Release of content through mechano-sensitive gates in pressurized liposomes

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Mechano-sensitive channels are ubiquitous membrane proteins that activate in response to increasing tension in the lipid membrane. They facilitate a sudden, nonselective release of solutes and water that safeguards the integrity of the cell in hypo- or hyper-osmotic shock conditions. We have simulated the rapid release of content from a pressurized liposome through a particular mechano-sensitive protein channel, MscL, embedded in the liposomal membrane. We show that a single channel is able to relax the liposome, stressed to the point of bursting, in a matter of microseconds. We map the full activation–deactivation cycle of MscL in near-atomic detail and are able to quantify the rapid decrease in liposomal stress as a result of channel activation. This provides a computational tool that opens the way to contribute to the rational design of functional nano-containers.
relatively high. At a similar submicrosecond loading rate (140 kN/m s), we observe the vesicular membrane of a pure lipid vesicle to rupture at an estimated membrane tension of (69 ± 1) mN/m, similar to the tension required for channel activation. Although the onset of both channel gating and membrane rupture are time scale dependent, the response of the bilayer appears faster; simulations performed with loading rates of 660 kN/m s do not lead to channel activation but rather to rupture of the membrane as in the case of the liposome without an embedded channel (Fig. S2). It is clear that in our simulations, MscL activates only at the very limits of membrane elasticity, in agreement with experimental findings (8, 17) for Tb-MscL.

Opening Mechanism Is Asymmetric. The observed mechanism of MscL opening follows roughly the proposed (9,11) iris-like model with the transmembrane helices reorienting more loosely and at a more pronounced angle from the membrane normal. Contrary to the beautiful, symmetric opening of the iris-like model, the free, nonbiased MscL seems to open in a distinctly asymmetric manner (Fig S3). The transmembrane helices tilt simultaneously, but independently, to accommodate the thinning of the membrane, while the N-terminal helices follow the movement of TM1, staying parallel to the membrane surface, and do not show any indication of either moving to block the channel or to line the rim of the channel as has been suggested (11). The surface area of the open channel (60 nm², including both protein and channel) is more than two times larger than that of the closed channel (26 nm²). The minimum radius of the open channel in the constricting region, estimated from the minimal distances between residues I14–V21 of opposing subunits, measures (11.6 ± 0.8) Å. This is smaller than the 15–20 Å estimated from conductance measurements including increasingly large “blocker” molecules (18, 19) and from thermodynamic consideration of in-plane area changes (8). It is plausible that the permeation of larger molecules occurs by forcing the channel to open even further than necessary for a pure water solvent, which would explain the difference.

Flow of Water Through the Channel Is Bidirectional. Our detailed model allows us to calculate the flow of water through the channel. The open channel conducts on average (4.4 ± 1.4) water molecules per ns in the outward direction. This is in disagreement with an earlier geometrical estimate (4) of 0.22 ns⁻¹, when ΔP = 0.1 bar, or >40 ns⁻¹ when applied to the current system with ΔP = 138 bar. It should be noted that their analytical estimate is based on a macroscopic theory of solvent flow through a cylindrical pipe, which is likely to break down for a nano-sized protein channel. Interestingly, we observe a bidirectional, competitive

Fig. 1. Mechano-sensitive channel of large conductance. X-ray crystal structure (white licorice) overlaid with a coarse-grained protein model (backbones shown in red; side chains in yellow). (A) Single subunit showing transmembrane helices TM1 and TM2, N-terminal helix S1, and the C-terminal helix, (B) complete MscL with all five subunits, (C) periplasmic view inside the channel, and (D) cytoplasmic view at the C-terminal helix bundle.

Fig. 2. Response of a vesicle to increased interior pressure with or without a mechano-sensitive channel (white) embedded in the liposomal membrane (orangegray). (A–C) An osmotic shock causes a liposome to swell and ultimately to pop when the liposome reaches its elastic limits. (D–F) In contrast, a liposome equipped with a mechano-sensitive channel, such as MscL, can survive the osmotic shock through a controlled release of excess solvent and solutes (blue) via the membrane channel.

