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Rate-dependent response of axonal microtubules and tau proteins under shear forces

Luca Bellino[1, a*], Giuseppe Florio[1,3,b], Alain Goriely[2,c] and Giuseppe Puglisi[1,d]

[1]Dipartimento di Ingegneria Civile, Ambientale, del Territorio, Edile e di Chimica (Dicatech), Politecnico di Bari, Bari, Italy

[2]Mathematical Institute, University of Oxford, OX2 6GG, UK

[3]INFN, Sezione di Bari, I-70126 Bari, Italy

[a*] luca.bellino@poliba.it, [b] giuseppe.florio@poliba.it, [c] goriely@maths.ox.ac.uk, [d] giuseppe.puglisi@poliba.it

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Abstract. The brain tissue is a very complex biological material exhibiting viscoelastic-type properties at the macroscopic level, arising from a hierarchical multiscale structure. Thus, to describe such interesting features at the molecular level, we introduce a model mimicking the coupling of microtubules and tau proteins inside the neuronal axon and we study the rate-dependent response under different conditions of applied load, rate, and temperature.

Introduction

One of the most complex biological systems is the brain tissue, which is made of billions of neurons, the fundamental component of nervous tissue, responsible for transmitting electrochemical signals. It has been addressed that mechanical fields have crucial importance for the correct functioning of these cells [1]. Specifically, it has been found that external forces, impacts or traumatic accidents acting on the brain can generate damage causing severe consequences at the level of the axons, also years after the event, a class of pathologies known as Traumatic Brain Injury (TBI) [2]. This phenomenon has first been observed in U.S.A. boxers because a great percentage of them suffered Alzheimer's or Parkinson's diseases even years after their retirement [3]. In these patients, there is evidence that the damage acting on the brain can cause scattered lesions at the level of the axons, a pathology known as Diffuse Axonal Injury (DAI) [4]. These lesions are responsible for the axonal failure, a phenomenon that can happen abruptly (primary axotomy) or with a progressive degradation of the internal components of the axons (secondary axotomy) [5], finally resulting in the malfunction of the whole tissue. As a matter of fact, these multiscale features are due to the damage mechanisms and to the structure of the complex energy landscape at the microscale. As a consequence, the comprehension of these phenomena requires to understand the behaviour of the material at this length scale.

In this paper, we study the effect of forces and loading rates at the level of the axons, considering two main components of the axonal cytoskeleton *i.e.,* the microtubules (MT) and the tau proteins [6]. MTs are arranged in bundles and are kept together by the crosslinking tau proteins. They do not cover the whole length of the axon and their length may vary in the range from 2 to 100 μm. There can be found almost 20 MTs per section radially spaced at about 23 – 38 nm, with a Young Modulus of about 1.9 GPa [7]. On the other hand, the main function of the tau proteins is to stabilize the microtubules and maintain the correct arrangement along the axonal length [8]. It is important to stress that another clinical evidence is that agglomerates of tau proteins are found inside the brain's white matter of patients affected by neurodegenerative diseases. This observation suggests the possibility that damages caused by mechanical fields may break these proteins depending mainly on the force and the loading rate. Indeed, three main regimes can be identified.

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When the strain and the strain rate are low, the microtubules may slide through each other, and the tau proteins can detach and reattach in other vacant sites on the same MT or on adjacent ones. If the strain increases, the crosslink tau proteins start to break and are no longer able to reattach. When also the strain rate is large, there exists the possibility that the MTs break, causing malfunction and irreversible damage [9,10]. From a theoretical point of view, the challenge of describing such small systems considering both mechanical and thermal fields at varying rates is widely tackled using discrete or continuous models and numerical methods such as Molecular Dynamic (MD) simulations [11]. To mention some examples, it is possible to approach the direct modelling of the inertial dynamics [12,13] or to describe the evolution of the system using kinetic theories [14,15]. On the other hand, the possibility of investigating the microscopic characterization of the complex energy landscape of such systems obtaining analytical results is still to be thoroughly investigated, possibly considering phenomena such as hysteresis, damage, and residual stresses. In this spirit, we refer to recent papers where the study of different biological problems has been approached by analytical methods, such as the unfolding and misfolding of proteins materials [16-18], the effect of nucleation and propagation stresses in memory shape materials taking into account also thermal fields [19,20], focal adhesion [21,22], spider silks and new bio-inspired materials [23] or the mechanics of double-stranded polypeptide chains [24].

