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NUMERICAL SIMULATION OF NANOINDENTATION AND PATCH CLAMP EXPERIMENTS ON MECHANOSENSITIVE CHANNELS OF LARGE CONDUCTANCE IN ESCHERICHIA COLI

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Abstract

A hierarchical simulation framework that integrates information from all-atom simulations into a finite element model at the continuum level is established to study the mechanical response of a mechanosensitive channel of large conductance (MscL) in bacteria *Escherichia Coli* (*E.coli*) embedded in a vesicle formed by the dipalmitoylphosphatidylcholine (DPPC) lipid bilayer. Sufficient structural details of the protein are built into the continuum model, with key parameters and material properties derived from molecular mechanics simulations. The multi-scale framework is used to analyze the gating of MscL when the lipid vesicle is subjected to nanoindentation and patch clamp experiments, and the detailed structural transitions of the protein are obtained explicitly as a function of external load; it is currently impossible to derive such information based solely on all-atom simulations. The gating pathways of *E.coli*-MscL qualitatively agree with results from previous patch clamp experiments. The gating mechanisms under complex indentation-induced deformation are also predicted. This versatile hierarchical multi-scale framework may be further extended to study the mechanical behaviors of cells and biomolecules, as well as to guide and stimulate biomechanics experiments.

Keywords

mechanosensitive channel; nanoindentation; patch clamp; finite element simulation

1. INTRODUCTION

Many fundamentally important biological processes rely on the mechanical response of biomolecules and their assemblies. An important example is the gating of mechanosensitive (MS) channels, which are important in the transduction of signals related to touch and hearing in animals and osmotic sensing in bacteria [1–4]. Mechanosensitive channels have been identified in more than 30 cell types [5]. For example, a much studied system is the Mechanical sensitive channel of the Large conductance (MscL) in *E.coli*, whose conformation is sensitive to tension in the membrane; opening of the channel allows exchange of small molecules (including water) and ions between cytoplasm and the environment, which releases the potentially devastating osmotic stress.

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To elucidate the molecular mechanism for the gating of MscL, diverse experimental techniques have been applied. The experiments that directly probe the gating threshold are patch-clamp measurements, which simultaneously monitor the membrane tension and ion current through the channel (thus opening probability). The activities of the pressure-active ion channels in giant *E.coli* spheroplasts were documented using patch clamp electrical recording techniques twenty years ago [6]. For *E.coli*-MscL, careful patch-clamp studies have been carried out by Sukharev and colleagues on lipid vesicles [4,7]. An important clue from these studies on purified lipid layers is that the mechanical property of the membrane plays a central role in gating. A five-subconductance-states model was established, in which the tension-dependent conformational transition is primarily attributed to the pore-area variation that occurs between the fully closed state and the first subconductance (opened) state. Structural models for the close-open transition were constructed [8,9] based on geometrical constraints provided by various measurements and the crystal structure of MscL in mycobacterium tuberculosis (Tb) [10]. The configurations of the S3 helix bundle in the modeled intermediate states were recently argued to be inaccurate, and revised models were subsequently proposed where the conformational changes of the S3 helices are much smaller in scale [11]. Other experimental studies have also been used to probe the conformational transition pathway, which include electron paramagnetic resonance spectroscopy (EPR) with site-directed spin labeling (SDSL) [12–14] for monitoring the structural rearrangements and cysteine scanning for identifying residue contacts in the transmembrane helices [15].

To properly assign structural features to important intermediate states along the close-open transition identified in the previous studies based on patch clamp experiments, it is important to be able to simulate the structural response of the channel to mechanical perturbation consistent with the experimental protocol. In the foreseeable future, this is difficult to do with atomistic simulations despite the rapid progress being made [16,17]. For example, even with a steered molecular dynamics protocol [18] the pore radius reached only 9.4 Å after more than 10 ns, which is significantly smaller than the experimentally estimated radius (~19 Å) for the fully opened state. [9]. Approximate open-close transition trajectory can be obtained with targeted molecular dynamics simulations [19] which, however, requires the detailed knowledge of both the closed and open states [8,9]. In addition, in these biased all-atom simulations the “pulling force” on the protein is large in magnitude and artificial since the simulations were short and lipid bilayer membrane was completely ignored in the TMD simulations.

Motivated by these considerations, a simplified continuum model of *E.coli*-MscL has been constructed in our recent work [20]; the model is hierarchical in nature because the material properties of and interactions among the continuum elements were estimated based on all-atom simulations. Despite the simplicity of the model in Ref. [20], which only included the transmembrane helices and the lipid membrane as elastic rods and sheet, respectively, the simulated gating behavior of the channel when the membrane was subjected to stretching was consistent with previous MD simulations [18,19] and experimental results [8,9]. More importantly, it was found that the structural response of the channel was very different when the membrane was subjected to bending rather than biaxial stretching, which illustrated the unique value of the continuum model.

