain Injury

Introduction

Mechanical behavior of biomolecular materials is of interest for biomedical and mechanical engineers and scientists, especially for the last two decades, as they are instrumental to characterize many relevant biophysical and mechano-chemical phenomena. In this regard, several experimental and computational approaches have provided instrumental insights into mechanical behavior of protein materials, such as failure criteria of biological materials in general [1, 2], multiscale behavior of protein from single strand level to assembly level [3], molecular level contraction and expansion criteria of biomolecular materials [4], mechanical response of biological materials in vitro and in silico [5], etc. Specifically, in brain research, region- or component-specific research have been rampant to determine the threshold of damage [6]. However, research in axonal cytoskeletal components lacks significantly in terms of mechanical behavior of the constituents. This manuscript attempts to point out some of the most conspicuous limitations in this regard.

There are three (3) major axonal cytoskeletal components in neuron: microtubules (MT) [7] supported by microtubule associated proteins such as MAP1B and tau [8, 9], neurofilaments (NF) [10], and microfilaments (MF) [11]. Among these, the structural units of MFs are globular or G-actin, which form filamentous or F-actin. However, these three components do not comprehensively define the axonal structure, as recent seminal super-resolution microscopy study has shown that axonal diameter is determined by periodic arrays of actin ring formed by short F-actin filaments [12]. The literature differs regarding the opinion, but it can be comprehended from the difference on the opinion about periodic actin ring formation that generally 12–14 actin monomer conformation is referred as short, while significantly larger than that (when 1 filament is long enough to encompass the circumference of the ring) is referred as long actin filament [12, 13].

Furthermore, spectrin, which is structurally and functionally important component of axonal cytoskeleton, forms heterodimers and eventually, tetramers—which generate a lattice by connecting the periodic actin rings [14]. Furthermore, spectrin-membrane association is established by several membrane-associated proteins—such as adducin which caps one end of F-actin and promotes actin-spectrin bond [15, 16], ankyrin which binds spectrin to membrane [14], etc. The overall mechanical behavior of axon and extent of mechanical support to the cytoskeleton are determined by significant contribution of actin-spectrin network.

Therefore, mechanical behavior of (i) lone F-actin, (ii) lone spectrin, and (iii) actin-spectrin interaction are required.
Computational Modeling Techniques to Extract Mechanical Properties of Protein Materials (Especially Axonal Cytoskeletal Components)

As this manuscript reports substantial number of computational studies which have contributed significantly to modeling and extracting mechanical properties of axonal cytoskeletal components, a summary of available modeling techniques is provided here. The relevant modeling schemes relevant to this manuscript are:

i) Fully atomistic modeling,
ii) Coarse-grained modeling,
iii) Multiscale modeling.

i) **Fully atomistic modeling:** It consists of modeling a representative biological system where each atom is explicitly defined, including their bonds, angles, dihedrals, and non-bonded interactions. A number of force fields, i.e., functional form to define energetic states and interactions have been developed e.g., CHARMM, AMBER, OPLS, GROMACS packages, etc. The scheme is to use an initial structure for a reasonable extent to obtain an equilibrated (stable) structure, and then apply viable mechanical loading schemes (such as a velocity field). At frequent interval, the displacement of the group of atoms of interest is tracked, along with per atomic stress. In case of application of steered molecular dynamics (SMD), force versus displacement can be tracked. The pulling criteria, in conventional molecular dynamics, is defined by applying a velocity field to the regions of interest, or in case of SMD, by tethering the group to a virtual spring with pre-defined stiffness [176]. Recently, axonal cytoskeletal components such as microtubules and tau proteins have been modeled by using atomistic simulation [118, 170, 172]. Actin failure mechanisms have also been tested by atomistic simulation (conventional and SMD) [61, 86].

ii) **Coarse-grained modeling:** When the model of interest is substantially large, fully atomistic simulation is highly expensive in terms of computational cost (machine power implemented and simulation run time). Therefore, a simplification is adopted as a reasonable compensation. While the interaction and behavior cannot be captured like fully atomistic simulation, the repetitive groups of atoms are modeled as a “bead”, and therefore, the resulting number of interactions decreases substantially—resulting in facilitation of modeling a bigger system and larger timescale of simulation [177, 178]. Recently, successful implementations of coarse-grained MD simulation have been depicted for actin [20, 49, 55] and spectrin [134, 165].

iii) **Multiscale modeling:** While fully atomistic simulation and coarse-grained simulations are viable at nanoscale and mesoscale, even bigger models need appropriate parameterization and adoption of continuum scheme. In this regard, the advantages from both approaches (atomistic and continuum) are considered, while the limitations of each method can be avoided to substantial extent. A prominent example is atomistic-based continuum modeling, which uses atomistic results from simulation on axonal cytoskeletal components to build on bigger model of axon by incorporating continuum homogenization method, i.e., by defining representative volume spheres and their interaction in a plane-plane manner [173, 179]. Furthermore, such multiscale modeling can also couple cellular level damage to the tissue level damage by adopting constitutive modeling technique for the corresponding length scale [119]. Lately, multiscale modeling has been able to address the anisotropy of brain damage at different length scale [122, 180].

Structure of Actin, Spectrin, and Periodic Actin-Spectrin Network

**Actin: Structure and Mechanical Behavior**

**Actin Structure**

Actin, being one of the most conserved biological structures found in nature has been explored in numerous experimental and computational ways. Although actin has diversified functionality, interaction with cell membrane is one of the most significant attributes of this cytoskeletal component. Actins can be related to several neuropathological disorders. For example, cancer-affected cytoskeleton properties depend on actin filaments to microtubule content [19]. Also, AFM experiment shows that affecting MTs reduces stiffness of axons greatly, while in case of affecting actins the effect is not that severe [11]. However, the later part of this section depicts that due to importance of actin and other constituents of axonal cytoskeleton in regulating overall axonal behavior,
numerous experimental and computational studies have been undertaken.

F-actins contain right handed double helices of actin monomer with around 18nm² cross sectional area and several micrometers of length [20]. It has an asymmetric structure with ~6 nm long monomer [21, 22]. The monomeric subunit (G-actin) has four subdomains, the conformation of which strongly depends on ATP or ADP-bound states [23, 24] (Fig. 1). X-ray diffraction experiments show that globular to stable, flat, fibrous actin transition can occur by rotation of two major domains [25].

Electron microscopy shows different structural states of actin, especially from the perspective of subunit to subunit connection, and therefore, it should be studied as an ensemble of different structures [26]. Cryo-EM technology has facilitated direct visualization of secondary structure of actin [27]. Immunofluorescence has also been applied for examining actin network structure [28]. Actin networks and bundles should be studied along with the properties of individual filaments, as they show the holistic characteristics of the cytoskeletal component. There are additional dynamic aspects of actin with respect to intermolecular bonding in F-actin, modifiers of the structure, and differences within different monomeric forms of actin—which are thoroughly worked out in recent studies [29]. To maintain focus on mechanical behavior of actin in filament level, discussions on these aspects are not shown in this review.

Furthermore, actin binding proteins play an instrumental role to the assembly and parallel branching network of actins [30]. Also, it is asserted in multiple studies that actin filaments are crosslinked by actin binding proteins (ABP) such as filamin, and a single type of ABP can lead to formation of different actin microstructures [31]. F-actin, being crosslinked by bundling proteins such as actinin, can assemble into frequently branching 3D network [32]. Additionally, divalent cation binding sites induce F-actin polymerization [33]. Therefore, the growth and network formation of actin is highly dynamic—regulated by not only ABPs but also localization of binding sites.

It is worth mentioning that actin network is associated with various cross-linking mechanism which may lead to tightly packed bundles, or loosely connected network. Tightly packed bundles are created by crosslinks of fascin or fimbrin [34, 35], while loose networks can be facilitated by crosslinkers like filamin, actinin, or spectrin (for both erythroid and axonal networks) [36, 37] or bundles like fimbrin, villin, dematin, etc. [38, 39]. However, discussing the mechanical aspects of all of them is out of the scope of the manuscript, and in the later sections, the review will be limited to axonal networks only (short actin interaction with mostly α2-β2 spectrin).

Fig. 1 Globular or G-actin (A), and filamentous or F-actin (B). A Every monomer of G-actin can be divided into four sub-domains (marked by “SD” and four different colors: red, blue, green, and purple), with polarity at the ends (barbed (+ve) end and the pointed (-ve) end). B The polarity of the monomeric structure is retained at the filamentous level, as depicted by cryo-EM representation of F-actin (PDB ID: 3G37). All the monomers are marked by distinctive colors to conveniently visualize the coordination of monomers in a filament. The F-actin is believed to contribute to the stability of axonal cytoskeleton. Ref.: [17, 18]
Mechanical Properties of Actin

Mechanical properties, especially the persistence length of actin filament have been studied in earlier works [40, 41]. The persistence length with all subunits in ATP state (F-ATP) is ~17 µm, although it can vary to a large extent. ATP bound, unfolded actin DB-loop persistence length has been found to be twice of ADP bound, folded DB-loop in actin [42]. Further studies revealed that unfolding of subunit structures may lead to alteration of persistence length [25, 43]. Evidently, stable state of actin is dependent on the folded DB-loop, which is a result of low free energy. In other words, folded DB-loop leads to formation of softer actin filament, eventually leading to a shorter persistence length [43–45].

Due to having helical structure, bending and twisting of F-actin have significant effects, especially on short ones [46]. However, the structural responses like extension, bending and twisting might be the result of strictly mechanical loading, strictly induced by biochemical parameters, or a combination of both. From that perspective, mechanical behavior of actins become complicated, and it is difficult to decide which aspect to consider for a specific case. For example, in high strain rate loading (TBI scenario), it might be assumed that due to the extreme mechanical loading, biochemical aspects might be overlooked. However, this is not realistic, as in axonal cytoskeletal component, mechanically induced injury may lead to biochemical implications (one example is hyper-phosphorylation of tau after TBI) [47]. For actin, there is a specific example in the literature, which suggests that structural changes occur in actin during motor activity, implying the contribution of filament bending flexibility for actinmyosin function [48]. Therefore, mechanical loading leading to biochemical implication and biochemical phenomena leading to mechanical property alteration are possible for axonal cytoskeletal components like actin.