Specifically, following [24], we propose a micromechanical-based model describing the response of the microtubules and tau proteins to different loading rates (including temperature effects) when a shear force is applied to the system, as shown in Fig. 1. The analysis of rate effects can be performed by using different approaches such as the Fokker-Planck formulation [25], numerical FEM simulation [26] or Langevin dynamics [27]. To highlight the role of energy barriers, we will tackle the problem by adopting a Bell-type theory [28] based on Kramer's rate equation [29,30], as widely developed in recent works [24,31]. In particular, based on the possibility of evaluating the energy barriers separating the metastable states, we obtain the rate-dependent response of a system composed of two MTs linked by tau proteins and study the mechanical response of such a system under shear forces for different loading rates and temperatures.

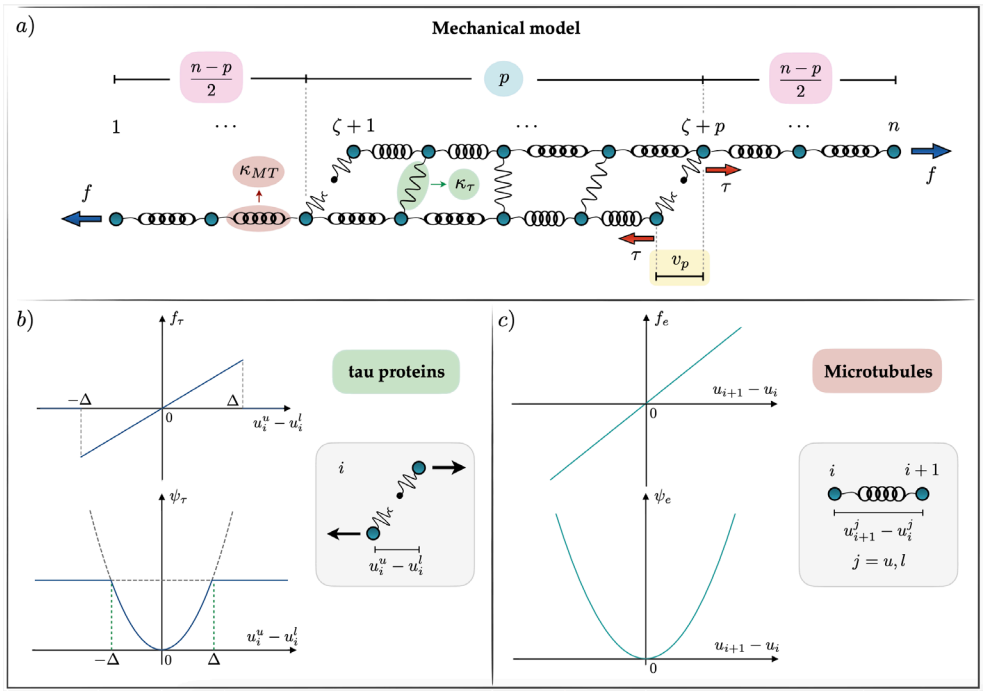

Fig. 1: Panel a): Mechanical model of the system describing microtubules and tau proteins. The energies used for the two microscopic elements are displayed in panel b) for the tau protein and in panel c) for the microtubule.