In the present work, as an important step towards making the continuum model more realistic, cytoplasmic helices and loops are included. Comparing the results to those for the simpler model [20] allows us to probe the importance of these structural motifs in gating. Another important motivation of this work is to further demonstrate the unique flexibility of the continuum mechanics framework in dealing with complex loading conditions. Specifically, we explicitly simulate the structural response of MscL to realistic mechanical loading patterns found in two common types of experiments. First, we simulate the patch-

clamp experiment on a lipid vesicle, which is widely used in channel studies. Second, we simulate a hypothetical indentation experiment on the same liposome. Nanoindentation is another fundamental experimental technique to explore the mechanical behaviors of small organisms. The mechanotransduction pathways of a single cell were identified in an atomic force microscopy (AFM) indentation test [21], including the activation of stretch-activated ion channels due to contact and the relevant stress. Although indentation is arguably one of the most efficient ways of measuring the mechanical properties of small structures, it has not yet been widely employed to investigate the gating of MscL channels. The potential of such technique needs to be explored. Indeed, interesting differences are found in the structural response of MscL in the current numerical experiments, which we hope will stimulate new experimental studies for verification.

Regarding the quantitative value of the current simulations, we caution that many potentially important factors, such as the effect of solvation, have not been included, which makes the direct comparison to experiments difficult. However, once a physically complete model is established in the future, explicitly comparing simulation and experiment with the same loading conditions (e.g. patch clamp) is a powerful way to validate and refine the computational model, as well as to stimulate new experiments.

2. THEORY AND METHODS

In this section, the continuum framework for the whole protein-lipid system is described, and the relevant parameterization and simulation techniques are summarized. The hierarchical model is employed to simulate the gating behaviors of *E.coli*-MscL when a nanoindentation experiment or a patch clamp experiment is carried out on a spherical vesicle that crudely mimics a liposome used in patch clamp experiments or a bacterium.

2.1 Continuum Model of the Whole Protein-Lipid System

2.1.1 The Protein—Based on the determined crystal structure of MscL in mycobacterium tuberculosis (*Tb*-MscL) [10], Sukharev et al. proposed a model structure for the *E.coli*-MscL by incorporating a series of constraints observed from experiments. The top and side views of the structure are shown in Figs. 1a and 1b, respectively. Several basic components of the protein include: the transmembrane (TM1, TM2) helices, the cytoplasmic (S) helices, and various loops.

Two types of transmembrane helices with five-fold symmetry exist in *E.coli*: the five identical TM1 helices (orange) form an inner gate, and the five identical TM2 helices (sky blue) form a larger outer gate that is adjacent to the lipid bilayer (and interact with the lipid via non-bonded interactions) [11]. Each long TM1 helix and its nearest neighbor, a short TM2 helix, are assembled into a pair through periplasmic loops. A closer view of the TM1 shows that there is a break near the top of the helix due to Pro-43, thus the segment above Pro-43 is also referred to as the S2 helix. The cytoplasmic region contains two different types of helix bundles, referred to as the S1 helices (light green) and S3 helices (green), respectively; each bundle contains five subunits, and each subunit of the S1 or S3 bundles is connected to a TM1 or TM2 helix through a loop. The TM1 helices, TM2 helices, S1 helices, and S3 helices correspond to residues Asn-15-Gly-50, Val-77-Glu-107, Ile-3-Met-12, and Lys-117-Arg-135, respectively.

In the hierarchical continuum model, the main components of the *E.coli*-MscL are modeled by three-dimensional continuum structures, whose geometry match exactly to that of the model structure in the closed state [11]. Each helix (TM helices or S helices) is simplified as an elastic cylinder with a diameter of 5 Å [20]. In our previous proof-of-concept study [20], only the transmembrane helices were modeled for simplicity; in the present work, all

important structural details of the protein are taken into account, where the cytoplasmic helices and the loops connecting the transmembrane helices and cytoplasmic helices are also incorporated. The top and side views of the continuum model of *E.coli*-MscL are shown in Figs. 1c and 1d, respectively, where the color codes for different continuum components are given.

Assuming that the mechanical properties of a helix vary little with respect to sequence, each helix is assumed to be homogeneous and isotropic, and its elastic properties remain constant during the gating transition. The only exception is the above mentioned break between S2 and TM1 helices (Pro-43), which is illustrated by the dark blue segment whose elastic stiffness is lower than the rest. The moduli of different helical segments are calibrated from atomistic simulations. According to previous all-atom MD simulations, the Young's moduli of alpha-helices are found in the range of 60–180GPa [22]. In our continuum model, a Young's modulus of 100GPa and a Poisson's ratio of 0.3 are adopted; both are fitted such that the lowest eigenvalues from a normal mode analysis (NMA) using the continuum model match those from an all-atom NMA using the CHARMM22 force field. The modulus of the Pro-43 segment is taken to be 25GPa, which is also obtained based on matching NMA results for that segment at the continuum and all-atom levels.