One viable approach to address such dilemmas (by characterizing modeling the response to deformation) mentioned in the previous paragraph and study mechanical aspects of such filaments is atomistic simulation e.g., using molecular dynamics (MD) simulation which, however, cannot capture biological phenomena at large length scale. Therefore, an alternative has been coarse-grained (CG) modeling [20, 49]. There are several theories regarding behaviors of F-actin, such as elastic rod buckling theory and filament severing by cofilin theory [50, 51], while some models have examined mechanics and chemistry interplay [52]. Yogurtcu et al. [53], however, has proposed an intermediate scale model by ignoring conformational changes strongly associated with biochemical parameters. For filament length much longer than the helical actin pitch (> 1 µm), the filament mechanically deforms as a semiflexible rod. Another model worthy to mention considers buckling of actin due to compressive loading [49].

Earlier works have found that bending and torsional rigidities may differ strongly based on ATP or ADP-bound states [40, 51, 54]. Coarse grain MD simulations validated the findings about the properties like bending and torsional rigidities [55], strengthening the earlier statement in this chapter that biochemical phenomena can affect the mechanical properties of actin [48]. From the force-extension experiment in CG, Chu et al. [49] found the stretching stiffness of F-ATP actin and F-ADP actin as 37pn/nm and 31pn/nm, respectively. It is to be mentioned that ATP hydrolysis in actin is not the focus of this study, but the ATP states of actin affects the structure and behavior of actin. For specific aspects ATP hydrolysis, readers may refer to some earlier studies [56, 57].

Furthermore, the response to different mechanical loading is also important for actin, as this sheds light on mechanical properties such as stiffness. In relevance, effect of tensile force on actin filaments has been studied earlier [58], and it is shown that tensile force causes decrement of twist angle, leading to increased extensional and torsional stiffness (1.1 ± 0.1×10–26 Nm²/rad). According to earlier works, torsional rigidity per unit length of actin filament ranges from 2.3×10–27 to 8×10–26 Nm² [41, 59, 60], and extension-torsion coupling is important to comprehend the mechanical behavior of actin. Actin-actin bond breaking force is also measured in some studies, and they showed that turning of filaments greatly reduce the required force (Tsuda et al. 1996). In separate studies, strain dependent behavior of actin networks has been investigated to show that at high strain the elasticity ceases to be linear (Gardel et al. 2004).

Moreover, role of ABPs (which incorporate conformational changes to the structure of actin) on mechanical properties such as stiffness of actin has been investigated by steered molecular dynamics (SMD) simulations [61]. Formation of cofilactin (cofilin bound with each actin monomer) is found to reduce the stiffness of actin filament, especially actin filament with partially bound cofilin (refer to Fig. 2). The severing mechanism of cofilin on actin filaments is described in some studies, and found that mechanism of cofilin activity is promoting stress concentrations in junctions of filaments, which is similar to grain boundary fracture of crystalline materials or shear transformation of colloidal materials [62]. Regulatory severing protein (cofilin) has been found to increase bending and twisting compliance of actin in some atomistic and continuum models [45], because buckled cofilactin (actin decorated with cofilin) accelerates severing. Experimentally determined Young’s Modulus of actin is reported to be 400 MPa–2.5 GPa [63–67], to which the MD simulation results of Kim et al. [61] and Matsushita et al. [58] match closely. Torsional stiffness value obtained from MD simulation performed by Matsushita et al. [60] also agreed with the experimental value (0.23 ± 0.1×10–26 Nm²/
rad), assuming that difference can occur depending on the initial conformational state.

Going further into mechanical properties, range of obtained rheological parameters of actin, such as shear modulus and storage modulus from different experiments have been investigated as the reported values differ from 0.01 Pa to tens of Pa, and it is concluded that mechanical properties depend on initial length of filament as well as preparation, polymerization condition and storage methodology [68, 69]. It is also found in a study we have already mentioned that stiffness of 1 µm long actin is higher in case of association with tropomyosin [64].

In the discussion of mechanical behavior of actin, inclusion of viscoelastic behavior is relevant, especially in network level, which has been studied in different works [31, 70–72] and it is found that the response depends on several parameters, such as (i) cross-linker off rate, (ii) binding energy, (iii) characteristic bond length depending on actin-actin or actin-ABP interaction [31]. The response of the network is a combination of elasticity of the network and force-induced cross-linker unbinding and rebinding [73]. Both oscillatory and shear type experiments showed viscoelastic nature for filamentous and non-filamentous actins [74]. Viscoelastic behavior of actin network has been studied by modeling single filament, cross-linking and incorporating Maxwell properties in FEM, and validated by large strain experiments [75]. The authors would also like to mention that specific protein (such as filamin) induced crosslinking may also lead to significant change to mechanics of actin [76], the detailed discussion of which is out of the scope of this manuscript.

After going through structure, mechanical properties, and mechanical behavior, it is relevant to explore the dynamic aspects and mechanics of actin at axonal cytoskeletal level—as the studies on mechanics and dynamics of actin shed light on their response to deformation and filament-to-filament interaction. Mechanics of F-actin cytoskeleton is dictated by diverse mechanical dynamics of F-actin networks and bundles, and it has been reviewed thoroughly [77]. Specifically, cross-linking dynamics has been strongly related to mechanical properties of actin [78]. Rate of deformation also affects the difference between the stiffness of pure actin and actinin—at higher deformation rate, actinin is found to be stiffer than pure actin [79]. Actin, when associated with cross-linking proteins, act as around 40 times stiffer than pure actin under high deformation rate (strain rate), although the difference becomes indistinguishable under small deformation rate [79], which is justified by multiple rearranging of cross-link hypothesis.

**Computational Aspects of Actin Modeling**

Computationally, there have been numerous approaches to model and characterize actin. For example, atomistic studies on MFs have used Oda [25] or Holmes [29] model. Due to the nanometer length scale, multiscale approaches have also been proven effective from modeling perspective [80].

The structure, network, and stiffness properties have been determined by multiple studies. However, recently published review study, after performing a comprehensive literature exploration has concluded that the stiffness of microfilaments (including actins) can vary significantly, from a few hundred megapascals to 2.5 gigapascals [81], as actin has been studied under the application of a wide range of strains and strain rates. However, most of the stiffness data come from typical long filament of F-actin, not the short filament present in axonal periodic actin-spectrin network. In order to avoid redundancy in the discussion, the stiffness aspect of actin is limited to Table 1 in the manuscript.

Atomistic and CG studies have facilitated obtaining significant insight regarding mechanical behavior of actin including the effect of actin-severing protein [82], effect of crosslinking [83], and dynamic attributes [20]. Aside from the stiffness and strictly mechanical insights, computational studies have also provided with critical information regarding mechnanochemical attributes [84], deformation criteria [53], persistence length [55], torsional mechanism [55], viscoelastic characteristics [75], etc. In this section, however, emphasis will be given to the findings of modeling study.
| Axonal cytoskeletal component | Mechanical property or attribute found | Numeric value/description | Method | Reference |
|---|---|---|---|---|
| Actin (Mostly for typical long F-actin. Quantitative comparison between short and long actin filaments is currently unavailable in the literature) | Persistence length | 18 ± 1 µm (can be increased two-fold by using phalloidin) | Fluorescence optical video microscopy | [40] |
| | 16 µm | | Fully atomistic simulation | [42] |
| | Structural and unfolding characteristics | Globular to fibrous transition due to major domain rotation | Cryo-electron microscopy | [25] |
| | Polymerization leads to change to cleft region | | Fully atomistic simulation | [43] |
| | Mechanics of F-actin bundles | Mechanics of F-actin bundle is regulated by a diversified set of biochemical phenomena, such as interaction with ADP | Review | [77] |
| | Stiffness and torsional flexibility | 60% of elastic free energy of the filament originates from twist-bend coupling | Analytical | [46] |
| | | Mg²⁺ and Ca²⁺ ions regulate torsional flexibility of actin filament (filament with ions at active cleft sites are more flexible); quantified by optical anisotropy (0.051–0.077) | Steady-state phosphorescence emission spectroscopy | [54] |
| | | Torsional stiffness = 0.47–0.65 × 10⁻²⁶ Nm²/rad | Coarse-grained atomistic simulation | [55] |
| | | Average torsional stiffness = 0.25–0.5 × 10⁻²² Nm²/rad, average extensional stiffness = 0.05–0.1 N/m | Fully atomistic simulation | [60] |
| | Severing mechanism and response to tensile loading | Actin filament strain facilitates dissociation from cofilactin (actin-severing protein) | Fully atomistic simulation | [45] |
| | | Actin filament is highly deformable under compressive loading | Coarse-grained atomistic simulation | [49] |
| | | Actin buckles due to cooperative effect of formin and myosin-II | Total internal reflection fluorescence microscopy | [50] |
| | | Cofilin increases flexibility of actin filament facilitating easy severing | Fluorescence microscopy | [51] |
| | | 200pN of pulling force can invoke large structural changes in actin filament (due to rotation of − 2° per subunit) | Fully atomistic simulation (steered molecular dynamics) | [58] |
| | | Cofilin decoration for the actin filament incorporates flexibility due to incorporation of discontinuity in the structure | Coarse-grained atomistic simulation (steered molecular dynamics) | [61] |
| | | Cofilin decoration for the actin filament incorporates flexibility due to incorporation of discontinuity in the structure and stress concentration at the junctions | Review | [62] |
| | Mechanics + chemistry aspects | Interaction with ADP regulates the mechanical behavior | Coarse-grained atomistic simulation | [82] |