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Mathematical framework

Following the theoretical model developed in [24], we consider a bundle of two elastic chains representing the microtubules connected by n breakable units spaced by a length ℓ, describing the tau proteins. The total length of the system is $L = n\ell$. The upper and the lower chains are described by elastic springs, as shown in Fig. 1c). On the other hand, the behaviour of each breakable unit is described by introducing a discrete variable χ_i (a "spin" variable) such that the condition $\chi_i = 1$ ($\chi_i = 0$) corresponds to an attached (detached) configuration. Thus, the quantity $p = \sum_{i=1}^{n} \chi_i$ represents the number of attached proteins (see Fig. 1b). By considering the rescaled end-points displacement $\delta = \frac{u_n^u}{\Delta} = -\frac{u_1^l}{\Delta}$ (Δ representing the breaking threshold displacement of the tau proteins, see Fig. 1b) and introducing the total stiffness of the system κ^t, the free energy and the force-displacement relation at the equilibrium read (see Ref. [24] for a detailed calculation)

$$g_{eq}(f) = -\frac{f^2}{\kappa^t} + \mu^2\left(1 - \frac{p}{n}\right), \qquad \delta(f) = \frac{f}{\kappa^t}, \tag{1}$$

where

$$\kappa^t = \frac{4n}{2n-p-1+4\gamma(p)}, \quad \gamma(p) = \frac{\sinh(\lambda)+\sinh(p\lambda)}{2\sinh[(p+1)\lambda]-\sinh(\lambda)-\sinh(p\lambda)}, \quad \lambda = \cosh^{-1}\left(1 + \frac{2\mu^2}{n^2}\right). \tag{2}$$

In the previous formula, we introduced the main non-dimensional parameter of the system

$$\mu^2 = \frac{\kappa_\tau}{2\kappa_{MT}}\frac{L^2}{\Delta^2}, \tag{3}$$

where κ_τ and κ_{MT} are the stiffnesses of the tau proteins and the of MTs, respectively,

Energy Barriers

In our approach, the loading rate affects the probability of jumping from a metastable equilibrium solution to another, and it depends on the height of the energy barriers. The ability of overcoming these barriers depends, among other effects, on the mechanical and thermal fields. This approach allows the possibility of exploring different locally stable solutions with partially detached elements. To evaluate $g_b(f)$, *i.e.* the height of the barrier for a certain value of the applied shear force f, we initially consider the elements of the system in equilibrium and we study the effect of changing the position of the last attached element p from its equilibrium value to the breaking threshold, by imposing a force τ (see Fig. 1a). The resulting mechanical quantities obtained by studying this variational problem represent the equilibrium conditions of the system when the energy is maximized (the analogous of a saddle point when smooth non-convex energies are considered [31,32]). Thus, we can evaluate the height of the barriers as

$$g_b(f) = g_\tau(f) - g_{eq}(f), \tag{4}$$

where $g_\tau(f)$ is the maximized energy and $g_{eq}(f)$ is given in Eq.(1) (see Ref. [31] for detailed analysis and calculation).

Rate effects

Following [29,32], we consider the time-dependent force $f(t)$. The loading rate reads

$$v(t) = v_0 e^{-\beta g_b(f(t))}, \tag{5}$$

where the energy barrier is given by Eq. (4), v_0 is a constitutive parameter and $\beta = 1/(K_B T)$, with T the absolute temperature and K_B the Boltzmann constant. Following [32], we define the probability $P_p(t)$ that the system exhibits p attached units a time t. The evolution equation for this probability is a first-order differential equation of the form

$$\frac{d\,P_p(t)}{d\,t} = -v_{p\to p-1}(t)P_p(t) + v_{p+1\to p}(t)P_{p+1}(t). \tag{6}$$

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By Eq. (6) we can obtain the value of $P_p(t)$ at time t. In particular, it depends on the process where the configuration of the system can pass from the state with p attached elements to that with $p-1$ (with rate $\upsilon_{p\to p-1}(t)$) and the process where the system goes from a configuration with $p+1$ attached elements to p (with rate $\upsilon_{p+1\to p}(t)$). Two configurations must be considered separately *i.e.,* $\upsilon_{n+1\to n}(t)=0$ and $\upsilon_{0\to -1}(t)=0$. Starting the analysis at time $t=0$, the probability of being in the fully attached configuration ($p=n$) is $P_n(t)=1$. Following the action of the applied force, the tau proteins start to detach and the probability of finding the system in the initial state decreases while the probability of being in the configuration with $p=n-1$ increases. This process goes on until the system completely breaks (corresponding to the configuration with $p=0$).
Eventually, the total displacement is

$$\langle\delta(t)\rangle=\sum_{p=0}^{n}P_p(t)\delta(p), \tag{7}$$

with $\delta(p,t)$ given by Eq. (1).

