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Vibrational Spectra of a Mechanosensitive Channel

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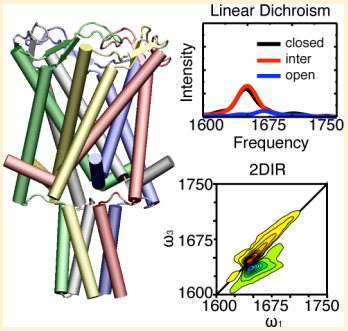
Supporting Information

ABSTRACT: We report the simulated vibrational spectra of a mechanosensitive membrane channel in different gating states. Our results show that while linear absorption is insensitive to structural differences, linear dichroism and sum-frequency generation spectroscopies are sensitive to the orientation of the transmembrane helices, which is changing during the opening process. Linear dichroism cannot distinguish an intermediate structure from the closed structure, but sum-frequency generation can. In addition, we find that two-dimensional infrared spectroscopy can be used to distinguish all three investigated gating states of the mechanosensitive membrane channel.



SECTION: Biophysical Chemistry and Biomolecules

Mechanosensitive (MS) channels, which respond to the physical forces applied on a cell membrane, belong to a specialized class of membrane proteins.[1] Once the inner pressure of the cell grows, these channels are sensitive to the change in surface tension in the cell membrane, and start to open a pore. Thus, this mechanism rapidly reduces the inner pressure and ultimately prevents the cell from lysis. During the opening process, MS channels undergo large conformational changes, where a pore existing in the open state is formed by tilting and sliding of the transmembrane helices.[2] Following the solution of its crystal structure,[3] the *Mycobacterium tuberculosis* MS channel of large conductance (Tb-MscL) has become well-known in the family of MS channels. There are five identical protein chains forming a pentamer structure in Tb-MscL, with each subunit containing two bundles of transmembrane helices known as TM1 and TM2 (Figure 1). The TM1 helix lies around the channel pore forming a constriction near the pore entrance in the cytoplasmic domain. The TM2 helix connects to TM1 through a loop lying on the water/membrane interface of the extracellular domain, and it surrounds the TM1 bundle outwardly.

It has been proposed that chemically modified versions of MS channels may be used for targeted drug delivery.[4] For such applications, a better understanding of the gating mechanism in these channels is needed. This has already been investigated both theoretically[5−7] and experimentally.[8−11] However, it is currently not possible to connect experimental observations with microscopic structural changes during the gating process. To achieve this, experiments that are fast enough to be applied during the opening process are needed. Currently applied patch clamp techniques are not fast enough to catch the dynamics during an opening event.[11] Vibrational spectroscopies are good candidates for this, as they can be applied with subnanosecond shutter speed. Vibrational spectra are, however, congested and challenging to interpret. They cannot be directly inverted to give a detailed atomistic picture. The analysis therefore has to rely on molecular dynamics (MD) simulations combined with spectral simulations. Here we will investigate whether vibrational spectroscopies can indeed be expected to distinguish between different structures expected during the gating process.

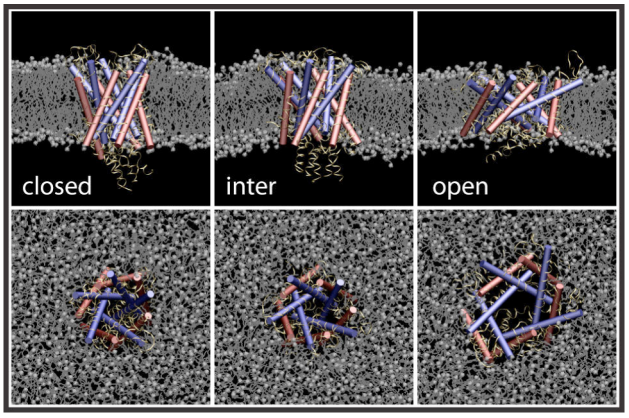


Figure 1. The three simulated structures. Proteins are shown as yellow ribbons. The transmembrane helices TM1 and TM2 are highlighted as blue and red cylinders, respectively. Lipid head groups and tails are shown as gray beads and lines, respectively. For clarity, water molecules in the simulation systems are not shown.