In the structural model proposed by Sukharev et al. [11], the loops are speculated to have important effects on the gating of MscL, thus they are also included in the continuum model here. The initial geometry of the loops connecting TM1 and TM2 helices, TM1 and S1 helices, and TM2 and S3 helices are measured from the closed state of structural model [11]. All loops are modeled as elastic springs and their spring constants are found via NMA by requiring the three lowest frequencies to be the same as that computed from an all-atom calculation. The geometrical parameters and material properties of the helices and the loops are listed in Table 1.

2.1.2 Lipid Vesicle—The elastic lipid membrane is taken to be homogenous and isotropic, and its Young's modulus is assumed to be stress-independent. The thickness of the representative dipalmitoylphosphatidylcholine (DPPC) bilayer is taken to be 35Å [18]. Based on previous experiments and MD simulations [23,24], the area compressibility of a DPPC bilayer is about 350mN/m, which is equivalent to a Young's modulus of $E=140\text{MPa}$ and is employed in the present study. A Poisson's ratio of 0.3 is used [20]. To implant the protein into the lipid membrane, a cavity of 10-petal flower shape is created in the membrane, where the distance of each petal to its relevant TM1/TM2 helix is set to be the equilibrium distance (5.5Å), measured in the model structure of the fully closed state [18]. The diameter of the cavity is about 44Å. Fig. 2 shows the zoom-in view of the embedded protein and surrounding lipid membrane in the continuum model; the size of the square membrane patch is 400Å by 400Å in this figure, and it can be subjected to non-equi-biaxial stress field, which is inherited from the overall deformation field of the vesicle depending on the loading mode (see below).

A realistic *E.coli* cell is of cylindrical shape with a diameter of 1μm and length of 2μm. In previous patch clamp experiments, spherical lipid vesicles were used to simplify the system, which was crucial for establishing that lipid tension was the sole factor that triggers the opening of MscL. Spherical cell geometry also helps to simplify the numerical analyses of both nanoindentation and patch clamp experiments, where the stress-strain fields may be obtained from an axisymmetric investigation.

In the continuum approach, the lipid vesicle is modeled as a spherical liquid-filled shell, assuming that the lipid membrane is not permeable [4]. According to Ref.[25], the bulk modulus of cytoplasm is similar to that of pure water when the cell is under high pressure,

therefore, a bulk modulus of 2.2GPa is chosen for the “cytoplasm”). The diameter of the vesicle is taken to be 5 μ m, which is in the middle of the size range of liposome used in patch clamp experiment [4]. For simplicity, we assume only one MscL is embedded in the cell and it is located at a position where gating may be maximized during subsequent deformation (i.e. patch clamp and nanoindentation in the present paper, refer to Figs. 3 and 4, respectively). Since the size of the protein ($\sim 40\text{\AA}$) is much smaller than the diameter of the vesicle, upon external loading, the stress-strain field of the vesicle is essentially unaffected by the presence of the protein, which can be computed by the deformation of a continuous, “defect-free” shell as elaborated below.

2.1.3 Non-bonded interactions—When a force is applied on the membrane, it is transmitted to the helices through non-bonded interactions between the lipid and helix, and that between the helices, and finally leads to the gating of the channel. The non-bonded forces include both electrostatic and Van der Waals components, and without losing generality, an effective pair-wise potential of Lennard-Jones form is used to describe such interactions. As the distance between two interacting components (α) is varied, the interaction energy $E_{\text{int}}(\alpha)$ between the surfaces of two continuum members varies as:

$$E_{\text{int}}(\alpha) = C \left[\frac{n}{m} \left(\frac{d_0}{\alpha} \right)^m - \left(\frac{d_0}{\alpha} \right)^n \right] \quad (1)$$

where for any given pair of interaction, d_0 is the initial equilibrium distance in the undeformed configuration, m , n , and C are parameters that can be fitted from atomistic simulations. The derivative of $E_{\text{int}}(\alpha)$ with respect to α leads to the pressure-distance relationship between the two deformed surfaces:

$$p(\alpha) = \frac{\psi}{6} \left[\left(\frac{d_0}{\alpha} \right)^{m+1} - \left(\frac{d_0}{\alpha} \right)^{n+1} \right] \quad (2)$$

where $p(\alpha)$ is the interaction pressure between the two surfaces, and $\psi = 6Cn/d_0$.

Specified for different interaction pairs between transmembrane helices, cytoplasmic helices, and that between lipid and transmembrane helices, d_0 is measured from the crystal structure of *E.coli*-MscL in the closed form, and m , n , and C are fitted from all-atom Molecular Mechanics (MM) analyses using CHARMM. As the distance between the two interacting surfaces is varied, m , n , and C are adjusted such that the $E_{\text{int}}(\alpha)$ computed from finite element (FEM) simulations matches with that from MM. The detailed fitting procedure was described in our earlier work [20], although the present analysis involves more pairs of non-bonded interactions with the incorporation of the full protein model. The fitted constants are presented in Table 2. In Fig. 5, the curves of interaction potential computed from the MM simulations and continuum model (by using the fitted constants in FEM simulations) are compared to each other, to illustrate the effectiveness of the current fitting procedure.

