Molecular dynamics analysis of axonal membrane during traumatic brain injury

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ABSTRACT

Diffuse axon injury (DAI) accounts for approximately half of traumatic brain injuries, yet its underlying mechanism remains unclear. Different theories exist about the mechanism of DAI, among which, one hypothesis states that membrane poration of the axons initiates DAI. To investigate the hypothesis, molecular models of axonal membranes, incorporating 25 different lipids distributed asymmetrically in the leaflets, were developed using molecular dynamics simulations and a coarse-grain description. Employing a bottom-top approach, these models were coupled with a finite element model representing the axon at the cell level. Different concentrations of proteins were embedded inside the model to represent the node-of-Ranvier and unmyelinated axonal membrane. The bilayers were investigated in equilibration and under deformation to characterize the structural and mechanical properties of the membranes and comparisons were made with other subcellular parts, particularly myelin. Results indicate that pore formation in the node-of-Ranvier occurs at a lower rupture strain compared to other axolemma models, whereas myelin poration exhibits the highest rupture strains among the investigated models. The observed rupture strain for the node-of-Ranvier aligns with experimental studies, indicating a threshold for injury at axonal strains exceeding 10-13% depending on the strain rate. This study enhances our understanding of DAI at a multi-scale level by revealing the mechanical properties of axonal membranes under both biological conditions and during injury.

Introduction

Traumatic brain injury (TBI) is the brain pathology caused by an external force applied to the brain in traffic or sport accidents, falls or violence[1]. Diffuse axonal injury (DAI) is a common type of TBI that affects the brain in cellular and sub-cellular levels. Mild to low-moderate DAI cannot often be diagnosed by conventional imaging techniques, though these injuries cause microscopic pathology in the brain tissue that can disrupt normal brain function[2]. Therefore, to gain more insights on the mechanism of this type of injury, it is important to use alternative techniques for investigating it in cellular and sub-cellular levels.

Swollen and disconnected axons are indicative of DAI at the cellular level[3,4]. Axons are elongated projections of nerve cells. They can be myelinated or un-myelinated. Myelinated axons are wrapped by a multi-layer sheath called myelin. Myelins do not stretch along the whole axon and gaps between myelin sheaths are called node-of-Ranvier. Figure 1 provides a schematic representation of a neural cell together with a sub-cellular representation of axonal membranes.

Experimental studies showed that lipid composition of membranes of different cells and sub-cellular elements varies a lot and affects the function and properties of the cells (Table 1)[5]. Various studies have investigated how lipid content and cholesterol concentration affect the mechanical and structural properties of lipid bilayers[6–12]. These studies have highlighted the significant impact of molecular composition on membrane properties, like for example area compressibility[6–9,11], but also the effect on processes like mechanoporation[9–12], interdigitation[8,10], and bilayer thinning[7,12] in non-equilibrium states. The high dependence of bilayer properties on lipid composition emphasizes the necessity for an accurate and specific molecular model that captures the unique composition of the axolemma. Such a model would enable a more comprehensive investigation of the axolemma's properties and behavior under deformation.

Axonal membrane or axolemma contains many membrane proteins, acting as ion channels and transporters, which play a vital role in the physiological functioning of cells. The concentration of these proteins is not uniform along the axon. This has been shown to affect how the axonal membrane reacts during an impact[13,14].

As shown in Fig. 1, the node-of-Ranvier exhibits a higher concentration of ion channel proteins in the membrane, while unmyelinated axons have on average a lower ion-channel concentration. Among the proteins that exist in the axolemma, we find voltage-gated sodium channels as one of the most abundant ones in the brain and axonal cells. Among this class of protein, Nav1.1 proteins are particularly prevalent in axon initial segments and the node-of-Ranvier[15,16].

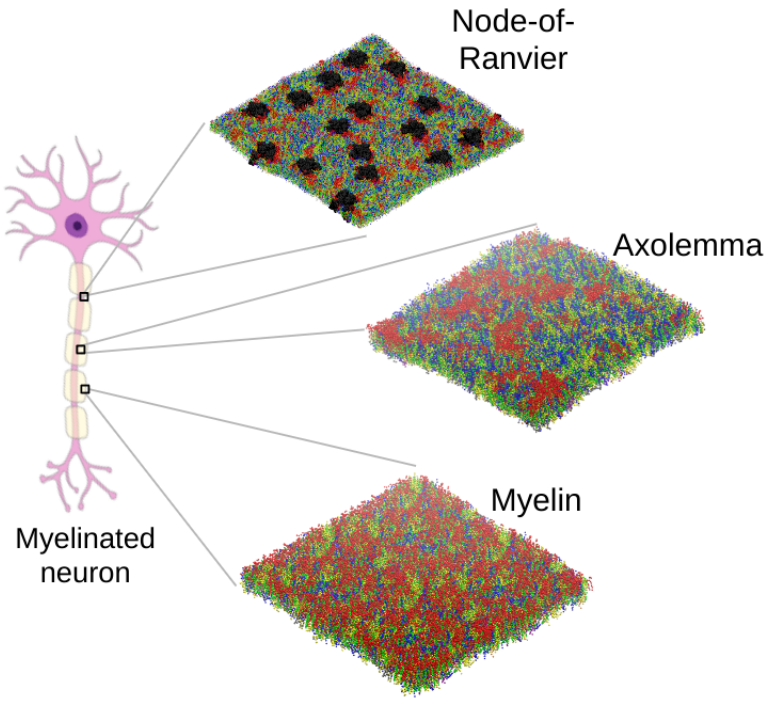


Figure 1. A schematic view of a myelinated neuron together with snapshot of the simulated bilayers. The arrow connect the bilayers to the corresponding element in the neural cell. Axolemma and the node-of-Ranvier have the same lipid composition. Lipids are illustrated as follows: PC lipids are in blue, PE in yellow, SM in orange, PS in purple, glycolipids in red and CHOL in green. The proteins are represented in black. Water and ions are not shown.