Sonic vibration and analytical | [52] |
| Axonal cytoskeletal component | Mechanical property or attribute found | Numeric value/description | Method | Reference |
|------------------------------|----------------------------------------|---------------------------|--------|-----------|
| Young’s modulus (E) and stiffness (K) | Tensile force incorporates rotation in every monomer unit of actin filament | **E** = 872–1256 MPa | Fully atomistic simulation (steered molecular dynamics) | [58] |
| Viscoelastic behavior (at actin network level) | Single type of actin binding protein such as filamin can incorporate different types of microstructures (Elastic modulus = 1–10 Pa, bulk modulus = 1–100 Pa) | **K** = 43.7 ± 4.6 pN/nm for 1 µm of length | Fluorescence microscopy | [64] |
| | Crosslinking proteins regulate viscoelastic behavior (Elastic modulus = 10–100 Pa) | **E** (per inverse area) = 4.4 × 10⁻⁸ N | Analytical | [65] |
| | Nonlinear actin deformation incorporates stiffening at network level | 1.3–2.5 GPa | Review | [81] |
| Cross-linking dynamics | From 8 to 25 °C, α-actinin interaction with actin facilitates actin bundle to lose its solid attribute | Increased strain rate stiffens actin for up to 40 times (dynamic modulus = 1–50 dyn/cm²) for deformation frequency of 10⁻¹–1 Hz | Stopped flow fluorescence | [78] |
| Rheological parameters (such as storage and lost modulus) at filament and network level along with shear modulus, persistence length, and stiffness | Elastic storage modulus (G’) of ~1 Pa at a deformation frequency of 0.1–1 Hz | Shear modulus = -100 Pa, persistence length = 10 µm | Oscillatory rheometry | [68] |
| Torsional rigidity, actin-actin bond breaking force | Torsional rigidity = 8.0 ± 1.2 × 10⁻²⁶ Nm², bond breaking force = 320–600 pN when filaments are twisted 90° | Stiffness = 65.3 ± 6.3 and 43.7 ± 4.6 pN/nm with and without tropomyosin, respectively | Dynamic light scattering and fluorescence microscopy | [69] |
| Elastic behavior at network level | Linear elastic modulus = 10–100 Pa | | Fluorescence microscopy | [41] |
| | | | | |

Reference: [58], [61], [64], [65], [66], [67], [68], [69], [70], [73], [75], [78], [79], [81].
| Axonal cytoskeletal component | Mechanical property or attribute found | Numeric value/description | Method | Reference |
|------------------------------|----------------------------------------|---------------------------|--------|-----------|
| Spectrin (mostly for erythroid spectrin. Only the “Forced unfolding mechanism” contains literature on axonal spectrin) | Shear modulus | Low shearing force (250–750 dynes cm⁻²) can sever erythroid spectrin-spectrin dimer | Electrophoresis | [103] |
| Elastic response (shear modulus, Young’s modulus) | Shear modulus μ₀ = 4.6–8.3 μN/m; uniaxial tension Young’s modulus E = 22.1 μN/m | Optical tweezer stretching and coarse-grained atomistic simulation | [108, 109] |
| Biochemical failure | Caspase-3 catalyzation leads to failure of αII-spectrin | Mass spectrometry and fluorescence microscopy | [112] |
| Forced unfolding mechanism | < 50 pN at 1 nm/ms extension rates leads to tandem unfolding in erythroid spectrin | Atomic force microscopy | [113] |
| Single spectrin repeat domain unfolds in a stepwise fashion when stretched | | Single molecule force spectroscopy | [125] |
| 25 to 35 pN is sufficient to unfold triple helical coiled coil conformation of spectrin repeats, and refolds in < 1 s upon removal of the pulling force | | Single molecule force spectroscopy | [126] |
| α-actinin (a protein in spectrin family) interchain binding properties reveal mechanical properties of spectrin | | Review | [127] |
| Wild-type domains of chicken brain spectrins (domains 15, 16, and 17) have same folding but different kinetic characteristics | | Fluorescence and stopped flow measurement | [128] |
| Erythroid spectrin unfolding and folding follows a distinctive pathway dependent on stretching | | Analytical and Monte Carlo simulation | [129] |
| Destabilization of the tertiary structure regulates the unfolding of the whole spectrin repeat unit, which is dependent on the linker protein | | Fully atomistic simulation (steered molecular dynamics) | [130] |
| Spectrin has intermediate stable structures when susceptible to stretching | | Atomic force microscopy and fully atomistic simulation (steered molecular dynamics) | [132] |
| Linker protein regulates unfolding of spectrin repeat unit | | Fully atomistic simulation (steered molecular dynamics) | [133] |
| Actin-Spectrin Network | Actin-Spectrin lattice organization (conflicting results regarding actin ring) | Short adducin-capped periodic actin ring vs long braided actin ring | Optical super-resolution microscopy | [12, 13] |
| Contribution of reversible unfolding of spectrin at low strain rate | Customized force measurement protocol to obtain reversible unfolding data | Confocal microscopy | [164] |
mechanical behavior of actin under moderate to extreme strain rate which are relevant to brain injury scenario.

From the modeling perspective, there have been numerous MD, coarse grained MD, FEM and continuum scale models on actin, and it is relevant to mention the aspects of some studies we have already cited in the earlier section. Fully atomistic models by fitting known structures of G-actin has been proposed earlier [25, 27, 33]. Some MD simulation studies have attempted to capture global structure as well as internal stereochromy of actin [33]. Coarse-graining from fully atomistic simulations further revealed holistic properties of F-actin [20]. Actin network properties has also been studied by continuum models, which highly emphasize cross-linking proteins [75, 85]. Oda model and Holmes model have been used extensively by others in MD simulations to further investigate F-actin network structure and properties [43]. Last but not the least, a very recent MD simulation study has found that when subjected to extreme strain rate, actin filaments can show high tensile stiffness [86]. Also, such actin filaments behave as stiffer material with the increase of the applied strain rate. Therefore, computational studies provide evidence that the most important parameters to dictate mechanical behavior of actin are the filament length and applied strain rate.

In this discussion on actin, it is clear that the literature is highly enriched on the mechanical behavior of actin, but most of the studies are relevant to typical long actin filaments. However, as it will be discussed in the later section on actin-spectrin periodic network, the current limitation will be conspicuous—that there are only a few studies that focus on the mechanical behavior of short actin filaments contributing to the actin-spectrin lattice, and quantitative comparison between the contribution of longer versus shorter actin filaments in axon stability is currently absent in the literature. Therefore, further studies on this topic will have high impact in the injury biomechanics and TBI research areas.

**Spectrin: Structure and Mechanical Insight**

**Structure of Spectrin**

As a significant axonal cytoskeletal component and a member of F-actin crosslinking superfamily, the functionality, structure, and attributes of spectrin have been explored in detail [14]. Structurally, spectrin forms α-spectrin and β-spectrin heterodimers [87] which lead to tetramers [88], eventually forming a hexagonal lattice when combined with periodic actin rings. Among the two α and five β isoforms, the α-Ⅱ (genetic encoding: SPTAN1) and β-Ⅱ (genetic encoding: SPTBN1) are the most relevant ones for axonal spectrins [89]. While the discussion on contribution of the individual α-Ⅱ and β-Ⅱ in axonal stability is not in the scope of this review, it is interesting to note that the comparative importance of them has been analyzed, and the loss of α-Ⅱ spectrin leads to more axonal degeneration than that of β-Ⅱ [89].

It is worth mentioning that due to being part of heterodimers, α and β shares only 30% similarity in structure. Furthermore, they have distinct function in axonal pathfinding [90]. The role of spectrin is recognized in maintaining axon stability and mechanical properties [91, 92]. Lack of spectrin has been marked as a source of axon instability, even breaking [93]. As the literature is enriched with numerous studies on erythroid spectrin and the current study focuses on the limitation on axonal spectrin behavior, the term “spectrin” will mean “axon spectrin” onwards in this manuscript unless otherwise stated specifically (like erythroid spectrin).

Recently proposed medium resolution zipper model of spectrin dimerization shows that α20-21 and β1-2 repeats create a dimer initiation site and close the dimer by utilizing electrostatic interaction. At the junction between α20-21 and β1-2 repeats, there are the actin binding domain, adducin binding spot, and Ca2+ binding EF hand. The determination of building blocks of spectrin i.e. α and β subunits have been performed experimentally in the early 80’s, mostly by using gel-filtration and ion-exchange chromatography, which laid out the groundwork of membrane associated actin-spectrin cytoskeleton [94, 95]. However, the function of spectrin was observed from biochemical perspective by incorporating phosphorylation and resulting change in association/dissociation from membrane which leads to specific stability states of the cytoskeleton, instead of response to mechanical loading perspective. It is to be mentioned that the actin-binding domain in spectrin subunits has also been determined in early chromatography researches [96, 97] and later green fluorescence microscopy [98], which led to quantification of specific domains and repeat regions later, even for non-erythroid spectrins [99, 100]. Additionally, the ~ 180 nm periodicity of spectrin, which is consistent with the periodicity of axon rings placed ~ 180 nm interval along the length of axon has been established by recent nanoscopy studies—which provide concluding evidence that indeed axonal cytoskeleton contains periodic lattice of actin-spectrin network [101], while the exact details of the structure will be dependent on appropriate imaging method.

**Limited Mechanical and Modeling Insight into Spectrin**

Most of the published work on spectrin properties investigate erythroid spectrin and actin-spectrin biochemical interaction [102–107], large deformation and elastic response of erythroid spectrin [108], network level elasticity in erythrocytes [109], etc. These studies provided excellent validation of continuum models of red blood cells by providing reliable length scale relationships. The extent of progress
in erythrocyte related modeling which incorporates properties of spectrin are diverse and advanced in literature due to advancements in optical tweezer experiment methodologies and atomic force microscopy (AFM), while the literature lacks insight regarding axonal cytoskeletal modeling of spectrin [110, 111].

However, due to biochemical phenomena or mechanical loading, axonal spectrin may unfold and stretch, which leads to the failure of the filament. Essentially, such failures are relevant to traumatic brain injury scenario (TBI). As a biological material, the failure behavior of spectrin can be explained from different viewpoints, such as mechanical, biochemical, or a combination of both [112]. However, forced unfolding research studies are particularly relevant to the current work, as they provide insight regarding extensibility of multi-domain proteins such as spectrin (refer to Fig. 3). One example is forced unfolding of tandem spectrin repeats at low forces performed by AFM, which has suggested that tandem unfolding differs significantly from single unfolding [113]. It is to be mentioned that similar insight regarding axonal spectrin is not available in the literature, and it is expected that the mechanical behavior will be significantly different due to the difference in persistence length, stiffness, and packing mechanism of tandem spectrin and axonal spectrin. However, for modeling purpose of the injury scenario of axonal spectrin, one of the focal points will be the unfolding mechanism of spectrin under moderate to high strain rate. This implicit insight will hopefully invoke further molecular level studies on axonal spectrin and actin-spectrin periodic lattice. Only then, a quantitative comparison between the mechanical behavior of axonal spectrin and erythrocytic spectrin will be possible. It is worth mentioning that Experimentally, the applied strain rate in macroscale which is relevant to TBI lies in $10^{-2}$s$^{-1}$–$10^{2}$s$^{-1}$ range [114, 115]. However, cavitation bubble implosion scenario [116, 117] is also relevant to TBI and depends on bubble size [118], which facilitates different modes of axonal deformation, and at this scenario, extreme strain rate of $10^{3}$s$^{-1}$–$10^{9}$s$^{-1}$ can be realized. It is worth mentioning that the anisotropy of damage propagation leads to a dilemma of length scale, meaning that relevant damage scenario at macroscale, tissue scale, and cellular scale are different, i.e., mild head injury may lead to substantial damage in the tissue and irreversible damage to axon [119–123]. In short, the extreme strain rate simulated in molecular dynamics simulation studies are relevant to specific TBI scenario. The reason behind the difference between the strain rates that experimentalists tend to apply versus the ones computational modeling experts do lies behind inherent limitations of each methodology. For example, lower strain rate application is viable experimentally, but at higher strain rate, capturing the resulting nanoscale deformations and is often impossible. On the other hand, in atomistic simulation studies, higher strain rate can be applied, and the resulting nanoscale behavior can be captured without losing substantial accuracy. In balance, applying low strain rate requires running the simulation for a very long time, which renders impractical for large systems and for simulations greater than 1–2 microseconds. As a result, reasonable compensations are often made in this regard. However, such discrepancy can be taken care of by using mathematical theories which consider increased stiffness in intermolecular bonds e.g., Bell’s theory [124], i.e., predicting changed mechanical behavior at changed strain rate.