2.2 Decouple Deformation of Vesicle and Deformation of Protein

Since the protein is much smaller than the vesicle, the analyses of their deformation can be decoupled: during the first stage, only the lipid deformation is followed by computing the stress and strain fields of a homogeneous vesicle under external loading such as indentation or suction (Figs. 3 and 4). Such analysis is axisymmetric. The stress and strain evolutions at a material point where the MscL is implanted are obtained, and they are recorded and transferred to the next stage. During the second stage of numerical simulation, only the

protein deformation is followed explicitly. In each increment, the stress field recorded in the first stage is employed as a boundary condition on a much smaller lipid sheet embedded with a protein (Fig. 2); since the size of the lipid sheet (400Å by 400Å) is still much smaller than the dimension of the vesicle, the sheet can be assumed to be flat and it is subjected to in-plane stresses. The non-bonded interactions between the lipid and the helix bundle gradually pull the channel open, which can be in turn related to the load applied by the nanoindenter tip or pipette. There are multiple increments within each step, and the MscL structure is updated after each increment.

2.2.1 Nanoindentation on a vesicle—The axisymmetric configurations of indentation experiment are sketched in Figs. 3a and 3b, for undeformed and deformed cells, respectively. A rigid spherical indenter with a diameter of 2µm is applied to perform the experiment, and a rigid substrate supports the bottom of the vesicle during the test; an indenter of same dimension was used in a previous experiment on colloidosome particles of similar elastic properties [26], which can be readily obtained by melting and pulling the tip of a glass fiber. All contacts between the membrane, indenter, and substrate surface are assumed to be frictionless. The initial position of the *E.coli*-MscL is assumed to be at the same height as the center of the spherical vesicle (i.e. on the equator, Fig. 3). The indenter moves downwards by a prescribed displacement, and the maximum indentation depth is 80% of the cell diameter (further increase of the load may lead to excessive deformation of the vesicle beneath the indenter and damage the membrane). Upon indentation, the deformed vesicle shape becomes persimmon-like, with a concave surface in its center and protruded (bulged) perimeter as shown in Fig. 3b.

2.2.2 Patch Clamp on a vesicle—The axisymmetric geometry of patch clamp experiment is sketched in Figs. 4a and 4b, respectively, for undeformed and deformed configurations. The wall of the rigid pipette is represented as the inclined U-shaped line, with a thickness of 0.2µm, and an inclination angle of 10°; the diameter at the opening of the pipette is 1µm— such geometry was measured from the experiment by Sukharev et al. [7]. The contact between the cell and the pipette surface is frictionless. In the undeformed configuration, the bottom surface of pipette just touches the cell. Next, a suction pressure is applied on the portion of cell membrane within the pipette pore, to gradually pull the part of membrane upwards, which becomes a bulged hemisphere (Fig. 4b); with the increase of suction pressure, the area of the membrane cap sucked into the pipette also increases and the force boundary condition is continuously updated. We assume that the *E.coli*-MscL is located at the north pole of the vesicle during the experiment, which subjects maximum membrane tension that is equi-biaxial (Fig. 4). The suction pressure is increased until the stress needed for full MscL gating is reached.

2.2.3 Estimation of Stress and Strain required for Gating—To estimate the stress field in the vicinity of the protein, a plane stress model is adopted in which a flat membrane contains a circular cavity whose undeformed radius (a) is the same as the medium radius of the ten-petal flower-shaped lipid cavity hole in the fully closed state of the MscL (22Å). When a is much smaller than the size of the membrane patch, and when the membrane is subjected to an equi-biaxial load, the radial displacement fields in the lipid membrane is,

$$u_r = \frac{1}{E} [(1 - \nu)\sigma_o r + (1 + \nu)(\sigma_o - \sigma_i) \frac{a^2}{r}] \quad (3)$$

where r is the radial distance measured from the center of the cavity, σ_o and σ_i are the external (applied) membrane stress and interaction stress between the embedded helix and

lipid inner hole, respectively. E and ν are the Young's modulus and Poisson's ratio of membrane, respectively. The interactions between the lipid and protein is assumed to be much smaller than the external load [20], i.e., $\sigma_i \ll \sigma_o$. From Eq. (3) the cavity remains a circular shape after deformation and the radius increment is

$$\Delta a = u_r(a) = \frac{2a}{E} \sigma_o \quad (4)$$

Assuming that the lipid hole needs to be expanded to a critical area A^c , such that the enclosed TM1 pore (MS channel) area is large enough for gating, the equi-biaxial membrane required for gating is then