Investigations of injuries at the cellular level usually employ techniques such as atomic force microscopy[17], micropipette aspiration[18,19], laser-induced cavitation[20,21], transient pressurised fluid deflection[22], cell culture deformation[23], and computational modelings[24,25]. However, capturing sub-cellular mechanisms of injuries and data on local strains and stresses from experiments is challenging due to their time and length scales that are in order of nanoseconds and nanometers. Alternatively, to get more detailed data modeling techniques at different levels like finite element (FE) modeling and molecular dynamics (MD) simulations can be used to study brain injuries and elucidating their mechanisms in sub-cellular and molecular levels[26,27].

Studies investigating the axonal injury in sub-cellular level have lead to multiple hypotheses about the initial trigger of injury. One hypothesis regarding the primary mechanism of axonal injury is mechanoporation, which involves the formation of pores in the axonal membrane[10,28–32]. Another proposed hypothesis is cytoskeletal damage of the axon[17,33]. However, computational studies utilizing FE models of the axon, incorporating major cytoskeletal components and the axonal membrane, have rejected the cytoskeletal damage hypothesis[30]. These FE studies have demonstrated high levels of strain primarily on the axonal membrane rather than the microtubules, suggesting that the membrane is more susceptible to injury than the cytoskeletal elements.

On molecular level, different models are developed in-situ to investigate the properties of membranes from different brain cells[9–11,34]. For example, Ingolfsson et al. provided a generalized membrane model for the brain[34] and Saeedimasine et al. developed a model to describe the myelin sheath[11].

Here, we aim to gain a better understanding of whether the mechanoporation of axonal membranes can trigger axonal injury using molecular modelling. To achieve this, we first-of-all need to develop a model that accurately describe the axolemma composition. Then using molecular dynamics techniques, we can tackle which sub-cellular compartment of the axon undergoes poration first and how lipid composition and protein concentration affect mechanical properties of membranes. For this purpose, we have developed a coarse grained (CG) model of axolemma based on experimental data. To elucidate the effect of the protein in the membrane, we embed different concentrations of protein inside the bilayer. In total, we derived three models representing different parts of axon. The models were simulated in equilibrium and under deformation. Finally, to simulate an injury condition, we applied uniaxial deformation on the systems. According to the hypothesis, membrane poration was

considered as the time when axonal damage initiated. Therefore, in the last step, we combined the deformation results in molecular simulations with the equivalent deformation of axolemma in a FE model of an axon in cellular level under uniaxial tension[35].

Methods

Molecular model for axon

Axolemma is described as a bilayer consisting of different types of lipids distributed asymmetrically in the extracellular and cytoplasmic leaflet of the axon cells. We developed the model utilizing existing experimental data on the lipid composition of the axolemma, their distribution within the extracellular and cytoplasmic leaflets, and their saturation index[11,34,36,37]. Lipid composition and distribution between leaflets, saturation index, and tail length were determined using a combination of experimental data and information from established brain-type membrane models. While overall lipid composition of axolemma, fatty acid composition and unsaturation index of PC and PE were derived from experiments on human brain reported by DeVries et al.[36], neuron data reported by Fitzner et al. was used to derive chain length and saturation number in PS[37]. In case the experimental data on axolemma were missing, information on more general brain-type membrane models were used[11,34]. Namely, lipid disposition in the extracellular and cytoplasmic leaflet was derived from the brain membrane model developed by Ingólfsson et al.[34].

The cryo-EM structure of human Nav1.1, as presented by Pan et al.[38], available in the protein data bank (PDB ID: 7DTD), was utilized to describe the protein system. Three distinct models for the axon membrane were created with varying protein concentrations within the membrane to mimic the membrane in different sections of the axon. Experimentally the density of sodium channels in the axonal membrane differs across regions, ranging from 5 to 3000 $channels/\mu m^2$, with the highest concentration found in the node-of-Ranvier[39–41]. One model is without protein, called "Axolemma" model, one with only 1 embedded protein ("one-protein-axolemma") and one with 16 embedded proteins, with equivalent protein concentration of 2519 $channels/\mu m^2$, labelled as "node-of-Ranvier".

System setup

All the molecules were described at the coarse-grained (CG) level using Martini 2.2 force field[42]. The starting structure of the membrane without the protein was built using INSANE (INSert membrANE) bilayer builder[43] and Martinizer python code was employed to transform the protein's all-atom structure into a coarse-grained model[44].

A bilayer was first built using 1004 lipids and 820 cholesterol solvated by 52177 water beads, with 0.15 mol concentration of Na-Cl as ions in a box of size $20 \times 20 \times 20\ nm^3$. Then equilibration run was performed for the lipids to find their most suited neighboring configuration. The equilibrated system was then used to build a larger system $38 \times 38 \times 24\ nm^3$. This system was equilibrated under the same condition for 20 microseconds, before deformation.

In order to simulate the node-of-Ranvier, more concentration of proteins were embedded inside the membrane according the following procedure. First, one protein was embedded in an equilibrated axonal membrane model of size $20 \times 20 \times 20\ nm^3$ and the system was equilibrated again. Then, a larger system with a box size of $80 \times 80 \times 20\ nm^3$ containing 16 proteins was developed from a previuosly equilibrated model, representing a system with protein concentration of 2519 $channels/\mu m^2$.