Results and discussion

To approach the problem of the rate-dependent response of the microtubule bundles into the neuronal axon, we introduced a microscale-based model describing two MTs connected by crosslinking tau proteins. In Fig. 2a we show the effect of the loading rate υ_f (with the applied shear force being $f=\upsilon_f t$). It is possible to observe that as the rate grows the force breaking thresholds increase and the system is not able to explore metastable configuration, being confined in the solution $p=n$. On the other hand, for low rates, solutions with partially detached elements are found before rupture, which occurs at smaller values of the force. Thus, at increasing rates, a "Maxwell" behaviour is preferred, with the overall collapse of the system attained without exploring other solutions whereas for decreasing rates the sequential detachment of the tau proteins is observed, as described in many papers [10,33,34]. In Fig. 2b we show the effect of changing temperatures, affecting the possibility of overcoming the energy barriers. As the temperature increases, the system exhibits a ductile type of fracture attained at low forces and explores different metastable solutions. Conversely, when the zero-temperature limit is approached, *i.e.* $\beta\to\infty$, the system is 'frozen' in the configuration with all the elements attached, following the "Maxwell" path.

Eventually, we obtained the mechanical response of a simple bundle composed of two MTs and crosslinking tau proteins and we observed the effect of the loading rate and the temperature. Through our model, we obtained results previously described in the literature, where other methods such as numerical FEM simulations, Molecular Dynamics or continuum models have been used to approach the analysis of similar systems [8-11,33,34].

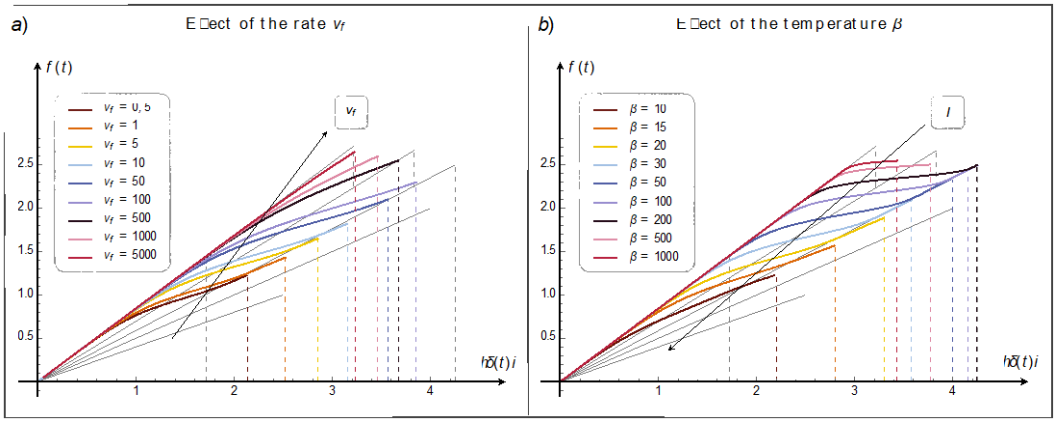

Fig. 2: Panel a) Effect of the loading rate. Panel b) Effect of the temperature.