$$\sigma_o^c = \frac{E \sqrt{A^c/\pi} - a}{2a} \quad (5)$$

From the structural model, the desired increment of the lipid hole radius is estimated to be 15Å in order to achieve full gating, thus the required equi-biaxial membrane tensile stress should be about 48MPa. While the stress around MscL is equi-biaxial in patch clamp experiment, the situation is different in indentation. Upon non-equi-biaxial loading, (σ_1 in x direction and σ_2 in y direction, c.f. Fig. 2), the radial displacement field can be readily derived,

$$u_r = \frac{1}{E} \left[(1-\nu)r + (1+\nu)\frac{a^2}{r} \right] \frac{(\sigma_1 + \sigma_2)}{2} + \frac{2\cos 2\theta}{E} \left[\frac{(\sigma_1 - \sigma_2)}{4} (1+\nu)r + (\sigma_1 - \sigma_2)a^2 \frac{1}{r} - \frac{(\sigma_1 - \sigma_2)a^4}{4} (1+\nu) \frac{1}{r^3} \right] \quad (5)$$

where θ is the angle measured counterclockwise from the x-axis. Thus, the increment of pore radius is,

$$\Delta a = u_r(a) = \frac{a}{E} [(\sigma_1 + \sigma_2) + 2\cos 2\theta(\sigma_1 - \sigma_2)] \quad (6)$$

If the area enclosed by the deformed lipid hole equals to that required for full gating under equi-biaxial tension, i.e.

$$\int_0^{2\pi} \frac{1}{2} [a + u_r(a)]^2 d\theta = \pi a^2 \left[1 + (2+2k) \frac{\sigma_1}{E} + (3 - 2k + 3k^2) \left(\frac{\sigma_1}{E} \right)^2 \right] = A^c \quad (7)$$

where $k = \sigma_2/\sigma_1$, then the required critical membrane stress for gating is

$$\sigma_1^c = \frac{-(1+k) + \sqrt{(-2k^2 + 4k - 2) + (3k^2 - 2k + 3)A^c/(\pi a^2)}}{3k^2 - 2k + 3} \quad (8)$$

2.3 Simulation Protocol

As mentioned in the last section, the numerical simulation is divided into two stages. In the first stage, a micro-scale simulation is carried out to study the vesicle deformation behavior upon indentation or patch clamp experiment (Figs. 3 and 4). Three-node axis-symmetric shell elements are used to discretize the membrane, and fluid link elements are used to describe the interior of the vesicle with prescribed fluid bulk modulus. Large deformation algorithm is invoked in the computation, and the indenter, substrate, and pipette are taken to be rigid surfaces.

In the second stage, simulation is carried out on the nano-scale membrane sheet containing the MscL (c.f. Fig. 2). Four-node tetrahedron finite elements are used to discretize the helices, with all nodes roughly equally spaced; two-node spring elements are used to mesh the loops with nodes uniformly distributed; four-node tetrahedron elements are used to simulate the lipid membrane where the mesh is more refined toward the boundary of the cavity. All simulations are carried out by using ABAQUS [27]. The non-bonded interactions are implemented by a user interaction subroutine (UINTER); more details of can be found in our previous work [20].

3. RESULTS AND DISCUSSION

In this section, the numerical simulations of nanoindentation and patch clamp experiments are presented, and the gating behavior of the *E.coli*-MscL is presented as a function of the external load.

3.1 Stress Field of Nanoindentation Experiment

The longitudinal and hoop stress contour plots are given in Fig. 6a, at the maximum penetration of 4 μ m. The evolution of the normalized hoop and longitudinal stresses at the equator, σ_1/E and σ_2/E , as a function of the normalized indentation depth (δ/R) and indentation load ($P/(E\delta^2)$), are presented in Fig. 7a. Here, δ is the indentation depth, P is the indentation load, and R is the cell diameter. Both longitudinal and hoop stresses are tensile at the equator and increase nonlinearly with indentation. At the maximum indentation depth used in this study, the hoop stress is about 15MPa higher than that in the longitudinal direction. Both stress components (as well as their evolutions) are carried over to the next stage of simulation where the gating of MscL is followed explicitly (see below).

3.2 Stress Field of Patch Clamp Experiment

In the patch clamp experiment, the normalized stress at the north pole increases nonlinearly with the normalized suction pressure, p/E . The suction pressure can be regarded as the difference between the pressure in the pipette and the external (atmosphere) pressure. The variation between the normalized equi-biaxial membrane stress σ_o/E at the north pole and p/E is given in Fig. 7b; the results are used to compute MscL gating at the relevant stress levels. In addition, the height of bulged membrane that has been sucked into the pipette is also given in Fig. 7b. To reach a maximum membrane stress of about 50MPa, a suction pressure of 0.09MPa is required. At the maximum pressure, the longitudinal and hoop stress contour plots are given in Fig. 6b.

3.3 Responses of the *E.coli*-MscL

The stress fields obtained from Sections 3.1 and 3.2 are applied as the boundary conditions in the nano-scale simulations of the conformational changes in MscL during the gating transition. The qualitative gating pathways and quantitative information of the evolution of MscL channel radius are presented and discussed.