Molecular dynamics simulation

All the simulations were performed in the GROMACS simulation package[45], version 2020 and 2021[46,47]. After energy minimization, all the systems were equilibrated at NPT condition. The pressure and temperature were kept constant at 1 bar using C-rescale barostat[48] $\left(\tau_p = 12.0\ ps,\ \kappa = 3 \times 10^{-4}\ bar^{-1}\right)$ and at 320 K using V-rescale thermostat[49] $\left(\tau_T = 1.0\ ps\right)$, respectively. The models containing proteins were first equilibrated with having position restraint on the proteins to relax lipids distribution around the protein and then, the position restraint on the proteins were removed to allow the proteins to move freely as well. For the one-protein-axolemma, the protein-lipid contacts and energy of the system did not change much after removing the position restraint of the protein (supplementary Fig. 1); therefore, the last frame of the system with restrain on the protein was used to run the deformation simulations. However, the proteins in the node-of-Ranvier model tend to move when they are free, so the deformation is applied to the system after being equilibrated for 11 μs with no position restraint. Table 2 and supplementary table S1 provides the information about how different systems were developed and simulated in more detail.

To explore pore formation under strain, we initially deformed the systems with rate of $1 \times 10^{-5}\ nm/ps$ in the x-direction until 30% of deformation. We then conducted two subsequent deformations with slower rates of $1 \times 10^{-6}\ nm/ps$ and $1 \times 10^{-7}\ nm/ps$ on the deformed model. This allowed us to observe and analyze the impact of deformation rate on pore formation. All the simulations were run for at least 3 times. In order to compare the properties of the developed systems with myelin model by Saeedimasine et. al, similar deformation runs were also applied to the equilibrated model for the myelin.

All the simulations were performed on a supercomputer with 128 CPU cores on each node. Each simulation takes on average 1300 hours (54 days) starting from the equilibration of the system to the pore formation for a total of 33 simulations(6

equilibration and production simulations and 27 deformation simulations). That would be a total of more than 5.4 million hours of simulation on each CPU core.

Equilibrium and mechanical properties

System in equilibration

In order to check the equilibration of the system and the lipid aggregation in equilibrium, enrichment factor was calculated and compared for the last 10 μs of equilibrium for each lipid type based on the method proposed by Corradi et al.[50]:

$$Enrichment\,factor\,(L) = \frac{Ratio\,(L)_x}{Ratio\,(L)_{bulk}} \tag{1}$$

In which: $Ratio\,(L)_x = \frac{no.\,(L)_x}{(total\ no.\ lipids)_x}$, is calculated by dividing the number of type L lipids within the distance of x from the given lipid by total number of lipids within the distance of x from the given lipid and $Ratio\,(L)_{bulk} = \frac{total\ no.\ L}{total\ no.\ lipids}$, is defined as the total number of type L lipids in the system divided by total number of lipids.

For the systems with protein, we use $Ratio\,(L)_x$ to define the protein neighboring. The cut-off distance was set to be 1.5 and 2.1 nm to calculate lipid-lipid enrichment factor in axolemma model and protein-lipid neighboring in one-protein-axolemma and the node-of-Ranvier, respectively, in line with the distance chosen by Saeedimasine et al.[11].

System under deformation

To derive the mechanical properties of the systems, such as Young's modulus, principal stress in x direction, σ_x, and strain, ε_x, values were calculated as follows:

$$\sigma_x = P_{xx} \tag{2}$$

$$\varepsilon_x = \frac{(x - x_0)}{x_0} \tag{3}$$

where P_{xx} is the lateral pressure in the x direction, the same direction from which the system is deformed[51], x is the bilayer dimension in x direction at different deformation points and x_0 is defined as the x dimension of the bilayer when the average surface tension of the system is zero.

The stress strain curve of a material under deformation acts differently at different stages of deformation. At small strains, called the elastic region, there is a linear relationship between stress and strain curve and the slope of the line in this part is representative of the stiffness or Young's modulus of the system. The strain in which the stress-strain curve becomes non-linear depends on the material and its mechanical properties. The elastic region for the simulated systems in this study was until the deformation of 15%. Equation 4 shows how the Young's modulus is calculated in the elastic region.

$$Young's\,modulus = \frac{\partial \sigma}{\partial \varepsilon} \tag{4}$$

in which σ is the uniaxial stress corresponding to the σ_x in our simulations, and ε is the strain of the system which is equal to ε_x in this study . The Young's modulus for the systems is derived from the stress-strain curve of the systems under deformation with the strain rate of $1 \times 10^{-5}\ nm/ps$ by employing the ordinary least squares regression method in Python to fit a line to the strain-stress curves and the slope of the line along with the standard error of the fit are presented.

Another mechanical property of the bilayer is the area compressibility modulus, K_A, which is defined as the derivative of the surface tension γ with respect to areal strain ε_A in constant temperature, experimentally[52,53].

$$K_A = \frac{\partial \gamma}{\partial \varepsilon_A} \tag{5}$$

Assuming that the surface area of the bilayer is equal to the surface area of the box, the areal strain was derived by dividing the area of the box at different deformations to the initial area of the box at strain of zero (ε_0). $\varepsilon_A = \frac{(x \times y - x_0 \times y_0)}{(x_0 \times y_0)}$. Surface tension can also be calculated as:

$$\gamma = l_z\left(P_{zz} - \frac{P_{xx}}{2} - \frac{P_{yy}}{2}\right) \tag{6}$$

in which P_{xx}, P_{yy} are the lateral pressure, P_{zz} is the normal pressure and l_z is the height of the box.

At small deformations, there is a linear relationship between surface tension and areal strain and consequently, area compressibility modulus is constant. Therefore, to calculate this parameter, we simulated the systems in constant pressure and areal strain condition, NP_zAT, in small areal strains of up to 4.5% for 6 μs and using the data of the last 4 μs of simulations to make sure the system is in equilibrium, the production data was processed every 1 μs and the average surface tension, γ, and strain values were used to calculate the area compressibility modulus to make the results more comparable with experimental data. K_A is calculated the same regression method used to calculate the Young's modulus.

Bilayer rupture occurs with formation of pore in the bilayer and corresponds to a sudden decrease of the surface tension and the strain at this point is considered as the rupture strain, ε_{pore}.