Investigating more into mechanical response of spectrin, single molecule force spectroscopy (SMFS) has demonstrated single unfolding of spectrin specifically, which showed that single spectrin unfolding occurs in a stepwise fashion when susceptible to stretching, substantiating the presence of multiple intermediate repeat region in the structure [125]. Also, AFM study has quantified that force required to unfold spectrin repeats ranges between 25 and 35pN [126]. In addition, biochemical analyses of spectrin folding and unfolding mechanism have attributed different folding mechanism to interchain binding aspects [127], different kinetic characteristics [128], and existence of critical extension [129].

Additionally, steered molecular dynamics (SMD) studies on chicken brain spectrin have examined forced unfolding of multiple repeat spectrin suggesting that α-helical linker can be ruptured if susceptible to forced unfolding, the propagation of which may lead to destabilization of the tertiary
structure [130]. Moreover, the same group has investigated the rupture criteria of spectrin specifically by implementing non-equilibrium MD simulation, which has shown that force-extension response changes significantly at ~0.4 nm extension, and spectrin behavior can make a transition from elastic to viscous material as suggested by force-extension curves [131]. Formation of stable intermediate unfolded structures has been substantiated by similar MD simulation works on spectrin [132, 133]. In this regard, a latest contribution is an MD simulation study that applies high strain rate on α-spectrin and β-spectrin, both of which are found to have high stretchability, which is manifested at high strain rate [86].

Furthermore, theoretical modeling of spectrin network in axonal cytoskeleton has led to insights regarding the extension and fluctuation characteristics, which suggested that spectrins, within the periodicity of axons, can fluctuate in sync. This study substantiated that a spectrin network can be considered as a slender structure which can be coarse-grained [134].

Lastly, among other mechanical properties of spectrin, the frequency dependence of shear response has been measured by multiple lumped resonating viscoelastometer, which suggested that spectrin dimer can extend at a specific ionic strength [135]. Moreover, viscoelastic and mechanochemical characteristics of spectrin gel have also been determined, but only for erythrocytic ones, and therefore, insight regarding axonal spectrin is still lacking to date [136, 137]. The limitation in the literature is therefore evident that the stiffness and mechanical response data are almost limited to erythrocyte related studies [138].

Periodic Actin-Spectrin Skeleton: Role in Axon and Strain Rate Dependent Scenario

The periodic actin-spectrin cytoskeleton structure (Figs. 4 and 5) as well as the difference between cytoskeleton structure in dendrites, synapse, axon initial segment, and axonal cytoskeleton have been substantiated only after 2010s by dint of super-resolution microscopy and fluorescence nanoscopy [12, 139–143]. The significant advancement in the microscopy front is understood by recent review work and remodeling study on periodic actin-spectrin network [144, 145]. Specifically for axonal cytoskeleton, the periodicity is stated to be formed at the early stages of development and extends from proximal to distal end of axon [146]. It is to be mentioned that the periodicity of cytoskeleton is found in different types of cells and across species [147] and even axonal actin structure may differ from rings to waves and trails [148]—but in this section, the discussion is limited to axonal cytoskeleton. For clarity, it is to be mentioned that the actin-spectrin network is a part of the actomyosin network, and therefore, myosin may have important role in determining axonal behavior [149–152]. Nevertheless, as only axonal damage is the focus of this review, the effect at myosin level is not included here. Rather, the contribution of the main individual elements is discussed here, as in preliminary modeling, only the connections between the main constituents such as actin and spectrin will likely to be considered.

Specifically, the periodic actin-spectrin network is found in relevant neuronal structures such as main longitudinal portion of axon, initial segment of axon, and necks of dendritic spines—which differs than the structure of actin found in dendrites changing the conformation as one moves forward along the length of dendrites [153, 154]. In this regard, earlier experimentations have investigated spectrin-actin gel elasticity as a function of protein concentration, which proposed actin fiber network crosslinked by spectrin networks at regular intervals [155]. The role of actin-spectrin network in maintaining axon diameter and MT stability have also been well-established by multiple studies including recent ones [15, 156–158]. Where it might be intuitive that as the periodic actin-spectrin lattice is constituted of short filaments of actin and longer filaments of spectrin, the mechanical response should be dictated by spectrin. However, the real axonal response is
a result of highly dynamic network with involvement of and contribution of multiple membrane-associated proteins and actin-capping proteins aside from actin, spectrin, and their interaction. Therefore, it is relevant to study the recent standings of the actin-spectrin interaction studies. However, the current understanding of axonal actin-spectrin network is enigmatic, as suggested by conflicting results from recent super-resolution microscopy studies [159]. While one study suggests that actin-spectrin network is consisted of short filaments of actin capped by α2-β4 tetramer of spectrin), actin and spectrin are stained green and magenta respectively, which clearly suggests the periodicity of actin-spectrin network and their non-uniformity along the length of axon. Panel D shows a live imaging sequence, while E shows actin and spectrin specifically (dotted circles)

\[\text{adducin [12], the opposing study asserts that adducin not only acts as an actin-capping protein, but it may attach itself to the side of actin to promote longitudinal interaction. Also, it suggests that rather than a bunch of short filaments of actin, the periodic ring consists of intertwined (braided) long filaments of actin [13]. As the mechanical behavior of actin depends on the length of the filaments, it can be inferred that the mechanical behavior and response to injury of actin-spectrin network is not conclusively defined yet. Therefore, axonal actin-spectrin network}\]
remains an interesting topic for further study. Also, it is worth mentioning that not only the actin-spectrin network provides mechanical support to axonal cytoskeleton, but also, they have functionalities related to signaling and axonal transport. Many of such functionalities are activated by receptors as depicted in a recent super-resolution microscopy study on RTK activation in neurons [160]. Such study suggests that membrane-associated cytoskeleton facilitates a dynamic framework of signaling pathways, and thus further investigation in this regard might generate interesting insights.

Spectrin-actin interaction and associated alteration of cytoskeleton (even axon degeneration) have been found to be manipulated by numerous biochemical agents and phenomena, such as effect of dematin [161], tropic deprivation (TD) [162], etc. Furthermore, continuous remodeling of neural cytoskeleton has been attributed to neural growth, which is dictated by actin-MT and actin-spectrin interaction [163]. However, studies focusing on spectrin-actin interaction or alteration of the interface between them due to mechanical loading is scarce in literature. Among the few recent studies relevant to actin-spectrin cytoskeleton, one has attributed the tension-buffering shock absorber mechanism of the cytoskeleton (especially reversible unfolding of the repeat domains of the spectrin tetramers) to the ability of axon to stretch significantly by performing stretching experiment on chicken dorsal root ganglion in a customized force apparatus [164]. In relevance, red blood cell (RBC) coarse grain modeling has revealed that spectrin contributes to shear stress at lower shear strain, but lipid membrane also contributes at higher strain rates [165].

The knowledge gap in the mechanical behavior of axonal actin-spectrin network is also admitted in another study which combines atomic force microscopy (AFM) and molecular dynamics (MD) simulation approaches as an attempt to distinguish among the somatic, dendritic, and axonal stiffness [166]. This study demonstrates that the axonal stiffness is significantly higher than those of the somatic and dendritic regions. The associated MD simulation shows that coarse graining of the cytoskeletal components can reliably reproduce experimental finding in this regard, and therefore, it can be one of the plausible approaches to address the ongoing limitations. However, the authors also admit that there is significant difference between red blood cell (RBC) associated and axonal actin-spectrin network, and no quantitative comparison between their mechanical properties or behavior does not exist till date. In this regard, a very recent MD simulation study attempts to shed light on axonal actin-spectrin interaction subjected to high strain rate, and finds that the mode of failure (separation of spectrin from actin) differs based on the applied strain rate: at lower strain rate, the actin-spectrin interface is susceptible to failure, while at higher strain rate, the likely scenario is failure of spectrin filament due to significant stretch [86].

In short, current literature lacks insight in mechanical behavior of axonal actin, spectrin, and actin-spectrin interaction. However, recent studies on axonal cytoskeletal components have focused on applicable strain rate on soft biomaterials [167–169] relevant to brain and different cytoskeletal components such as microtubules, tau proteins, and neuromfilaments [118, 170–172]. Such extreme high strain rate scenarios can be captured by undertaking atomistic computational approaches which can be extended to other axonal cytoskeletal components to provide novel insights regarding the specific mechanical behavior of them at extreme strain rate. However, to accomplish these objectives, different customized approaches are expected, such as atomistic based continuum modeling, coarse-graining, adopting comprehensive multiscale maneuver [173], etc. Optimistically, the possible future directions mentioned above will play an instrumental role in developing a bottom-up axon model focusing on moderate to high strain rate scenario and contribute to the existent computational axon models.

### Conclusion

In this study, current scenario and insights regarding actin, spectrin, and actin-spectrin combination are briefly. As biological materials, relevant literature from biochemical perspective is also represented. In this way, it can be asserted that several ongoing limitations from mechanical perspective have been pointed out, which can be summarized as:

A. **Actin:** Mechanical insights are mostly present for axonal actin. However, comparative mechanical behavior of short versus long actin filaments are not present in the literature.