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References

[1] S. B. Smith, D. F. Meaney, Axonal damage in traumatic brain injury, The neuroscientist, 6, 6, 483 – 495, (2000). https://doi.org/10.1177/107385840000600611
[2] M.D. Tang-Schomer, A. R. Patel, P. W. Baas, D. H. Smith, Mechanical breaking of microtubules in axons during stretch injury underlies delayed elasticity, microtubule disassembly and axon degeneration, The FASEB Journal, 24, 5, 1401 – 1410, (2010). https://doi.org/10.1096/fj.09-142844
[3] C. Ballatore, V. M. Y. Lee, J. Q. Trojanowski, Tau-mediated neurodegeneration in Alzheimer's disease and related disorders, Nature rev. neuroscience, 8, 9, 663 – 672, (2007). https://doi.org/10.1038/nrn2194
[4] Ya. Shi, W. Zhang, Y. Yang, A. G. Murzin, B. Falcon, A. Kotecha, M. van Beers, A. Tarutani, F. Kametani, H. J. Garringer et al, Structure-based classification of tauopathies, Nature, 598, 7880, 359 – 363, (2021). https://doi.org/10.1038/s41586-021-03911-7
[5] T. Hawkins, M. Mirigian, M. S. Yasar, J. L. Ross, Mechanics of microtubules, Journal of Biomechanics, 43, 1, 23 – 30, (2010). https://doi.org/10.1016/j.jbiomech.2009.09.005
[6] H. Ouyang, E. Neuman, R. Shi, Contribution of cytoskeletal elements to the axonal mechanical properties, Journal of biological engineering, 7, 1, 1 – 8, (2013). https://doi.org/10.1186/1754-1611-7-21
[7] D. S. Eisenberg, M. R. Sawaya, Taming tangled tau, Nature, 547, 7662, 170–171, (2017). https://doi.org/10.1038/nature23094
[8] K. J. J. Rosenberg, 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, PNAS, 105, 21, 7445 – 7450, (2008). https://doi.org/10.1073/pnas.0802036105
[9] R. De Rooij, K. E. Miller, E. Kuhl, Modeling molecular mechanism in the axon, Computational mechanics, 59, 3, 523 – 537, (2017). https://doi.org/10.1007/s00466-016-1359-y
[10] H. Van den Bedem, E. Kuhl, Tau-ism: the Yin and Yang of microtubules sliding, detachment and rupture, Biophysical Journal, 109, 11, 2215, (2015). https://doi.org/10.1016/j.bpj.2015.10.020
[11] E. Evans, K. Ritchie, Dynamic strength of molecular adhesion bonds, Biophysical Journal, 72, 4, 1541 – 1555, (1997). https://doi.org/10.1016/S0006-3495(97)78802-7
[12] T. Cohen, S. Givli, Dynamics of a discrete chain of bi-stable elements: a biomimetic shock absorbing mechanism, JMPS, 64, 426 – 439, (2014). https://doi.org/10.1016/j.jmps.2013.12.010
[13] Y. R. Efendiev, L. Truskinovsky, Thermalization of a driven bi-stable FPU chain, Continuum mechanics and thermodynamics, 22, 6, 679 – 698, (2010). https://doi.org/10.1007/s00161-010-0166-5
[14] S. Givli, K. Bhattacharya, A coarse-grained model of the myofibril: overall dynamics and the evolution of sarcomere non-uniformities, JMPS, 57, 2, 221 – 243, (2009). https://doi.org/10.1016/j.jmps.2008.10.013
[15] R. Raj, P. K. Purohit, Phase boundaries as agents of structural change in macromolecules, JMPS, 59, 10, 2044 – 2069, (2011). https://doi.org/10.1016/j.jmps.2011.07.003