Fig. 3. A MscL in a liposome, right at the point of channel activation. Excess solvent (blue) is released from the pressurized liposome interior through the protein that acts as a nano-valve. For clarity, external water is not shown, the protein is shown as a backbone trace on a transparent surface representation (white) with transmembrane helices depicted as cylinders (red), and some of the lipids are cut away to reveal the inside of the liposome.
flow in the simulation. The average unidirectional flows are found to be \((6.0 \pm 1.3) \text{ ns}^{-1}\) outward and \((1.7 \pm 0.3) \text{ ns}^{-1}\) inward, with peaks as high as \(40 \text{ ns}^{-1}\) outward and \(28 \text{ ns}^{-1}\) inward. It is remarkable that, despite the large interior pressure, both a substantial outward and inward solvent flux occurs. By comparing the average force pushing water molecules along the pressure gradient (22 pN) to the average force due to Brownian motion (121 pN), or the equivalent energies of 0.5 kJ/mol and 2.6 kJ/mol, respectively, it is clear that thermal noise still dominates the motion of water molecules.

Liposomal Stress is Relaxed on a Submillisecond Time Scale. After channel opening the simulation has been extended over a period of 40 μs to monitor the relaxation of the system. During this period the liposome undergoes a sustained venting of excess internal solvent that eventually relaxes the built-up liposomal stress. Fig. 4 quantifies the relaxation of liposomal stress, showing the temporal evolution of the liposome radius, the pressure difference between the inside and the outside of the liposome, the surface tension in the membrane, radius of the MscL channel, and details about the amount of solvent exchange. The radius of the liposome decreases from 9.7 nm to 8.4 nm and the inside volume from 2,195 nm\(^3\) to 1,071 nm\(^3\) approaching steadily the radius (8.0 nm) and volume (843 nm\(^3\)) of the initial, relaxed liposome. The amount of excess internal solvent (compared to the initial relaxed liposome) is reduced by 82%, and the level of mixing of internal and external solvent reaches 66%. During this time the membrane thickness (estimated by the average distance between the phosphate moieties in the lipid head groups) increases from 3.30 nm to 4.12 nm. For comparison, an equilibrated, tensionless DOPC membrane at 310 K has a thickness of (4.54 \pm 0.02) nm.

As more and more solvent is fluxed out of the liposome, the pressure difference across the membrane decreases from \((138 \pm 1) \text{ bar}\) to \((56 \pm 1) \text{ bar}\) toward the end of the simulation. At this point, the vesicular membrane is still under a considerable tension of \((23 \pm 1) \text{ mN/m}\), enough to keep the channel open. The radius of the channel fluctuates around 1.1 nm, a decrease of approximately 10% with respect to the largest opening observed during the first microseconds after gating. However, the channel is still in an active state with a total water flow of \((0.6 \pm 0.2) \text{ ns}^{-1}\) and unidirectional flows of \((1.2 \pm 0.2) \text{ ns}^{-1}\) outward and \((0.6 \pm 0.1) \text{ ns}^{-1}\) inward. The pressure difference as well as the surface tension decrease linearly after an initial, high-conductive period of approximately 8 μs. (Note the crossover from high-conductive state to a low-conductive state around 8 μs in Fig. 4.)
By extrapolating from this linear regime (Fig. S4), one can estimate that it takes \((86 \pm 4) \mu s\) to reach a tensionless state and \((93 \pm 4) \mu s\) to eliminate the pressure difference. These estimates are of course only valid if MscL stays open and continues to conduct at a steady pace. This, however, is questionable; in our simulation we already observe a partial dissociation of the C-terminal helix bundle, allowing the helices to intermittently enter the channel and to obstruct the free flow of solvent. By blocking the flux through the channel, the helices may give MscL an opportunity to constrict once again without the need of dewetting the channel first, pointing to a different role of the C-terminal helix than hitherto assumed (10, 11, 20) (see SI Text for details).