3.3.1 Gating Pathways of MscL—In both experiments, both the pentagons formed by TM1 and TM2 helix bundles expand in the radial direction. Snap shots for the conformational transitions of the *E.coli*-MscL upon nanoindentation and patch clamp are shown in Figs. 8a and 8b, respectively, for both top and side views. Here, the fractional time (t) varies linearly with respect to the indentation depth in Fig. 8a, with $t=1$ corresponds to the maximum penetration ($\delta/R=0.82$ and $P/(E\delta^2)=0.00037$); in Fig. 8b t varies in proportion to the applied suction pressure, with $t=1$ corresponds to the maximum pressure at $p/E=0.00064$.

During the gating process in both experiments, the loops connecting TM1 and TM2 helices impose constraints on the sizes of the pore. Due to the strong interactions between TM1 and S1 helices as well as through the loops connecting them, the S1 helix bundle also expands in the radial direction, which leads to an increment of the pore size formed by the S1 helix bundle on the cytoplasmic side. In addition, thanks to the loops that connect the TM2 and S3 helices, the pore enclosed by the S3 helix bundle also enlarges a bit. In the closed state ($t=0$), the tips of TM1 and TM2 helices protrude outside of the lipid and the S1 helices are almost normal to the membrane. With the increase of external load (indentation force or suction pressure), membrane tension is developed and the transmembrane region expands and its thickness shrinks uniformly near the vicinity of protein (i.e. dilatation); consequently, both TM1 and TM2 helices become more tilted, and their projections in the direction normal to the membrane surface become shorter than the lipid thickness. In the cytoplasmic region, the S1 helices become more horizontal and they are lifted up towards the transmembrane region, whereas the orientations of S3 helices are essentially unchanged, except that the S3 helices move upward a little due to the loops connecting TM2 and S3 helices.

Clear differences are observed between the gating pathways of MscL in the patch clamp and nanoindentation experiments. Since the biaxial membrane stress components resulting from indentation are not equal to each other ($\sigma_1 \neq \sigma_2$ in Fig. 7a), when they are imposed at the boundary of lipid patch during the second stage of simulation (c.f. Fig. 2), the lipid cavity deforms into an elliptical shape. Through the non-bonded interactions between lipid and TM2 helices and those between TM2 and TM1 helices, the shape change of the cavity leads to an asymmetrical pentagon-shaped pore formed by the TM1 helix bundle. The asymmetrical ratio between the long and short axes of the pore is about 0.68. Likewise, the gate enclosed by the five S1 helices is also asymmetrical, with an asymmetrical ratio of about 0.36. In addition, the tilting angles of the transmembrane helices in the indentation experiment are slightly different among different helices. Even at the indentation depth of 80% of cell diameter, due to the biased pore evolution, the area enclosed by the MS channel is still somewhat smaller than that required for full gating – this indicates that it is more difficult to achieve fully opened channel via nanoindentation experiment or other mechanical perturbations of the similar pattern.

By contrast, during the patch clamp experiment, since the stress state is equi-biaxial surrounding the MscL at the north pole, the lipid cavity expands radially in a more uniform fashion. Accordingly, the pores enclosed by the five TM1 helices and five S1 helices also expand in a symmetric fashion. The variations of the tilting angles of the transmembrane helices are also essentially the same among different helices. Since the stress field is equi-biaxial, it is straightforward to increase suction pressure until full gating is realized in the MscL. At the instant of full gating, the contour plot of Mises stress is given for the protein in Fig. 8b. The locations of higher stresses (red) correspond to either higher local curvature due to helix bending, or the “load bearing” part of protein structure in order to maintain equilibrium with respect to the membrane stress. In general, the “load bearing” parts are located closest to the neighboring protein or lipid surface. If the mechanical properties of helices are changed (e.g. take into account inhomogeneous and anisotropic elastic

properties), we expect that some local curvature details will change but the loading bearing points should be robust.

In Fig. 8b, the simulation results of the fully opened channel are also compared with the structural model when the membrane is subjected to an equi-biaxial tension [11], where good agreements are found. The main difference is that the expansion of the pore enclosed by the S1 helices is smaller in the structural model; this may be in part due to the neglect of the solvation effect in the current model.

We note that in a previous version of the structural model [9], the S3 helices are lifted up into the membrane and become horizontal in the open state, which implies that the S3 bundle also plays an indispensable role during the gating process. In a later model [11], however, the S3 helices are essentially static during gating. Interestingly, the current simulation supports the newer structural model [11] and shows that the S3 helices are essentially unresponsive during the gating transition.

The protein conformational transitions discussed above are reversible, i.e. the system goes back to the original closed conformation once the load is removed, which indicates that the deformation of the channel follows closely the load across the membrane and the mechanical deformation of membrane plays a key role in mechanotransduction – this is consistent with patch clamp experiment [7].