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The amide I vibrational band dominated by the backbone CO-stretch vibration is the dominant target of vibrational spectroscopy of proteins, as this band is strong due to the large CO-stretch transition dipole.[12] At the same time, the large transition dipole leads to quite strong coupling between different residues resulting in delocalized vibrations that depend on the structural arrangement.[12−14] We will thus in this letter consider the amide I region of the vibrational spectrum. Fourier transform infrared (FTIR) methods to measure the linear absorption are cheap and efficient. Such measurements can be performed on liquid samples where the proteins are inserted in liposomes. This type of spectroscopy is, however, often not very sensitive. Linear dichroism (LD) and sum-frequency generation (SFG) spectroscopies are more demanding experiments as they require ordered samples, where the proteins are inserted into thin membrane layers. This requires more complicated sample preparation and typically also longer measuring times, as fewer proteins will be present in the sample volume. These methods are, however, known to be very sensitive to differences in the tilting angles of the chromophores.[15−18] Two-dimensional infrared (2DIR) spectroscopy is a new type of infrared spectroscopy that involves the combination of pump and probe laser pulses[19] and is more sensitive to vibrational couplings and relative orientation and thus more sensitive to structural changes than linear spectroscopies.[16,18,20,21] Similar to FTIR, this method does not require ordered samples. In the following, we will simulate the signals for these different spectroscopies using recently developed methods to combine MD and spectral simulations.[22−24] We will do this for three different states predicted to be present during the gating process[25] to answer whether one can distinguish these with vibrational spectroscopies. Furthermore, we will predict the spectral differences that will be useful for guiding and interpreting future experiments.

We first performed all-atom MD simulations on three different states of the Tb-MscL channel. These states, denoted as the closed, intermediate (inter), and open structures, are reconstructed[26] from previous coarse-grained simulations[25] (see Figure 1). The closed state corresponds to the crystal structure relaxed in the membrane environment. The intermediate state is characterized by tension-induced expansion of the periplasmic side that, however, does not open the channel, and no water can pass through the channel in this state. In the open state, the cytoplasmic side is also expanded, the hydrophobic lock is broken, and the channel is able to conduct water, ions, and small solutes. For a more detailed description of these states, we refer to refs 25 and 27. After obtaining the atomistic structures, we modeled their FTIR, LDIR, SFG, and 2DIR spectra from 2 ns long production trajectories. All further simulation and modeling details are described in the Computational Details section of the Supporting Information.

The FTIR and LDIR spectra are shown in Figure 2. The FTIR spectra of the three states are very similar, with a slight blueshift of the main peak of the open state as the most significant difference. One can thus expect that it will be very difficult to distinguish between the different states of the MS channel using FTIR.

Where FTIR is an absorption measure on an isotropic ensemble, LDIR spectroscopy measures the difference between the absorption parallel ($A_{\parallel}$) and perpendicular ($A_{\perp}$) to the membrane surface normal. It is expected to be sensitive to the orientation of the amide I transition dipole which is mainly

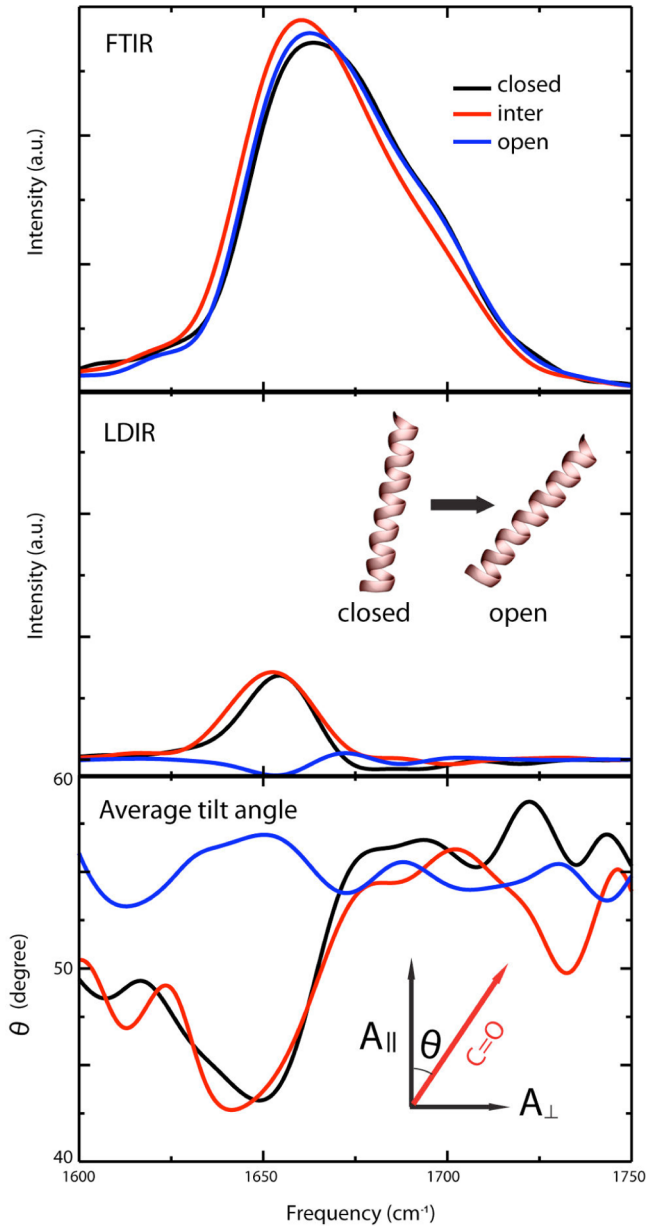

Figure 2. The FTIR, LDIR spectra, and average tilt angle.