In conclusion, it is quite remarkable that a single MscL is capable of releasing a 138-bar pressure difference in a matter of microseconds. This study demonstrates that the simulation of the tension-induced gating of a biomimetic nano-container is feasible and provides a computational tool for the rational design of programmable drug delivery vehicles.

**Materials and Methods**

**System Setup.** MD simulations of MscL in model liposomes were carried out using a modified version (21) of GROMACS (22) with mean-field force approximation (MFFA) boundary potentials (software available upon request). The MFFA boundary potential is an effective potential that mimics the bulk water surrounding the liposome, with the computational advantage that most of the external water can be removed without any adverse effect on the properties of the liposome (21). The MARTINI coarse-grained force field (15) was used in conjunction with its recently released extension (16) for protein models. In the MARTINI model, an average of four atoms and associated hydrogens are mapped to an effective interaction site. Likewise, a CG water bead represents four water molecules. Previously the tension-induced gating of MscL embedded in a lamellar membrane was simulated (23) using the same MARTINI model.

The initial system was obtained by immersing the crystal structure of Tb-MscL in its closed state (4) in a spontaneously formed lipid vesicle and allowing the system to equilibrate. The approximately 16 nm diameter liposome contained 2,108 DOPC lipids and 5,444 water beads with an additional 54-649 water beads forming an approximately 4-nm water layer around the vesicle, enclosed by the MFFA boundary potential. The temperature of the system was kept constant at 310 K and the external pressure at 1 bar. MscL activation was triggered by gradually increasing the internal pressure of the liposome during 0.5 μs, followed by a 40-μs simulation of the active channel. For further details see SI Text.

**Increasing Internal Pressure.** The internal pressure of the liposome was gradually increased by adding more and more water into the liposome. This was achieved by having an additional MFFA potential in the center of the liposome that acted as a water piston capable of creating a water-fillable cavity inside the vesicle. This cavity was then filled with water, and the process was repeated. MscL activation tension was reached after 480 ns of “pumping” in six cycles (Table S1). To prevent the rapid loss of pressure through basal water flux across the membrane, the parameters of the lipid tails were adjusted slightly without affecting other membrane properties (Fig. S5 and Table S2).

The pressure inside and outside the liposome was computed from the average 3D pressure field across the system, a method we have developed (24) recently. The surface tension in the membrane was estimated using the Laplace equation (25, 26)

\[
\Delta P = \frac{2\gamma}{r}
\]

where ΔP is the pressure difference, γ the surface tension, and \(r\) the radius of the liposome. A similar estimate of the tension was obtained from the mechanical definition of tension (Fig. 5G). See SI Text for details.

**Limitations of the Model.** To put our results into perspective, it is important to reflect on some of the limitations underlying our coarse-grained model. In the MARTINI force field, the secondary structure of the protein is constrained. Possible unfolding/folding events of MscL during the gating are therefore not considered. Tertiary structural changes, however, proceed in an unbiased way. The iris-like gating mechanism observed in our simulations results from the specific protein–protein and lipid-mediated interactions. These interactions have been parameterized in MARTINI based on reproduction of thermodynamic data for small building blocks. The four-to-one mapping scheme of MARTINI allows the keeping of chemical specificity, although the directionality of, e.g., hydrogen bonds is lost. The specificity retained in the model is good enough, for instance, to reproduce the dimeric structure of transmembrane helices (27, 28) and allows us to discriminate between several MscL mutants (Table S3 and Fig. S7) as described in detail in SI Text.

Another point warranting discussion is the time scale. Due to the coarsening of the interaction potentials, the dynamics is faster than in corresponding all-atom models. In most applications as well as in the current manuscript, a factor of four has been applied to provide an approximate time scale. This factor is based on the speedup of diffusion rates for water and lipids when modeled with MARTINI (15). However, this factor only accounts for the neglect of friction from the missing atomistic degrees of freedom. One should realize that the dynamics of MscL structural reorganizations is largely determined by the energy barriers of the transition states along the gating pathway, which are easily under- or overestimated by several kT, potentially leading to orders-of-magnitude shifts in the observed dynamics. Concerning dynamical aspects, the simulations should be considered as qualitative only.

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