Results and discussion

Equilibration and structure

The axolemma is described as a bilayer consisting of 26 lipid types and cholesterol(CHOL), distributed asymmetrically in the extracellular and cytoplasmic leaflet of the axon cells. The bilayer composition (% mol) is 17% phosphatidylcholine (PC), 18% phosphatidylethanolamine (PE), 3% phosphatidylserine (PS), 4% phosphatidylinositol(PI), 2% sphingomyelin (SM), 17% glycolipids and 45% CHOL. The weight and mol fractions are reported in Table 1 together with experimental values. The extracellular leaflet primarily consisted of 20% PC, 3% SM, 21% glycolipids, 44% CHOL and 1% other lipids , while the cytoplasmic leaflet was rich in 14% PC, 26% PE, 5% PS, 7% PI, 46% CHOL and 2% other lipids in % mol. The developed model had a lipid composition in the range of the reported experimental values, except for a slightly higher weight fraction of CHOL in the model compared to the experimental values.

The axolemma differ in lipid composition compared to an average plasma membrane, but also other membranes in the brain. In general, it has high weight fraction of CHOL followed by PC and PE phospholipids. A high CHOL content in the axolemma is expected since membranes of the brain cells are usually more enriched in CHOL compared with other cell membranes in the body[54,55]. The axolemma model is more enriched in PC lipids, less in glycolipids and CHOL than the myelin and average brain white matter membranes[11,34]. In comparison with plasma membrane, axolemma has higher content of glycolipids while the average plasma membrane model is more enriched in PC and SM.

Tail length and saturation index also differs in different membrane models. Tail length in all the models varies between 16 to 26. While axolemma and plasma membrane models have around 80% of short tail lengths of 16-18 in both extracellular and cytoplasmic leaflets, this value is 48% for myelin in cytoplasmic leaflet and 95% in the extracellular leaflet. This difference in the tail length between the models and the leaflets for the myelin can contribute to its mechanical properties. Saturation index is also different in the models and between leaflets. While the axolemma model has unsaturation index (saturated tails/unsaturated tails) of 0.47 and 0.42 in extracellular and cytoplasmic leaflets, respectively, these values are 0.66 and 0.68 for plasma membrane model and 0.44 and 0.34 for the myelin.

To describe the axon sub-cellular membranes, we use 3 models: axolemma, one-protein-axolemma and node-of-Ranvier (see **Molecular model for axon** section in methods for details). All the models were equilibrated and analysed in normal physiological conditions and under deformation to mimic an accidental TBI. To check the convergence of the simulations, total energy and surface area of the bilayers were calculated over time. Both properties reached equilibrium within the first 10 μs for all models (supplementary Fig. 2). In addition, lipid distribution in the bilayer in the axolemma model was checked and compared with the small axolemma system in the last 10 microseconds by calculating the lipid neighboring. We assumed that the system has reached equilibrium when the average number of lipid neighboring along with the surface area of the bilayer and energy of the system converged (see supplementary Fig. 3).

During the production run and under physiological conditions, lipid distribution was investigated for the developed models and enrichment factor for each lipid type was calculated and the results are shown in Fig. 2.The results show that the distribution of lipids inside the membranes in not uniform; however all axonal membrane models follow the same trend of lipid aggregation and depletion. While phospholipids in the extracellular leaflet tend to aggregate with each other, they tend to deplete the glycolipids. The same trend can be seen for glycolipids that aggregate most with each other. In the cytoplasmic leaflet where no glycolipid exists, lipids are distributed more uniformly; however, the PS lipids tend to deplete each other. Comparing different bilayers, it can be seen that the aggregation of glycolipids decreases in the presence of proteins in the extracellular leaflet when compared to the axolemma model. This change may be attributed to the fact that these lipids aggregate more with proteins as will be explained later which decreases the total lipids in their neighboring. In the cytoplasmic leaflet, SM lipids tend to aggregate more with other lipids in the one-protein-axolemma compared to the axolemma and the node-of-Ranvier models. However, as the number of SM lipids in the system is not much (only 2% of the composition), this change will not affect the properties of the system. Comparing the results with other models, it can be seen that the model of an average brain membrane developed by Ingolfsson et al.[34] also has similar trend in the extracellular leaflet where the glycolipids co-localize and other lipids tend to aggregate with each other and deplete the glycolipids. Similar behavior can be seen for the glycolipids in the

model of the plasma membrane[56], but in this model, other lipids also aggregate with glycolipids and less localization of the glycolipids can be seen. In the cytoplasmic leaflet, the distribution of lipids in the plasma membrane and brain membrane models[34,56] are similar to the axolemma; however, the depletion of PS lipids is not seen in these models.

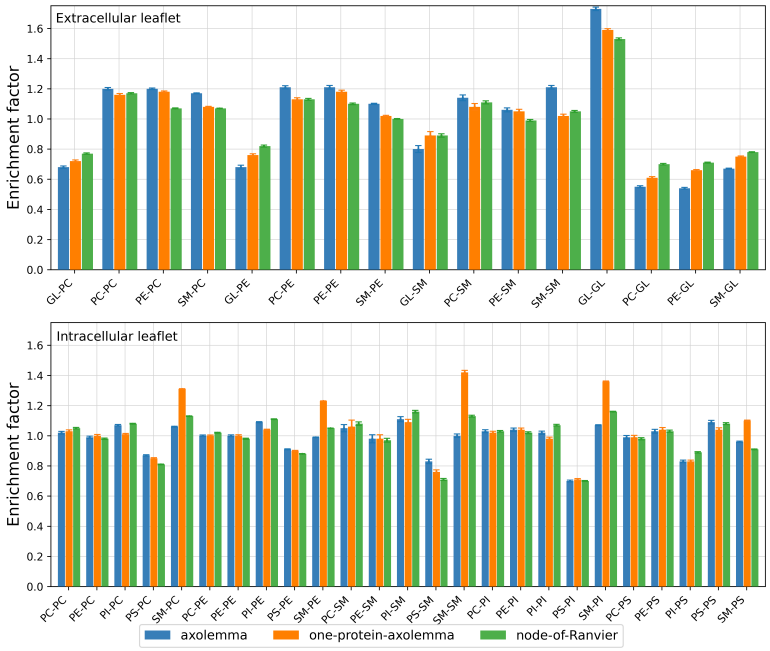


Figure 2. Average Lipid-Lipid enrichment of different systems in the last 10 μs of equilibration along with their standard deviation shown as error-bars. lipid head-groups are abbreviated as follows: cholesterol (CHOL), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), sphingomyelin (SM)