B. **Spectrin:** Little mechanical insight is present, but mostly for erythroid spectrin, not axonal ones. Erythroid spectrin structure significantly differs from the axonal ones, and therefore, the existent literature does not shed light on mechanical behavior such as forced unfolding maneuver in axonal spectrin.

C. **Actin-Spectrin Network:** Little mechanical insight is present, although response to high strain rate is relevant to brain injury scenario. The organization of actin ring is disputed—short adducin-capped actin filaments versus long braided actin filaments in the ring.

Especially, the existent literature fails to cover the high strain rate response of actin-spectrin network. However, the limitations can certainly be attributed to lack of advanced imaging techniques and customized mechanical experimentation as well as modeling. Over the next few years, it can
be hoped that the current limitations will be overcome to a significant extent—as structural insights will be more substantiated and microscopy methodologies will be advanced further. Due to the nanometer length scale, MD simulation and associated computational approaches will also serve valuable complementary purpose.

In short, in this study, we have identified some prominent knowledge gaps regarding actin, spectrin, and periodic axonal actin-spectrin network which require immediate attention. Clearer insight regarding such structures and their mechanical behavior awaits further improvement (or additional studies) of super-resolution microscopy. In the meantime, the computational (modeling) approaches will be able to provide further insight regarding their behavior. This study has given emphasis particularly on computational modeling as recent studies has strengthened the view that atomistic simulation is a viable approach to obtain insight regarding axonal cytoskeletal components in traumatic brain injury (TBI) scenario. The culmination of such studies is a comprehensive bottom-up modeling of axon which provides detailed insight of axonal level response to deformation when susceptible to injury. Therefore, this study not only points out the state of the art of the field, but also suggests viable approach for improvement.

There are both promising prospects and challenges for the topic discussed in the manuscript. While there has been significant advancement in the super-resolution microscopy and modeling technique of the axonal cytoskeletal components, the methodologies are not devoid of limitations. For example, in terms of visualization, actin and spectrin are explored to required extent to date, and interesting results regarding the associated pathways of brain pathology are being investigated, which will provide significant therapeutic and structural edge for both structural biologists and neuroscientists [174]. However, due to the complexity and being accompanied with so many membranes and membrane-associated proteins [164], the question marks regarding actin-spectrin network remains—meaning that this field is both a proving ground for spectroscopists and computational biologists. Specifically, from the computational modeling point of view, advancements in force fields and acquiring superior computational power facilitates simulating bigger and bigger systems. Nevertheless, the intricacy of the systems, especially at cellular level will continue to be a concern, as axonal actin structure and functionality not only depends on actin-spectrin interaction, but also on membrane-associated proteins [15]. In this regard, specialists in scaling and parameterization of biological structure need to provide insightful contribution to the field to dissolve the discrepancy in terms of translating mechanical deformation from nanoscale to macroscale [119, 120, 122], meaning that the most conspicuous challenge will be to address the multiscale aspect of mechanical behavior and deformation of biological materials.

Table 1 summarizes the mechanical properties, behavior, or insight available in the literature—substantiating that there is sufficient literature focusing on actin behavior, but not for spectrin and actin-spectrin periodic lattice.

Acknowledgements This work has been funded by the Computational Cellular Biology of Blast (C2B2) program through the Office of Naval Research (ONR) (Award # N00014-18-1-2082: Dr. Timothy Bentley, Program Manager).

Author contributions MIK collected the literature, interpreted the current scenario, and wrote the manuscript. SF and AA revised the manuscript. All the authors reviewed the manuscript.

Data availability No customized code or program was used in this review study.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. M.J. Buehler, S. Keten, Colloquium: Failure of molecules, bones, and the Earth itself. Rev Mod Phys 82, 1459 (2010)
2. Buehler MJ. Atomistic modeling of materials failure. Springer Science & Business Media; 2008.
3. K. Eom, Computer simulation of protein materials at multiple length scales: From single proteins to protein assemblies. Multiscale Sci Eng 1, 1–25 (2019)
4. Batt L. Molecular dynamics analysis of supercontraction in spider dragline silk 2013.
5. Sotomayor M, Schulten K. Single-molecule experiments in vitro and in silico. Science (80- ) 2007;316:1144–8.
6. Elkin BS, Morrison B. Region-specific tolerance criteria for the living brain. SAE Technical Paper; 2007.
7. J. Avila, Microtubule dynamics. FASEB J 4, 3284–3290 (1990). https://doi.org/10.1096/fasebj.4.15.2253844
8. K.J. Rosenberg, J.L. Ross, H.E. Feinstein, S.C. Feinstein, J. Israelachvili, Complementary dimerization of microtubule-associated tau protein: Implications for microtubule bundling and tau-mediated pathogenesis. Proc Natl Acad Sci 105, 7445–7450 (2008)
9. Lee G, Neve RL, Kosik KS. The microtubule binding domain of tau protein. Neuron 1989;2:1615–24. https://doi.org/10.1016/0896-6273(89)90050-0
10. P.A. Janney, J.-F. Leterrier, H. Herrmann, Assembly and structure of neurofilaments. Curr Opin Colloid Interface Sci 8, 40–47 (2003)
11. H. Ouyang, E. Nauman, R. Shi, Contribution of cytoskeletal elements to the axonal mechanical properties. J Biol Eng 7, 21 (2013). https://doi.org/10.1186/1754-1611-7-21
12. Xu K, Zhong G, Zhuang X. Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons. Science (80- ) 2013;339:452–6.
13. S. Vassilopoulos, S. Gibaud, A. Jimenez, G. Caillet, C. Leterrier, Ultrastructure of the axonal periodic scaffold reveals a braid-like organization of actin rings. Nat Commun 10, 1–13 (2019)
14. R. Zhang, C. Zhang, Q. Zhao, D. Li, Spectrin: structure, function and disease. Sci China Life Sci 56, 1076–1085 (2013)
15. S.C. Leite, P. Sampaio, V.F. Sousa, J. Nogueira-Rodrigues, R. Pinto-Costa, L.L. Peters et al., The actin-binding protein α-adducin is required for maintaining axon diameter. Cell Rep 15, 490–498 (2016)
16. N.G. Naydenov, A.I. Ivanov, Spectrin-adducin membrane skeleton: A missing link between epithelial junctions and the actin cytoskeleton? BioArchitecture 1, 3506–3517 (2011)
17. S. Kumar, A. Månsson, Covalent and non-covalent chemical engineering of actin for biotechnological applications. Biotechnol Adv 35, 867–888 (2017)
18. K. Murakami, T. Yasunaga, T.Q.P. Noguchi, Y. Gomibuchi, K.X. Ngo, T.Q.P. Uyeda et al., Structural basis for actin assembly, activation of ATP hydrolysis, and delayed phosphate release. Cell 143, 275–287 (2010). https://doi.org/10.1016/j.cell.2010.09.034
19. Pachenari M, Seyedpour SM, Jamaleki M, Shayan SB, Taranjose S, Hosseinikhani H. Mechanical properties of cancer microtubules content: Investigating different grades of colon cancer cell lines. J Biomech 2014;47:373–9. https://doi.org/10.1016/j.jbiomech.2013.11.020.
20. M.A. Deriu, A. Shkurti, G. Paciello, T.C. Bidone, U. Morbiducci, E. Ficarra et al., Multiscale modeling of cellular actin filaments: from atomistic molecular to coarse-grained dynamics. Proteins Struct Funct Bioinforma 80, 1598–1609 (2012)
21. H.P. Erickson, Co-operativity in protein-protein association: the structure and stability of the actin filament. J Mol Biol 206, 465–474 (1989)
22. Howard J. Mechanics of motor proteins and the cytoskeleton 2001.
23. Otterbein LR, Graceffa P, Dominguez R. The crystal structure of uncomplexed actin in the ADP state. Science (80-) 2001;293:708–11.
24. P. Graceffa, R. Dominguez, Crystal structure of monomeric actin in the ATP state structural basis of nucleotide-dependent actin dynamics. J Biol Chem 278, 34172–34180 (2003)
25. T. Oda, M. Iwasa, T. Aihara, Y. Maeda, A. Narita, The nature of the globular-to fibrous-actin transition. Nature 457, 441 (2009)
26. V.E. Galkin, A. Orlova, G.F. Schröder, E.H. Egelman, Structural polymorphism in F-actin. Nat Struct Mol Biol 17, 1318 (2010)
27. T. Fuji, A.H. Iwane, T. Yanagida, K. Namba, Direct visualization of secondary structures of F-actin by electron cryomicroscopy. Nature 467, 724 (2010)
28. E. Lazarides, Immunofluorescence studies on the structure of actin filaments in tissue culture cells. J Histochem Cytochem 23, 507–528 (1975)
29. R. Dominguez, K.C. Holmes, Actin structure and function. Annu Rev Biophys 40, 169–186 (2011)
30. R. Niederman, P.C. Amrein, J. Hartwig, Three-dimensional structure of actin filaments and of an actin gel made with actin-binding protein. J Cell Biol 96, 1400–1413 (1983)
31. K.M. Schmoller, O. Lieleg, A.R. Bausch, Structural and viscoelastic properties of actin/filamin networks: cross-linked versus bundled networks. Biophys J 97, 83–89 (2009)
32. Pelletier O, Fokidisheva E, Hirst LS, Bouxsein N, Li Y, Safinya CR. Structure of actin cross-linked with α-actinin: a network of bundles. Phys Rev Lett 2003;91:148102.
33. T. Splettstoesser, K.C. Holmes, F. Noé, J.C. Smith, Structural modeling and molecular dynamics simulation of the actin filament. Proteins Struct Funct Bioinforma 79, 2033–2043 (2011)
34. R.S. Sedeh, A.A. Fedorov, E.V. Fedorov, S. Ono, F. Matsumura, S.C. Almo et al., Structure, evolutionary conservation, and conformational dynamics of Homo sapiens fascin-1, an F-actin crosslinking protein. J Mol Biol 400, 589–604 (2010)
35. M.G. Klein, W. Shi, U. Ramagopal, Y. Tseng, D. Wirtz, D.R. Kovar et al., Structure of the actin crosslinking core of fimbrin. Structure 12, 999–1013 (2004)
36. Esue O, Tseng Y, Wirtz D. α-Actinin and filamin cooperatively enhance the stiffness of actin filament networks. PLoS One 2009;4:e4411.
37. Y. Tseng, K.M. An, O. Esue, D. Wirtz, The bimodal role of fimlin in controlling the architecture and mechanics of F-actin networks. J Biol Chem 279, 1819–1826 (2004)
38. Brown JW, Structure, function, dynamics, and cellular localization of villin-type headpiece domains 2010.
39. S.P. George, A. Esmaeiliakosoghi, S. Roy, S. Khurana, F-actin-bundling sites are conserved in proteins with villin-type headpiece domains. Mol Biol Cell 31, 1857–1866 (2020)
40. Isambert H, Venier P, Mauggs AC, Fattoum A, Kassab R, Panta- loni D, et al. Flexibility of actin filaments derived from thermal fluctuations. Effect of bound nucleotide, phalloidin, and muscle regulatory proteins. J Biol Chem 1995;270:11437–44.
41. Y. Tsuda, H. Yasutake, A. Ishijima, T. Yanagida, Torso-nal rigidity of single actin filaments and actin–actin bond breaking force under torsion measured directly by in vitro micromanipulation. Proc Natl Acad Sci 93, 12937–12942 (1996)
42. J.-W. Chu, G.A. Voth, Allostery of actin filaments: molecular dynamics simulations and coarse-grained analysis. Proc Natl Acad Sci 102, 13111–13116 (2005)
43. Pfaendtner J, Branduardi D, Parrinello M, Pollard TD, Voth GA. Nucleotide-dependent conformational states of actin. Proc Natl Acad Sci 2009;106:12723 LP—12728. https://doi.org/10.1073/pnas.0902092106.
44. Schramm AC, Hocky GM, Voth GA, Blanchoin L, Martiel JL, De La Cruz EM. Actin Filament Strain Promotes Severe and Cotin Dissociation. Biophys J 2017;112:2624–33. https://doi.org/10.1016/j.bpj.2017.05.016.
45. M. Enrique, J. Roland, B.R. McCullough, L. Blanchoin, J.-L. Martiel, Origin of twist-bend coupling in actin filaments. Biophys J 99, 1852–1860 (2010)
46. J. Cao, F. El Gaamouch, J.S. Meabon, K.D. Meeker, L. Zhu, M.B. Zhong et al., ApoE4-associated phospholipid dysregulation contributes to development of tau hyper-phosphorylation after traumatic brain injury. Sci Rep 7, 1–12 (2017)
47. P.G. Vikhorev, N.N. Vikhoreva, A. Månsson, Bending flexibility of actin filaments during motor-induced sliding. Biophys J 95, 5809–5819 (2008)
48. J.-W. Chu, G.A. Voth, Coarse-grained modeling of the actin filament derived from atomistic-scale simulations. Biophys J 90, 1572–1582 (2006)
49. J. Berro, A. Michelot, L. Blanchion, D.R. Kovar, J.-L. Martiel, Attachment conditions control actin filament buckling and the production of forces. Biophys J 92, 2546–2558 (2007)
50. B.R. McCullough, E.E. Grintsevich, C.K. Chen, H. Kang, A.L. Hutchison, A. Henn et al., Colillin-linked changes in actin filament flexibility promote severe. Biophys J 101, 151–159 (2011)
51. S. Asakura, M. Taniguchi, F. Oosawa, Mechano-chemical behaviour of F-actin. J Mol Biol 7, 55–69 (1963)
52. O.N. Yogurtcu, J.S. Kim, S.X. Sun, A mechanochemical model of actin filaments. Biophys J 103, 719–727 (2012)
53. C.A. Rebello, R.D. Ludescher. Influence of tightly bound Mg2+ and Ca2+, nucleotides, and phalloidin on the microsecond torsional flexibility of F-actin. Biochemistry 37, 14529–14538 (1998)
54. J. Fan, M.G. Saunders, G.A. Voth, Coarse-graining provides insights on the essential nature of heterogeneity in actin filaments. Biophys J 103, 1334–1342 (2012)
56. S. Vorobiev, B. Strokopytov, D.G. Drubin, C. Frieden, S. Ono, J. Condeelis et al., The structure of nonvertebrate actin: implications for the ATP hydrolytic mechanism. Proc Natl Acad Sci 100, 5760–5765 (2003)