[16] L. Bellino, G. Florio, G. Puglisi, The influence of device handles in single molecule experiments, Soft Matter, 15, 43, 8680 – 8690, (2019). https://doi.org/10.1039/C9SM01376H
[17] M. Benedito, S. Giordano, Isotensional and isometric force-extension response of chains with bistable units, PRE, 98, 5, 052146, (2018). https://doi.org/10.1103/PhysRevE.98.052146
[18] I. Benichou, S. Givli, the hidden ingenuity of titin structure, Applied Physics Letters, 99, 091904, (2011). https://doi.org/10.1063/1.3558901
[19] L. Bellino, G. Florio, S. Giordano, G. Puglisi, On the competition between interface energy and temperature in phase transition phenomena, Application of Engineering Science, 2, 100009, (2020). https://doi.org/10.1016/j.apples.2020.100009
[20] A. Cannizzo, L. Bellino, G. Florio, G. Puglisi, S. Giordano, Thermal control of nucleation and propagation transition stresses in discrete lattices with non-local interactions and non-convex energy, EPJ Plus, 137, 569, (2022). https://doi.org/10.1140/epjp/s13360-022-02790-9
[21] A. Cannizzo, G. Florio, G. Puglisi, S. Giordano, Temperature controlled decohesion regimes of an elastic chain adhering to a fixed substrate by softening and breakable bonds, Journal of Physics A, 54, 445001, (2021). https://doi.org/10.1088/1751-8121/ac2a07
[22] S. Di Stefano, G. Florio, G. Napoli, N. M. Pugno, G. Puglisi, On the role of elasticity in focal adhesion within the passive regime, International Journal of Nonlinear Mechanics, accepted, (2022). https://doi.org/10.1016/j.ijnonlinmec.2022.104157
[23] V. Fazio, D. De Tommasi, N. M. Pugno, G. Puglisi, Spider Silks Mechanics: Predicting Humidity and Temperature Effects. JMPS, in press, (2022). https://doi.org/10.1016/j.jmps.2022.104857
[24] L. Bellino, G. Florio, A. Goriely, G. Puglisi, Role of interlinker stiffness in melting and unfolding cooperativity and toughness of double stranded peptide chains, Preprint, (2022).
[25] H. Herrmann, U. Aebi, Intermediate filaments: molecular structure, assembly mechanism, and integration into functionally distinct intracellular scaffolds, Annual review of biochemistry, 73, 1, 749 – 789, (2012). https://doi.org/10.1146/annurev.biochem.73.011303.073823
[26] R. De Rooji, K. E. Miller, E. Kuhl, Modeling molecular mechanism in the axon, computational mechanics, 59, 3, 523 – 537, (2017). https://doi.org/10.1007/s00466-016-1359-y
[27] H. B. Da Rocha, L. Truskinovsky, Rigidity-controlled crossover, from spinodal to critical failure, PRL, 124, 1, 015501, (2020). https://doi.org/10.1103/PhysRevLett.124.015501
[28] G. I. Bell, Models for the specific adhesion of cells to cells, Science, 200, 4342, 618 – 627, (1978). https://doi.org/10.1126/science.347575
[29] P. Hanggi, P. Talkner, M. Borkovec, Reaction-rate theory: fifty years after Kramers, Reviews of modern physics, 62, 2, 251, (1990). https://doi.org/10.1103/RevModPhys.62.251
[30] H. A. Kramers, Brownian motion in a field of force and the diffusion model of chemical reactions, Physica, 7, 4, 284 – 304, (1940). https://doi.org/10.1016/S0031-8914(40)90098-2
[31] L. Bellino, G. Florio, A. Goriely, G. Puglisi, Effects of rate and temperature on the mechanical response of microtubules and tau proteins, Preprint, (2022).
[32] I. Benichou, Y. Zhang, O. K. Dudko, S. Givli, The rate dependent response of a bistable chain at finite temperature, JMPS, 95, 44 – 63, (2016). https://doi.org/10.1016/j.jmps.2016.05.001
[33] H. Ahmadzadeh, D. H. Smith, V. B. Shenoy, Mechanical effects of dynamic binding between tau proteins on microtubules during axonal injury, Biophysical Journal, 106, 5, 1123 – 1133, (2015). https://doi.org/10.1016/j.bpj.2014.01.024
[34] R. De Rooij, E. Kuhl, Physical biology of axonal damage, Frontiers in cellular neuroscience, 12, 144, (2018). https://doi.org/10.3389/fncel.2018.00144