For one-protein-axolemma model and the node-of-Ranvier, the lipid neighboring around the proteins were also calculated and the results are presented in the Table 3. From the lipid-protein aggregation data in this table, it is indicated that in both systems, about half of the lipids surrounding the protein are glycolipids in the extracellular leaflet, while SM lipids tend to be depleted from the proteins. In the cytoplasmic leaflet where no glycolipid is present, PE phospholipids are more aggregated around the protein while SM lipids deplete from the protein. Aggregation of glycolipids in the extracellular leaflet and PE phospholipids in the cytoplasmic leaflet along with SM depletion are also reported by Saeedimasine et al.[11] for the neighboring of two different plasma membrane models with different protein concentrations.

Deformation

To investigate the behaviour of the models during injury, the bilayers were deformed at different strain rates in x direction (Table 2 and supplementary table S1). First, the system was deformed until 30% with the rate of 1×10^{-5} nm/ps and then, two subsequent deformations with slower rates of 1×10^{-6} nm/ps and 1×10^{-7} nm/ps were applied to the deformed model. Based on the results, calculated stiffness of the bilayer, rupture strain and other mechanical properties of the bilayer under deformation are reported in this section.

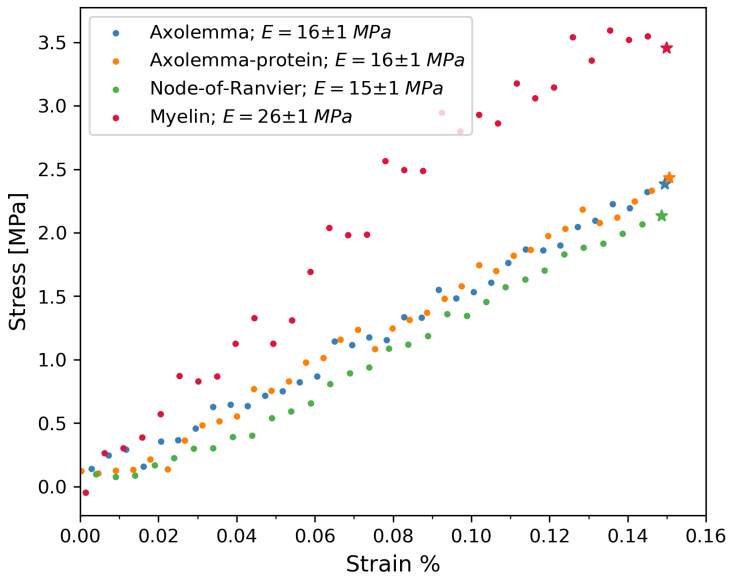
The values of Young's modulus representing the stiffness of the bilayers are reported in Table 4 and the stress strain points used for the fitting are plotted in Fig. 3. This value can give us more insight about the effect of protein concentration and lipid composition on the resistance of the bilayer against an applied force and deformation. The results show that all the models with axolemma composition have similar stiffness of 15-16 MPa, while the myelin model has higher stiffness(26 MPa). This suggests that the protein concentration has little effect on stiffness of the system and this value is largely influenced by the lipid composition. We also calculated the Young's modulus of each system using resulted strain-stress curves when the systems were simulated in equilibration at constant areal strains. Calculated stiffness values are 16.1, 14.9, 7.1 and 25.1 MPa for axolemma, one-protein-axolemma, node-of-Ranvier and myelin, respectively. While all the values are close to those derived from the stress-strain curve of the system under deformation, the Young's modulus derived for node-of-Ranvier is twice lower when calculated by this method. This difference is because all systems have lower stiffness in strains of less than 3% (5-9 MPa) and then start to get stiffer. By the strain of 5% all the models are already at the reported stiffness but the stiffness of the node-of-Ranvier increases slower than the other systems and reaches the reported value at the strain of 12%. This behavior can

be due to the fact that all models have some curvatures and the time it will take for them to get completely flat differs.

A direct comparison between our results and the experiments is not possible, as to the best of our knowledge, no experimental data has measured value of the Young's modulus for the axonal membranes under uniaxial deformation or if the presence of protein changes the stiffness of the membrane. Nevertheless, we try to compare the results with stiffness of other lipid bilayers. Jaing et al. performed tensile tests on two different silicone bilayers[57] and stress-strain data until 200% were reported. We calculated the Young's modulus of the membranes based on the reported data for the first 15% of deformation using linear regression method and derived the stiffness of 30 ± 1 and 37 ± 2 MPa for these bilayers.

Other studies applying compression to lipid bilayers using atomistic force microscopy calculate a Young's modulus of 19.3 to 28.1 MPa for a bilayer of DOPC/DPPC lipids[58], 27 to 31 MPa for POPC and PDPC bilayers[59] and 50 MPa for artificial lipid bilayers of DPhPC lipids[60]. The calculated Young's modulus in the presented study are in the corridor of the data derived for other membranes and lipid bilayers.