57. Akola J, Jones RO. Density functional calculations of ATP systems. 2. ATP hydrolysis at the active site of actin. J Phys Chem B 2006;110:8121–9.

58. S. Matsushita, Y. Inoue, M. Hojo, M. Sokabe, T. Adachi, Effect of tensile force on the mechanical behavior of actin filaments. J Biomech 44, 1776–1781 (2011)

59. S. Matsushita, Y. Inoue, T. Adachi, Quantitative analysis of extension–torsion coupling of actin filaments. Biochem Biophys Res Commun 420, 710–713 (2012)

60. S. Matsushita, T. Adachi, Y. Inoue, M. Hojo, M. Sokabe. Evaluation of extensional and torsional stiffness of single actin filaments by molecular dynamics analysis. J Biomech 43, 3162–3167 (2010)

61. Kim JL, Kwon J, Baek I, Na S. Steered molecular dynamics analysis of the role of cofilin in increasing the flexibility of actin filaments. Biophys Chem 2016;218:27–35. https://doi.org/10.1016/j.bpc.2016.08.002.

62. E.M. De La Cruz, How cofilin severs an actin filament. Biophys Rev 1, 51–59 (2009). https://doi.org/10.1007/s12551-009-0008-5

63. Huxley HE, Stewart A, Sosa H, Irving T. X-ray diffusion measurements of the extensibility of actin and myosin filaments in contracting muscle. Biophys J 1994;67:2411–21. https://doi.org/10.1016/S0006-3495(94)80728-3.

64. Kojima H, Ishijima A, Yanagida T. Direct measurement of stiffness of single actin filaments with and without tropomyosin by in vitro nanomanipulation. Proc Natl Acad Sci 1994;91:12962 LP—12966. https://doi.org/10.1073/pnas.91.26.12962.

65. ben-Avraham D, Tirion MM. Dynamic and elastic properties of F-actin: a normal-modes analysis. Biophys J 1995;68:1231–45. https://doi.org/10.1016/S0006-3495(94)80729-5.

66. Wakubayashi K, Sugimoto Y, Tanaka H, Ueno Y, Takezawa Y, Amemiya Y. X-ray diffraction evidence for the extensibility of actin and myosin filaments during muscle contraction. Biophys J 1994;67:2422–35. https://doi.org/10.1016/S0006-3495(94)80729-5.

67. Higuchi H, Yanagida T, Goldman YE. Compliance of thin filaments in skinned fibers of rabbit skeletal muscle. Biophys J 1995;69:1000–10. https://doi.org/10.1016/S0006-3495(95)79075-1.

68. Xu J, Schwarz WH, Käs JA, Stossel TP, Janmey PA, Pollard TD. Mechanical Properties of Actin Filament Networks Depend on Preparation, Polymerization Conditions, and Storage of Actin Monomers. Biophys J 1998;74:2731–40. https://doi.org/10.1016/S0006-3495(98)77979-2.

69. Janmey PA, Hvidt S, Käs J, Lerche D, Maggs A, Sackmann E, et al. The mechanical properties of actin gels. Elastic modulus and filament motions. J Biol Chem 1994;269:32503–13.

70. Lieleg O, Schmoller KM, Claessens MMAE, Bausch AR. Cytoskeletal Polymer Networks: Viscoelastic Properties are Determined by the Microscopic Interaction Potential of Cross-links. Biophys J 2009;96:4725–32. https://doi.org/10.1016/j.bpj.2009.03.038.

71. Efremov YM, Dokrunova AA, Efremenko A V, Kirpichenkov MP, Shaitan K V, Sokolova OS. Distinct impact of targeted actin cytoskeleton reorganization on mechanical properties of normal and malignant cells. Biochim Biophys Acta (BBA)-Molecular Cell Res 2015;1853:3117–25.

72. H. Lee, J.M. Ferrer, F. Nakamura, M.J. Lang, R.D. Kamm. Passive and active micro rheology for cross-linked F-actin networks in vitro. Acta Biomater 6, 1207–1218 (2010)

73. Gurumessa B, Ricketts S, Robertson-Anderson RM. Nonlinear Actin Deformations Lead to Network Stiffening, Yielding, and Nonuniform Stress Propagation. Biophys J 2017;113:1540–50. https://doi.org/10.1016/j.bpj.2017.01.012.

74. M. Sato, G. Leimbach, W.H. Schwarz, T.D. Pollard. Mechanical properties of actin. J Biol Chem 260, 8585–8592 (1985)

75. Unterberger MJ, Schmoller KM, Wurm C, Bausch AR, Holzapfel GA. Viscoelasticity of cross-linked actin networks: Experimental tests, mechanical modeling and finite-element analysis. Acta Biomater 2013;9:7343–53. https://doi.org/10.1016/j.actbio.2013.03.008.

76. K.L. Weirich, S. Banerjee, K. Dasbiswas, T.A. Witten, S. Vai- kuntanathan, M.L. Gardel. Liquid behavior of cross-linked actin bundles. Proc Natl Acad Sci 114, 2131–2136 (2017)

77. J. Stricker, T. Falzone, M.L. Gardel. Mechanics of the F-actin cytoskeleton. J Biomech 43, 9–14 (2010)

78. J. Xu, D. Wirtz, T.D. Pollard. Dynamic cross-linking by α-actinin determines the mechanical properties of actin filament networks. J Biol Chem 273, 9570–9576 (1998)

79. M. Sato, W.H. Schwarz, T.D. Pollard. Dependence of the mechanical properties of actin/α-actinin gels on deformation rate. Nature 325, 828–830 (1987). https://doi.org/10.1038/32582a0.

80. Li T, Gu Y, Feng X-Q, Yarlagadda PKD V, Oloyede A. Hierarchical multiscale model for biomechanics analysis of microfilament networks. J Appl Phys 2013;113:194701.

81. Khan MI, Hasan F, Mahmud KAH Al, Adnan A. Recent Computational Approaches on Mechanical Behavior of Axonal Cytoskeletal Components of Neuron: A Brief Review. Multiscale Sci Eng 2020;2:199–213. https://doi.org/10.1007/s42493-020-00043-4.