3.3.2 Evolution of the Pore Radius—The size of the channel pore is a critical parameter, which determines the ion flux that passes through and can be estimated through measuring the electric current experimentally [7]. In principle, three valves can be formed by the TM1 helix bundle, S1 helix bundle and S3 helix bundle, respectively (Fig. 1), the transmembrane pore enclosed by the TM1 helix bundle is the most important one and once its radius reaches a critical value, the conductive state is changed into one that allows ion entry [7]. The transmembrane channel is of pentagon shaped when projected onto the membrane plane, and an effective pore radius is defined as the radius of a circle with the same area as this pentagon-shaped pore. In Fig. 9a, the increment of effective pore radius is plotted as a function of the fractional experimental time. Although the relationship between the pore radius increment and membrane strain is almost linear [20], due to the nonlinear coupling between membrane stress and indentation load (or suction pressure), the pore radius varies nonlinearly with fractional time.

The averaged tilting angle of TM1 helices (with respect to the membrane plane) is given in Figure 9b. The tilting angle decreases with the dilatation of membrane. Again, the variation is nonlinear with respect to fractional time because the membrane stress evolves nonlinearly.

3.3.3 Comparison between Simulation and Patch Clamp Experiment—In the previous patch clamp experiment [7], the Young's modulus of the membrane is about 18MPa; the maximum membrane tension is about 3.4MPa (measured when the suction pressure is about 60mmHg) and thus the membrane strain is about 13% when the gating probability is 50% [7]. In the present simulation, at a maximum suction pressure of 675mmHg, the maximum membrane stress is about 50MPa; since the Young's modulus of the DPPC membrane used in our simulation is 140MPa, the maximum strain is about 25% that corresponds to the fully opened state. Therefore, the membrane strain at which gating may be measured via experiment agrees well, at least qualitatively, between the simulation and patch clamp experiment.

No nanoindentation experiment on liposome is available in the literature. Nevertheless, since nanoindentation experiment is arguably the most convenient test on small structures, we hope the numerical simulations presented in this work will stimulate new experiments.

4. Concluding Discussions

A continuum mechanics simulation framework has been established to study the structural response of *E. Coli*-MscL to complex mechanical perturbations applied to the lipid membrane. In this framework, the protein and lipid membrane are described with continuum elements such as elastic rods, elastic springs and elastic sheets, which makes it possible to simulate long-time and large-scale processes such as the gating transition of the *E. Coli*-MscL. The framework is intrinsically hierarchical because the material properties and interaction parameters for the continuum elements are derived from atomistic studies. Compared to our previous proof-of-concept study [20], all essential structural elements have been included for the *E. Coli*-MscL, thus comparison to the previous results based on a simpler model makes it possible to investigate the role of specific structural elements. For example, upon membrane tension the gating is primarily realized due to iris-like expansion of TM1/TM2 helices in the radial direction, as well as tilting of these motifs. In addition, the S1 and S2 helices move into the transmembrane region, and S1 pore is also pulled open. The cytoplasmic S3 helices, however, only move toward the membrane slightly and the geometry of the bundle is essentially unchanged; this indicates that S3 helices play only a minor role during mechanotransduction. The loops connecting TM1/TM2 helices impose constraints on the pores enclosed by these two bundles, as well as regulating the tilting angles of the transmembrane helix subunits. Most of these observations from the continuum simulation, as well as the configuration in fully opened state, agree with the structural model [11].

The main advantage of the continuum mechanics approach is that it can effectively study the structural response of biomolecules or even cells under complex mechanical perturbation. Such advantage is demonstrated in this paper through the numerical simulation of the gating pathways of *E.coli*-MscL upon nanoindentation and patch clamp experiments, which are difficult to study by all-atom simulations. In both experiments, the membrane stress in the vicinity of the channel induced by external load (indentation load in nanoindentation or suction pressure in patch clamp experiment) is the most critical: once the lipid is deformed, through the non-bonded interactions between the lipid and helices, and that between the helices, the gating transition of MscL is observed and the pore radius increases nonlinearly with external load. The pore expands more uniformly in the patch clamp experiment, whereas during nanoindentation, due to the different membrane stress components, the pore evolves into an asymmetric pentagon shape.

Although the present study involves only one MscL located at a particular position in the spherical lipid vesicle, the versatile approach can be readily extended to scenarios involving the protein at different locations, deformation of a cell/vesicle containing multiple MS channels or different species of proteins and more complex loading configurations such as during cell-cell adhesions. In specific applications, it should also be straightforward to consider cells/vesicles of non-spherical shape and the viscoelastic properties of the cell membrane and cytoplasm; adhesion between the indenter, substrate, pipette, and cell can also be readily built into the continuum framework.

In terms of future developments, a relatively straightforward improvement is to incorporate inhomogeneity and anisotropy of the helices, since it is known that the stretching and bending stiffness of alpha-helix are independent of each other[22], the longitudinal and transverse elastic properties are different [28], and mechanical properties of different amino

acids may vary. In the continuum model, the deformation of MscL is primarily dominated by the interactions among various components (lipid, helices, etc.), evidenced by the iris-like expansion of the TM1 and TM2 bundles during gating, and the details of their elastic properties are less important although they could certainly change the local curvature of deformed helices. Incorporating such improvements does not impose a significant challenge other than carrying out more numerical fittings involving more material parameters, and we expect the main conclusions of this paper still hold following such improvements.