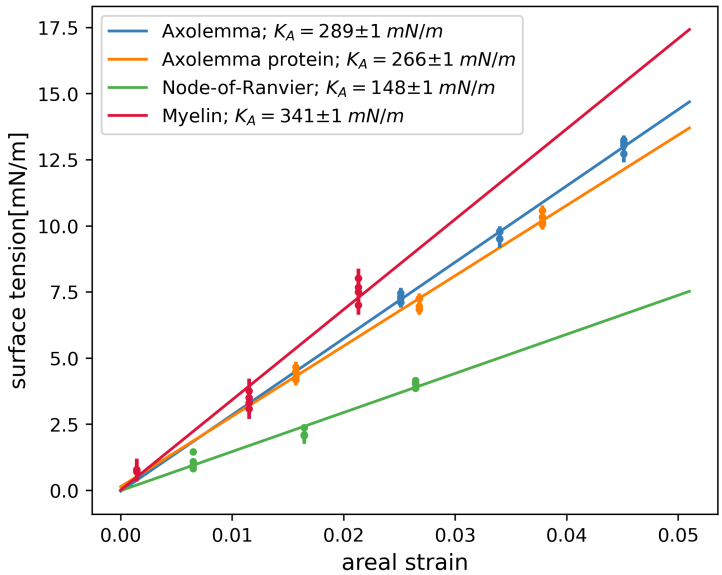
Figure 3. Strain Stress points of the systems in the elastic part of deformation (first 15% strain) and the corresponding fitted line using linear regression method. Stress value for each system at 15% strain representing the yield stress is shown by Stars.



Another mechanical parameter representing the response of the bilayer area under tension is areal compressibility modulus, K_A. This value was calculated for the systems at three different areal strain values of 0.1 to 4.5%. The reason for investigating the system under small deformations is that at higher strains, the surface tension will not grow linearly with strain while the areal compressibility modulus is defined as the linear relationship between these values. The average surface tension along with standard errors in each μs is plotted as a function of areal strain in Fig. 4 and the results for the calculated areal compressibility modulus and the corresponding standard error are reported in Table 4. Despite the Young's modulus that was mostly dependent on the lipid composition of the membrane, K_A is also affected by the concentration of proteins, as the node-of-Ranvier has almost half the compressibility modulus of the axolemma model with protein. Similar results were reported in previous studies on the model of plasma membrane[11]. The myelin has the highest compressiblity modulus compared to other systems. The reported experimental values for K_A are from 179 to 450 mN/m for red blood cell membrane at 25 °C[52,61,62] which are in the range of values reported in this study. However, no experimental data is available for the models of axonal membranes for more accurate comparison.

Assuming that the axonal injury is initiated by the pore formation of the axolemma, we deformed the systems until rupture at two different strain rates of 1×10^{-6} nm/ps and 1×10^{-7} nm/ps. The rupture in the system corresponds with formation of a pore in the bilayer that will lead to a jump in the surface tension. Therefore, by monitoring the surface tension, the rupture can be detected. Strain in which the rupture occurs depends on both the system and the deformation rate. Table 4 displays the mean rupture strain and standard deviation across various replicas for each system for different deformation rates. The node-of-Ranvier model exhibits a lower rupture strain compared to other systems. Additionally, as the deformation rate decreases, the rupture strain also decreases. Investigating the pore in the membranes, it can be seen that the pore forms in parts where there are more PE lipids present. For the models of one-protein-axolemma and node-of-Ranvier, some PC lipids can also be seen around the pore. Notably, in all models, no glycolipids are found in the vicinity of the pore.

Figure 4. Values of surface tension (mN/m) and the corresponding areal strain of different systems. Data is reported as the average and standard error of the surface tension vs. areal strain in time windows of 1 μs for each system when equilibrated in constant areal strain. Areal compressibility modulus of the systems is calculated as the slope of the line fitted to the data.



Comparing the results of mechanoporation in the node-of-Ranvier with experimental studies is not possible. But a similar MD simulation on the model of plasma membrane with similar concentration of proteins has done by Saeedimasine et al[11]. This model has been simulated with similar force-field and settings of the node-of-Ranvier in this study and deformed in x durection with the rate of $2.5 \times 10^{-6} nm/ps$. The results suggest a strain rupture of 36%, which is 22% lower than the strain rupture of the node-of-Ranvier. This difference stems from the differences in the lipid composition and the tail length and saturation level of the models. The difference in rupture strain reveals how structural properties affects the mechanical responses of membranes.

Furthermore, the systems' average tension at 0.1 μs before pore formation is calculated, providing both the mean value and standard deviation. Moreover, the failure stress, corresponding to the average stress right before poration, is computed as the average pressure along the x-direction at 0.1 μs before pore formation. The stress before rupture which corresponds with the ultimate stress in the membrane can give us data about the maximum stress each system can withstand before rupture.

Notably, the average surface tension before pore formation remains relatively similar for axolemma models, both with and without proteins, across various strain rates. However, the average stress before pore formation increases proportionally with the rupture strain. The reported values for myelin surpass those for axolemma models, likely due to myelin's higher stiffness, higher K_A, and higher rupture strain.

So far, the molecular dynamics results of the mechanical properties of different membrane systems are reported. Specifically, we investigated the areal compressibility modulus and stiffness of the systems and strain in which the membrane rupture occurs along with surface tension and ultimate stress in different deformation rates. In the next part, we couple the MD results with FE model of the axon to calculate the axon strain in which the axonal injury might occur. We use the results of the slowest deformation rate for the systems when coupling with FE results. The deformation rate of 1×10^{-7} nm/ps correspond to strain rate of 3000 s^{-1} for axolemma, one-protein-axolemma and myelin models and 1500 s^{-1} for the node-of-Ranvier. It is crucial to note that the rates mentioned here are 30 to 300 times faster than the strain rates observed in experimental studies on brain tissue damage and real-world scenarios. This notable difference arises from the inherently high computational cost and time associated with each simulation.