82. T. Kim, W. Hwang, R.D. Kamm, Computational analysis of a cross-linked actin-like network. Exp Mech 49, 91–104 (2009). https://doi.org/10.1007/s11340-007-9091-3

83. M.M.A.E. Claessens, M. Bathe, E. Frey, A.R. Bausch. Actin-binding proteins sensitively mediate F-actin bundle stiffness. Nat Mater 5, 748–753 (2006). https://doi.org/10.1038/nmat1718

84. Zheng X, Diraviyam K, Sept D. Nucleotide Effects on the Structure and Dynamics of Actin. Biophys J 2007;93:1277–83. https://doi.org/10.1529/biophysj.107.109215.

85. Holzapfel GA, Unterberger MJ, Ogden RW. An affine continuum mechanical model for cross-linked F-actin networks with compliant linker proteins. J Mech Behav Biomed Mater 2014;38:78–90. https://doi.org/10.1016/j.jmbbm.2014.05.014.

86. Khan MI, Ferdows SF, Adnan A. Mechanical behavior of actin and spectrin subjected to high strain rate: A molecular dynamics simulation study. Comput Struct Biotechnol J 2021;19:1738–49. https://doi.org/10.1016/j.csbj.2021.03.026.

87. H. Kusunoki, G. Minasov, R.I. MacDonald, A. Mondragón. Independent movement, dimerization and stability of tandem repeats of chicken brain α-spectrin. J Mol Biol 344, 495–511 (2004)

88. S.L. Harper, D. Li, Y. Maksimova, P.G. Gallagher, D.W. Speicher. A fused α-β “mini-spectrin” mimics the intact erythrocyte spectrin cytoskeleton spectrin head-to-head tetramer. J Biol Chem 285, 11003–11012 (2010)

89. C.-H. Liu, M.N. Rasband, Axonal spectrin: nanoscale organization, functional domains and spectrinopathies. Front Cell Neurosci 13, 234 (2019)

90. J. Hülsmeier, J. Pielage, C. Rickert, G.M. Technau, C. Klämbt, A.J. Baines, Evolution of spectrin function in cytoskeletal and membrane networks. Biochem Soc Trans 37, 796–803 (2009)

91. M. Hammarlund, E.M. Jorgensen, M.J. Bastiani, Axons break in animals lacking β-spectrin. J Cell Biol 176, 269–275 (2007)
94. R. Calvert, P. Bennett, W. Gratzer, Properties and structural role of the subunits of human spectrin. Eur J Biochem 107, 355–361 (1980)
95. N.R. Burns, V. Ohanian, W.B. Gratzer, Properties of brain spectrin (fodrin). FEBS Lett 153, 165–168 (1983)
96. Frappier T, Regnouf F, Pradel LA. Binding of brain spectrin to the 70-kDa neurofilament subunit protein. Eur J Biochem 1987;169:651–7.
97. T. Frappier, J. Derancourt, L. Pradel, Actin and neurofilament binding domain of brain spectrin β subunit. Eur J Biochem 205, 85–91 (1992).
98. Nestor MW, Cai X, Stone MR, Bloch RJ. Thompson SM. The actin binding domain of β-spectrin regulates the morphological and functional dynamics of dendritic spines. PLoS One 2011;6:e16197.
99. Moon RT, McMahon AP. Generation of diversity in nonerythroid spectrins. Multiple polypeptides are predicted by sequence analysis of cDNAs encompassing the coding region of human nonerythroid alpha-spectrin. J Biol Chem 1990;265:4427–33.
100. Nagase T, Ishikawa K, Nakajima D, Ohira M, Seki N, Miyajima N, et al. Prediction of the coding sequences of unidentified human genes. VII. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro. DNA Res 1997;4:141–50.
101. D’Este E, Kamin D, Götzfert F, El-Hady A, Hell SW. STED Nanoscopy Reveals the Ubiquity of Subcortical Cytoskeleton Periodicity in Living Neurons. Cell Rep 2015;10:1246–51. https://doi.org/10.1016/j.celrep.2015.02.007.
102. L. Pan, R. Yan, W. Li, K. Xu. Super-resolution microscopy reveals the native ultrastructure of the erythrocyte cytoskeleton. Cell Rep 22, 1151–1158 (2018).
103. X. An, M.C. Lecomte, J.A. Chasis, N. Mohandas, W. Gratzer, Shear-response of the spectrin dimer-tetramer equilibrium in the red blood cell membrane. J Biol Chem 277, 31796–31800 (2002).
104. D.E. Discher, P. Carl, New insights into red cell network structure, elasticity, and spectrin unfolding—a current review. Cell Mol Biol Lett 6, 593–606 (2001).
105. D.W. Speicher, V.T. Marchesi, Erythrocyte spectrin is comprised of many homologous triple helical segments. Nature 311, 177–180 (1984).
106. M.A. De Matteis, J.S. Morrow, Spectrin tethers and mesh in the biosynthetic pathway. J Cell Sci 113, 2331–2343 (2000).
107. S. Manno, Y. Takakuwa, K. Nagao, N. Mohandas, Modulation of erythrocyte membrane mechanical function by β-spectrin phosphorylation and dephosphorylation. J Biol Chem 270, 5659–5665 (1995).
108. M. Dao, J. Li, S. Suresh, Molecularily based analysis of deformation of spectrin network and human erythrocyte. Mater Sci Eng C 26, 1232–1244 (2006).
109. M. Hoore, F. Yaya, T. Podgorski, C. Wagner, G. Gompper, D.A. Fedosov, Effect of spectrin network elasticity on the shapes of erythrocyte doublets. Soft Matter 14, 6278–6289 (2018).
110. J. Li, M. Dao, C.T. Lim, S. Suresh, Spectrin-level modeling of the cytoskeleton and optical tweezers stretching of the erythrocyte. Biophys J 88, 3707–3719 (2005).
111. D. Li, S.L. Harper, H.-Y. Tang, Y. Maksimova, P.G. Gallagher, D.W. Speicher, A comprehensive model of the spectrin divalent tetramer binding region deduced using homology modeling and chemical cross-linking of a mini-spectrin. J Biol Chem 285, 29535–29545 (2010).
112. Witek MA, Fung LW-M. Quantitative studies of caspase-3 catalyzed αII-spectrin breakdown. Brain Res 2013;1533:1–15. https://doi.org/10.1016/j.brainres.2013.08.010.
113. R. Law, P. Carl, S. Harper, P. Dalhaimer, D.W. Speicher, D.E. Discher, Cooperativity in forced unfolding of tandem spectrin repeats. Biophys J 84, 533–544 (2003).
114. M. Hosseini-Farid, M. Ramzanpour, M. Ziejewski, G. Karami, Estimating the brain strain rates during traumatic brain injury. Biomed Sci Instrum 54, 361–368 (2018).
115. M. Hosseini-Farid, M. Amiri-Tehrani-Zadeh, M. Ramzanpour, M. Ziejewski, G. Karami, The strain rates in the brain, brainstem, dura, and skull under dynamic loadings. Math Comput Appl 25, 21 (2020).
116. Wardlaw A, Goeller J. Cavitation as a possible traumatic brain injury (TBI) damage mechanism. 26th South. Biomed. Eng. Conf. SBEC 2010, April 30-May 2, 2010, Coll. Park. Maryland, USA: Springer; 2010, pp 34–7.
117. Brennen CE. Cavitation in biological and bioengineering contexts 2003.
118. Y.-T. Wu, A. Adnan, Damage and failure of axonal microtubule under extreme high strain rate: an in-silico molecular dynamics study. Sci Rep, 8, 12260 (2018).
119. R.M. Wright, A. Post, B. Hoshizaki, K.T. Ramesh, A multiscale computational approach to estimating axonal damage under inertial loading of the head. J Neurotrauma 30, 102–118 (2013).
120. R.J.H. Cloots, J.A.W. Van Dommelen, S. Kleiven, M.G.D. Geers, Multi-scale mechanics of traumatic brain injury: predicting axonal strains from head loads. Biomech Model Mechanobiol 12, 137–150 (2013).
121. R.J.H. Cloots, H.M.T. Gervaise, J.A.W. Van Dommelen, M.G.D. Geers, Biomechanics of traumatic brain injury: influences of the morphologic heterogeneities of the cerebral cortex. Ann Biomed Eng 36, 1203 (2008).
122. C. Giordano, R.J.H. Cloots, J.A.W. Van Dommelen, S. Kleiven, The influence of anisotropy on brain injury prediction. J Biomech 47, 1052–1059 (2014).
123. R.J.H. Cloots, J.A.W. Van Dommelen, M.G.D. Geers, A tissue-level anisotropic criterion for brain injury based on microstructural axonal deformation. J Mech Behav Biomed Mater 5, 41–52 (2012).
124. Bell GI. Models for the specific adhesion of cells to cells. Science (80-) 1978;200:618–27.
125. P.F. Lenne, A.J. Rae, S.M. Altman, M. Saraste, J.K.H. Hörber, States and transitions during forced unfolding of a single spectrin repeat. FEBS Lett 476, 124–128 (2000).
126. M. Rief, J. Pascual, M. Saraste, H.E. Gaub, Single molecule force spectroscopy of spectrin repeats: low unfolding forces in helix bundles. J Mol Biol 286, 533–561 (1999).
127. A. Viel, K. Actinin and spectrin structures: an unfolding family story. FEBS Lett 460, 394 (1999).
128. K.A. Scott, S. Batey, K.A. Hooton, J. Clarke, The folding of spectrin domains I: wild-type domains have the same stability but very different kinetic properties. J Mol Biol 344, 195–205 (2004).
129. Zhu Q, Asaro RJ. Spectrin Folding versus Unfolding Reactions and RBC Membrane Stiffness. Biophys J 2008;94:2529–45. https://doi.org/10.1529/biophysj.107.119438.
130. S. Paramore, G.A. Voth, Examining the influence of linkers and tertiary structure in the forced unfolding of multiple-repeat spectrin molecules. Biophys J 91, 3436–3445 (2006).
131. Paramore S, Ayton GS, Voth GA. Extending a spectrin repeat unit. II: rupture behavior. Biophys J 2006;90:101–11.
132. S.M. Altman, R.G. Grünberg, P.F. Lenne, J. Yläne, A. Rae, K. Herbert et al., Pathways and intermediates in forced unfolding of spectrin repeats. Structure 10, 1085–1096 (2002).
133. V. Ortiz, S.O. Nielsen, M.L. Klein, D.E. Discher, Unfolding a linker between helical repeats. J Mol Biol 349, 638–647 (2005).
134. Lai L, Cao J. Spectrins in axonal cytoskeletons: dynamics revealed by extensions and fluctuations. J Chem Phys 2014;141:07B601_1.
135. M.L. Sandvold, A. Mikkelsen, A. Elgsaeter, Frequency dependence of the shear moduli of spectrin studied using a multiple
lumped resonator viscoelastometer. Acta Chem Scand 43, 783–786 (1989)

136. Stokke BT, Mikkelsen A, Elgsaeter A. Some viscoelastic properties of human erythrocyte spectrin networks end-linked in vitro. Biochim Biophys Acta (BBA)-Biomembranes 1985;816:111–21.