Another immediate task is to taking into account the effect of solvent since once the channel opens water may solvate the pore; solvation is expected to make a major contribution to the energetics of the gating transition [7]. In addition, thermal fluctuations need to be considered to make the gating behavior more realistic. Once a physically complete model is established, more quantitative results can be obtained in the continuum simulation framework, which we believe will not only uniquely supplement atomistic simulation studies but also stimulate new experimental investigations. We anticipate that this hierarchical continuum simulation framework will be an important addition to the tool sets that allow us to tease out the mechanistic details of a broad range of fascinating biomechanical processes that span multiple length and time scales.

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Fig. 1. Structural model [11] and continuum model of the *E. coli*-MscL. (a) Top view and (b) side view of the structural model in the closed state, (c) Top view and (d) side view of the continuum model in the close state.

Fig. 2. Biaxial tension of a small patch of membrane that embeds a MscL (400Å by 400Å, to be used in the second stage of simulation), plus a zoom-in view of the 10 petal flower-shaped lipid hole surface that hosts the protein. The tension stress can be equi-biaxial (σ_o) or non-equi-biaxial (σ_1 and σ_2).

Fig. 3. The vesicle indentation experiment: (a) before and (b) after deformation. The vesicle radius is $R=5\mu\text{m}$, and the vesicle thickness is $t_{memb} = 35\text{\AA}$. The indenter radius is $r=1\mu\text{m}$.

Fig. 4.

The path clamp experiment: (a) before and (b) after deformation. The vesicle radius is $R=5\mu\text{m}$, and the vesicle thickness is $t_{memb} = 35\text{\AA}$. The pipette dimension is $r=0.5\mu\text{m}$, $t_{pipette}=0.2\mu\text{m}$, and $\alpha=10^\circ$.

Fig. 5. Non-bonded interactions: comparison between atomistic molecular mechanics (MM) simulations with fitted parameters in Table 2. The x-axis is the normalized separation between the continuum components (with 1.0 being the equilibrium spacing).

Fig. 6. Normalized longitudinal and hoop stress contour plots for (a) nanoindentation and (b) patch clamp. Both shown in their maximum deformation state.

Figure 7 (a)

Figure 7 (b)

Fig. 7.

(a) The longitudinal and hoop stress variations at the cell equator upon nanoindentation, as a function of normalized indentation load and depth. (b) The equi-biaxial stress at the north pole of the cell upon patch clamp experiment, as a function of the normalized suction pressure and bulge height.

Figure 8(a)

Figure 8(b)

Fig. 8. The gating pathways of *E.coli*-MscL obtained from continuum simulation. (a) the indentation experiment. (b) the patch clamp experiment. The fractional time t is in proportion to the external load (it equals to 1 at the maximum load). In (b) the opened configuration of structural model [11] is also shown, which agrees well with that of the continuum simulation. Also shown in (b) is the Mises stress field of the helices at maximum gate opening, with red denotes higher stresses.

Figure 9(a)

Figure 9(b)

Fig. 9.

(a) The increment of effective MS channel radius, and (b) The averaged tilting angle of TM1 helices, as a function of the fractional time (or external load) during nanoindentation and patch clamp experiments.

Table 1

Material properties and geometry of the *E.coli*-MscL used in the continuum model.

Properties	Helices				Loops			Lipid
	TM1	TM2	Pro-43	S1	S3	TM1-TM2	TM1-S1	TM2-S3
Length (Å)	55.631	45.613	5.368	14.832	21.520	103	17	35
Thickness or diameter (Å)	5	5	5	5	5			35
Young's Modulus <i>E</i> (GPa) or Spring Constant <i>k</i> (N/m)	100	100	25	100	100	28	41	0.14
Poisson's ratio <i>v</i>	0.3	0.3	0.3	0.3	0.3			0.3

Table 2

Fitted parameters for the non-bonded interactions between different pairs*

Interaction parameters	Lipid-TM1	Lipid-TM2	Lipid-S1	TM1-TM1	TM1-TM2	S1-TM1	S1-TM2	S1-S1	S3-S3
d_0 (Å)	5.5	5.5	7.0	1.5	5.0	4.0	5.5	4.0	7.0
ψ (GPa)	3.2	3.2	0.05	6.5	3.4	3.0	0.8	9.0	6.0
m	9	7	4	2	9	8	10	9	11
n	3	3	1	1	3	3	3	4	6

*The interactions between TM2-TM2 is ignored because the TM2 helices are far apart ($>17\text{\AA}$); the interactions between S3-TM1, S3-TM2, S3-S1, and lipid-S3 are neglected because the S3 helices are far away from other components and such interactions play minor roles in the gating process.