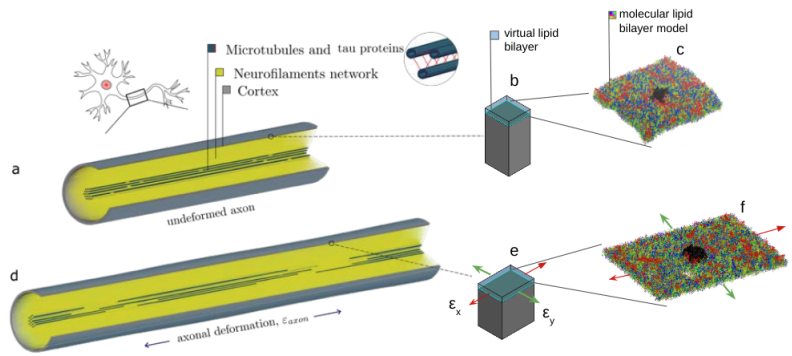
Multi-scale coupling

To investigate the membrane poration and how it leads to axonal damage in cellular level, we have coupled the molecular results with a generic FE model of the axon under stretch. The axon model used for the multi-scale approach is developed and completely described in Montanino et al.[30] and the multi-scale approach is explained and used in Montanino et al.[10]. Briefly, the axon model is modeled as an 8 μm cylinder consisting of microtubules, neurofilaments and tau proteins connecting the fibers together and an axolemma-cortex compartment surrounding the whole model. To minimize the effect of microtubule discontinuity on axonal behavior, 5 different axonal models with different microtubule discontinuity locations were stretched.

Subsequently, the axon undergoes deformation under uniaxial strain at varying rates.

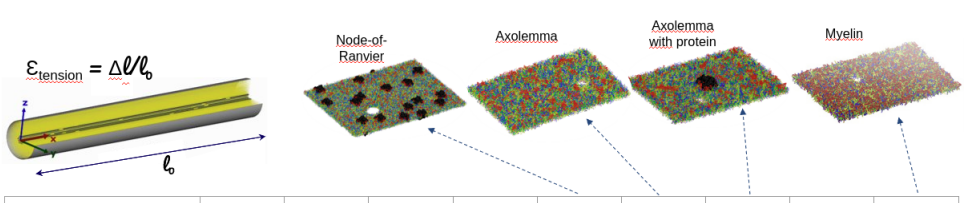
The strain applied to the axon leads to non-uniform deformation across its sub-cellular components, even within the same part. Employing a bottom-up approach, we assume that each element of the cortex corresponds to the membrane systems developed at the molecular level. Therefore, the deformation observed at the MD scale corresponds to the local strain experienced by each element of the cortex in the FE model. Figure 5 shows how the FE and MD models are coupled together.

Figure 5. Coupling FE model of the axon in cellular level with molecular systems representing the axonal membrane[10]. a) FE model of the undeformed axon in cellular level; A quarter of the axon model is removed for better representation of the sub-cellular elements. b) A single element on the cortex in an undeformed state corresponding with the membrane models in molecular level. c) Undeformed lipid bilayer of the axolemma with one embedded protein. d) FE model of the axon under uniaxial deformation in cellular level. e) The cortex element with the maximum local strain during the axonal deformation is extracted. f) Lipid bilayer of the axolemma with one embedded protein undergoing the maximum local strains of the cortex element.(figure adapted and modified from[10])



To calculate the axonal strain at which membrane poration occurs, we identify the cortex element with the highest local strain and analyze the resultant total axonal strain. Figure 6 illustrates the maximum local strain within the cortex in the FE model, alongside the corresponding axonal strain under various strain rates. Furthermore, the strain in which the mechanoporation occurs in MD is marked with the corresponding membrane. The node-of-Ranvier has a rupture strain of 41% which corresponds to axonal strain of 9.8 to 13.2 % depending on the strain rate, while the myelin seems to form rupture at higher strains compared to other membranes.

Figure 6. Coupling the molecular dynamics results with finite element model of the axon under deformation. The axonal strain with the corresponding element with maximum local strain for different deformation rates are provided in the table and the strain in which each system undergoes mechanoporation is marked.



maximum local strain (element)	0	0.10	0.20	0.30	0.41	0.43	0.45	0.50	0.55
$\varepsilon_{axon}(\dot{\varepsilon} = 1\ s^{-1})$	0%	4%	7%	10%	13.2%	14%	14.5%	16%	17.6%
$\varepsilon_{axon}(\dot{\varepsilon} = 10\ s^{-1})$	0%	3.5%	6.6%	8.3%	9.8%	10.2%	10.5%	11.5%	12%
$\varepsilon_{axon}(\dot{\varepsilon} = 20\ s^{-1})$	0%	4%	7.2%	8.8%	10%	10.2%	10.5%	11%	11.5%
$\varepsilon_{axon}(\dot{\varepsilon} = 40\ s^{-1})$	0%	5%	8%	10.6%	12%	12.1%	12.3%	13.5%	13.4%

A direct comparison of the results with available experimental data is not possible as the threshold for axonal injury in the brain is difficult to define precisely due to the complex nature of TBI[63]. Moreover, the specific threshold for axonal strain in the

brain is influenced by various factors, including the location of the injury, the direction and magnitude of the forces applied, and the individual characteristics of the brain tissue. However, experiments using animal models and computational studies have provided some insights into the biomechanical thresholds associated with axonal injury. For example, animal studies have shown that strains ranging from 10% to 20% can lead to axonal injury in the brain[63]. In another study on the neurons of rat embryo it was shown that strains more than 10% would result in total break of axonal bundles[22]. Another study that applyed 10% lagrangian strain with the rate of 20 /s to organotypic hippocampal slice cultures showed a moderate brain damage that was detected 4 days after the injury but not 2 days after the injury[64]. In contrast to our results, an experiment on the guinea pig optic nerve done by Bain and Meaney, the range of critical strain for axonal damage was shown to be between 0.14 to 0.34 with strain rate of 30 to 60 /s, below which no nerve was injured[65]. This difference might be due to mechanical differences between animal and human axons, or due to the fact that the axon model that is used in this study is isolated, while it is shown that the axons can withstand larger strains when embedded inside the tissue[35].