137. B.T. Stokke, A. Mikkelsen, A. Elgsaeter. Spectrin, human erythrocyte shapes, and mechaenochemical properties. Biophysics J 49, 319–327 (1986)

138. S. Svetina, G. Kokot, T.Ś. Kebe, B. Žekš, R.E. Waugh, A novel strain energy relationship for red blood cell membrane skeleton based on spectrin stiffness and its application to micropipette deformation. Biomach Model Mechanobiol 15, 745–758 (2016)

139. Huang CY-M, Zhang C, Ho TS-Y, Oses-Prieto J, Burlingame AL, Lalonde I, et al. ß1 spectrin forms a periodic cytoskeleton at the axon initial segment and is required for nervous system function. J Neurosci 2017;37:11311–22.

140. F.M. Barabas, L.A. Masullo, M.D. Bordenave, S.A. Giusti, N. Unsain, D. Refojo et al., Automated quantification of protein periodic nanostructures in fluorescence nanoscopy images: abundance and regularity of neuronal spectrin membrane-associated skeleton. Sci Rep 7, 1–10 (2017)

141. B. Han, R. Zhou, C. Xia, X. Zhuang. Structural organization of the actin-spectrin-based membrane skeleton and soma of neurons. Proc Natl Acad Sci 114, E6678–E6685 (2017)

142. M. Koskinen, P. Hotulainen, Measuring F-actin properties in dendritic spines. Front Neurounaut 8, 74 (2014)

143. S.C. Sidenstein, E. D’Este, M.J. Böhm, J.G. Danzl, V.N. Belov, S.W. Hell. Multicolour Multilevel STED nanoscopy of Actin/ Spectrin Organization at Synapses. Sci Rep 6, 26723 (2016). https://doi.org/10.1038/srep26725

144. N. Unsain, F.D. Stefani, A. Cáceres, The actin/spectrin membrane-associated periodic skeleton in neurons. Front Synaptic Neurosci 10, 10 (2018)

145. N. Unsain, M.D. Bordenave, G.F. Martinez, S. Jalil, C. Von Bilderling, F.M. Barabas et al., Remodeling of the actin/spectrin membrane-associated periodic skeleton, growth cone collapse and F-actin decrease during axonal degeneration. Sci Rep 8, 1–16 (2018)

146. G. Zhong, J. He, R. Zhou, D. Lorenzo, H.P. Babcock, V. Bennett et al., Developmental mechanism of the periodic membrane skeleton in axons. Elife 3, e04581 (2014). https://doi.org/10.7554/ eLife.04581

147. J. He, R. Zhou, Z. Wu, M.A. Carrasco, P.T. Kurshan, J.E. Farley et al., Prevalent presence of periodic actin–spectrin–based membrane skeleton in a broad range of neuronal cell types and animal species. Proc Natl Acad Sci 113, 6029–6034 (2016)

148. S. Roy, Waves, rings, and trails: The scenic landscape of axonal actin. J Cell Biol 212, 131 (2016)

149. Costa AR, Sousa SC, Pinto-Costa R, Mateus JC, Lopes CDF, Costa AC, et al. The membrane periodic skeleton is an actomyosin network that regulates axonal diameter and conduction. Elife 2020;9:e55471.

150. A. Fan, A. Tofangchi, M. Kandel, G. Popescu, T. Saif, Coupled circumferential and axial tension driven by actin and myosin influences in vivo axon diameter. Sci Rep 7, 1–12 (2017)

151. S.L. Berger, A. Leo-Macias, S. Yuen, L. Khatri, S. Pfennig, Y. Zhang et al., Localized myosin II activity regulates assembly and plasticity of the axon initial segment. Neuron 97, 555–570 (2018)

152. G. Gallo, Myosin II activity is required for severing-induced axon retraction in vitro. Exp Neurol 189, 112–121 (2004)

153. Abouelezz A. The Structure and Dynamics of the Actin Cytoskeleton in the Axon Initial Segment. Diss Sch Dr Ad San Investig Univ Hels 2020.

154. J. Bär, O. Kobler, B. Van Bommel, M. Mikhaylova, Periodic F-actin structures shape the neck of dendritic spines. Sci Rep 6, 1–9 (2016)

155. E. Schanus, S. Booth, B. Hallaway, A. Rosenberg, The elasticity of spectrin-actin gels at high protein concentration. J Biol Chem 260, 3724–3730 (1985)

156. A.R. Costa, R. Pinto-Costa, S.C. Sousa, M.M. Sousa, The regulation of axon diameter: from axonal circumferential contractility to activity-dependent axon swelling. Front Mol Neurosci 11, 319 (2018)

157. S.C. Leite, M.M. Sousa, The neuronal and actin commitment: Why do neurons need rings? Cytoskeleton 73, 424–434 (2016)

158. Y. Qu, I. Hahn, S.E.D. Webb, S.P. Pearce, A. Prokop, Periodic actin structures in neuronal axons are required to maintain microtubules. Mol Biol Cell 28, 296–308 (2017)

159. C. Letertier. Putting the axonal periodic scaffold in order. Curr Opin Neurobiol 69, 33–40 (2021)

160. Zhou R, Han B, Xia C, Zhuang X. Membrane-associated periodic skeleton is a signaling platform for RTK transactivation in neurons. Science (80- ) 2019;365:929–34.

161. I. Koshino, N. Mohandas, Y. Takakuwa, Identification of a novel role for dematin in regulating red cell membrane function by modulating spectrin-actin interaction. J Biol Chem 287, 35244–35252 (2012)

162. Wang G, Simon DJ, Wu Z, Belsky DM, Heller E, O’Rourke MK, et al. Structural plasticity of actin-spectrin membrane skeleton and functional role of actin and spectrin in axon degeneration. Elife 2019;8:e38730.

163. C.H. Coles, F. Bradke, Coordinating neuronal actin–microtubule dynamics. Curr Biol 25, R677–R691 (2015)

164. Dubey S, Bhemre N, Bodas S, Veer S, Ghose A, Callan-Jones A, et al. The axonal actin-spectrin lattice acts as a tension buffering shock absorber. Elife 2020;9:e51772.

165. Li H, Lykotrafitis G. Two-Component Coarse-Grained Molecular-Dynamics Model for the Human Erythrocyte Membrane. Biophys. J. 2012;102:75–84. https://doi.org/10.1016/j.bpj.2011.11.4012.

166. Zhang Y, Abiraman K, Li H, Pierce DM, Tzingounis A V, Lykotrafitis G. Modeling of the axon membrane skeleton structure and implications for its mechanical properties. PLoS Comput. Biol. 2017;13:e1005407.

167. K.A.H. Al Mahmud, F. Hasan, M.I. Khan, A. Adnan, On the Molecular Level Cavitation in Soft Gelatin Hydrogel. Sci. Rep. 10, 1–13 (2020)

168. Hasan F, Al Mahmud KAH, Khan MI, Patil S, Dennis BH, Adnan A. Cavitation Induced Damage in Soft Biomaterials. Multiscale Sci. Eng. 2021:1–21.

169. Hasan F, Mahmud K Al, Khan MI, Kang W, Adnan A. Effect of Random Fiber Network and Fracture Toughness on the Onset of Cavitation in Soft Materials. ArXiv Prepr ArXiv201213446 2020.

170. Khan MI, Hasan F, Hasaan Al Mahmud KA, Adnan A. Domain focused and residue focused phosphorylation effect on tau protein: A molecular dynamics simulation study. J. Mech. Behav. Biomed. Mater. 2020:104149. https://doi.org/10.1016/j.jmbbm.2020.104149.

171. Khan MI, Gilpin K, Hasan F, Mahmud KAH Al, Adnan A. Effect of strain rate on single tau, dimerized tau and tau-microtubule interface: a molecular dynamics simulation study. J. Mech. Behav. Biomed. Mater. Rev. 2021.

172. Khan MI, Hasan F, Mahmud KAH Al, Adnan A. Viscoelastic response of neurofilaments: an atomistic simulation approach. Biomolecules. 2021:11:540. https://doi.org/10.3390/biom11040540.

173. A. Adnan, S. Qidwai, A. Bagchi, On the atomistic-based continuum viscoelastic constitutive relations for axonal microtubules. J. Mech. Behav. Biomed. Mater. 86, 375–389 (2018)

174. L.H. Teliska, M.N. Rasband, Spectrins. Curr. Biol. 31, R504–R506 (2021)
175. Gardel ML, Shin JH, MacKintosh FC, Mahadevan L, Matsu-
daira P, Weitz DA. Elastic Behavior of Cross-Linked and Bun-
dled Actin Networks. Science. (80-) 2004;304:1301 LP – 1305. 
https://doi.org/10.1126/science.1095087.

176. B. Isralewitz, M. Gao, K. Schulten, Steered molecular dynamics 
and mechanical functions of proteins. Curr. Opin. Struct. Biol. 
11, 224–230 (2001)

177. T. Aoyagi, F. Sawa, T. Shoji, H. Fukunaga, J. Takimoto, M. Doi, 
A general-purpose coarse-grained molecular dynamics program. 
Comput. Phys. Commun. 145, 267–279 (2002)

178. P.J. Bond, J. Holyoake, A. Ivetac, S. Khalid, M.S.P. Sansom, 
Coarse-grained molecular dynamics simulations of membrane 
proteins and peptides. J. Struct. Biol. 157, 593–605 (2007)

179. Vogt B. Atomistic-based continuum constitutive relation for 
microtubules: elastic modulus prediction 2008.

180. Cloots RJH, Van Dommelen JAW, Kleiven S, Geers MGD. 
Traumatic brain injury at multiple length scales: relating dif-
fuse axonal injury to discrete axonal impairment. Proc. IRCOBI 
Conf., 2010, p. 119–30.

Publisher’s Note Springer Nature remains neutral with regard to 
jurisdictional claims in published maps and institutional affiliations.