It is important to acknowledge certain limitations within this study. First, the rate of deformation in the MD models is significantly faster than the common injury events, leading to a rate mismatch. Additionally, while efforts were made to create more realistic models of axonal membranes and the proteins embedded within them, the simulations were conducted in a coarse-grained level which has some simplifications compared to all-atom simulations. Furthermore, the protein's overall shape was constrained using the ElNeDyn approach, rendering the protein semi-rigid and preventing it from accurately representing its true behavior under deformation.

Conclusion

This study aimed to illuminate the mechanism of axonal injury, specifically focusing on the membrane poration of the axolemma under strain. For this purpose, a molecular model of the axolemma was developed, taking into account its lipid composition and tail information derived from experimental data. Models with different protein concentrations were considered to replicate different regions of the axon. The developed models along with myelin developed by Saeedimasine et al.[11] were deformed until rupture and the mechanical properties were compared. Our findings revealed that among axonal membranes, myelin exhibited the highest stiffness and also had a greater rupture strain and stress at the point of pore formation. In contrast, the node-of-Ranvier had the lowest rupture strain and counted as the most vulnerable part of the axonal membrane. However, the tension and stress at the pore were not significantly different for the models of the axonal membrane, suggesting that these parameters are primarily influenced by the lipid composition of the membrane rather than the protein concentration.

We then combined the molecular dynamics (MD) simulation results with a finite element (FE) model of the axon to get more understanding of the mechanism of the axonal injury and its initial trigger and shed light to the behaviour of sub-cellular elements of the axons during an impact. We get the rupture strain for each membrane model from MD and the axonal strain at which local membrane mechanoporation occurs from FE simulations and then compared the injury threshold with experimental findings. The simulation results indicate that the mechanoporation of the membrane occurs in the range of 9.8 to 13 % of axonal strain which aligns with the experimental data and therefore supports the hypothesis stating that mechanoporation triggers axonal injury. In addition,this research highlights how combining FE and MD approaches can explore complex multi-scale phenomena that cannot be investigated by a single method.

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	PC	PE	SM	PS	Glycolipid	CHOL	Other
Experimental composition (% weight)							
Axolemma exp.[36,66,67]	21 ± 5	20 ± 5	3 ± 4	4 ± 2	20 ± 12	24 ± 3	4 ± 2
Myelin exp.[68]	11 ± 1	17 ± 1	6 ± 3	6 ± 1	28 ± 3	28 ± 1	4 ± 2
Brain white matter exp.[69]	12 ± 1	20 ± 2	-	9 ± 1	24 ± 5	27 ± 6	9 ± 2
MD models composition (% weight)							
Axolemma model	22	23	3	4	15	29	5
Myelin model[11]	12	22	3	9	23	29	2
Plasma membrane model[56]	30	17	17	6	5	17	8

Table 1. Lipid composition (weight fraction) of different membranes. For experimental data, the mean weight fraction is provided along with its corresponding standard deviation. Abbreviations are as followed: phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM), phosphatidylserine (PS), cholesterol (CHOL)

Model Name	Dimension (nm)	No. of Nav1.1 proteins	Equilibration time (μs)	No. of replicas at each deformation rate($\frac{nm}{ps}$)		
				10^{-5}	10^{-6}	10^{-7}
Axolemma (small)	$20 \times 20 \times 20$	0	25	No deformation		
Axolemma	$38 \times 38 \times 24$	0	20	1	3	3
One-protein-axolemma	$46 \times 38 \times 19$	1	30^a	1	3	3
Node-of-Ranvier	$80 \times 80 \times 20$	16	$15^a + 11.5$	1	3	3

^{a}: simulation performed by applying position restraint on the protein

Table 2. Information on performed molecular dynamics simulations

	Extracellular leaflet				Cytoplasmic leaflet				
System	PC	PE	SM	GL	PC	PE	SM	PI	PS
one-protein-axolemma	0.25	0.15	0.02	0.57	0.19	0.49	0.01	0.13	0.18
node-of-Ranvier	0.27	0.18	0.04	0.51	0.21	0.47	0.02	0.14	0.16

Table 3. Protein-lipid neighboring for the axolemma model with protein and the node-of-Ranvier at the last 10 μs of equilibrium. For definition see Section 3.4.1.

model	Rupture strain	Average surface tension before pore (mN/m)	Average stress before pore (MPa)	Young's modulus (MPa)	K_A (mN/m)
axolemma	0.51 ± 0.02^b; 0.43 ± 0.03^c	73.1 ± 0.5^b ; 70.1 ± 0.6^c	5.63 ± 0.04^b; 5.05 ± 0.03^c	15.5 ± 0.99^a	289 ± 7
one-protein-axolemma	0.50 ± 0.01^b; 0.45 ± 0.01^c	73.5 ± 0.4^b ; 71.4 ± 0.4^c	5.56 ± 0.03^b; 5.19 ± 0.03^c	16.4 ± 0.99^a	266 ± 8
node-of-Ranvier	0.44 ± 0.01^b; 0.41 ± 0.01^c	72.3 ± 0.2^b ; 70.2 ± 0.2^c	4.84 ± 0.02^b; 4.60 ± 0.02^c	15.3 ± 0.99^a	148 ± 10

Data calculated at different deformation rates of:
a: 10^{-5}; $b : 10^{-6}$; $c : 10^{-7}$ nm/ps
d: Model derived from[11]

Table 4. Mechanical properties of different systems under deformation, the average rupture strain and surface tension $0.1\mu s$ before the pore for different tests are provided along with their standard error.