parallel to the transmembrane helices. During the opening process, the tilt angles of the transmembrane helices increase, which results in decreasing the parallel absorption and increasing the perpendicular absorption with respect to the membrane surface normal. Therefore, the LDIR spectrum intensity of the open state is much lower than that of the closed and intermediate states. LDIR spectroscopy can therefore easily distinguish between the closed and open states. The average tilt angle θ of the amide I transition dipole can be extracted from the relationship between the FTIR and LDIR spectra (see the Computational Details section of the Supporting Information). It is shown as a function of frequency in Figure 2. At 1650 cm^{-1}, where the FTIR and LDIR peaks arising from the transmembrane helices are located, θ for the open state is about 15° larger than that of the closed and intermediate states. As the amide I transition dipoles are mainly parallel to the transmembrane helices, changes in θ should reflect the change of the tilt of the transmembrane helices. This correlates well

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with the structural changes observed in the simulations. Thus the LDIR spectra reveal the structural change of the transmembrane helices and can distinguish the open states from the closed and intermediate state. It can not, however, distinguish the closed and intermediate states from each other.

The resonant SFG response functions of the Tb-MscL channel are shown in Figure 3. These response functions

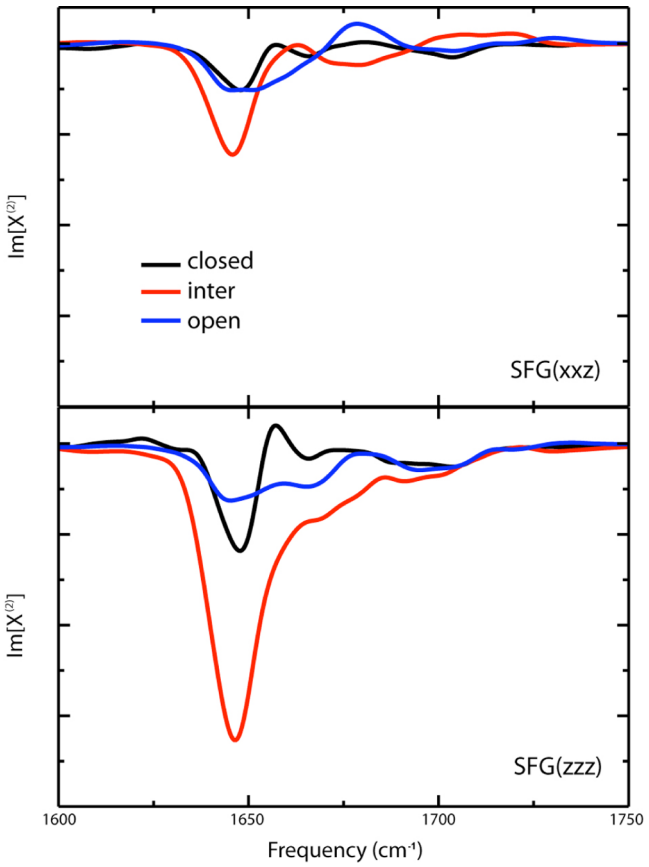

Figure 3. The SFG response functions of three states of Tb-MscL with two different polarization schemes (xxz and zzz).

determine the SFG spectra, which, however, depend on fine experimental details, such as angle of incidence and on the detection technique.[17] Here we present the resonant response functions that determine the spectral features and that are not restricted to a specific experimental setup. We note that the common homodyne-detection technique provides a highly convoluted signal, where the underlying response functions are challenging to disentangle, whereas the heterodyne-detection technique allows detecting a signal that quite closely reveals the underlying response functions.[28]

In general, the SFG response is negative in most of the frequency range, which shows that there are more amide I transition dipoles pointing downward along the channel axis than the other way. The SFG response of the intermediate state contains a sharp negative peak at 1645 cm^{-1}, while the negative signal at this frequency of the closed and open states is less pronounced. The high frequency peaks are complicated due to the cancellation between positive and negative signals, which depends on the orientation of the amide I transition dipoles. Spectra of the individual structural elements are presented in Figure S2 (see Supporting Information). The transmembrane helices TM1 and TM2 have strong positive and negative contributions around 1645 and 1655 cm^{-1}, respectively. The sign differences arise because the amide I transition dipoles along TM1 and TM2 helices are pointing in opposite directions. The SFG response of the separated TM2 in the intermediate state are red-shifted and have a stronger negative signal than that of the closed and open state, which results in stronger negative signal of the overall SFG response of the intermediate state, because the interference with the positive TM1 signal is less pronounced at 1645 cm^{-1} in the intermediate state. SFG spectroscopy is thus capable of distinguishing the intermediate state of the Tb-MscL channel from the closed and open structures, but the latter are harder to distinguish from each other.

The simulated 2DIR spectra with parallel and perpendicular polarization are shown in Figures 4 and 5, respectively. For a

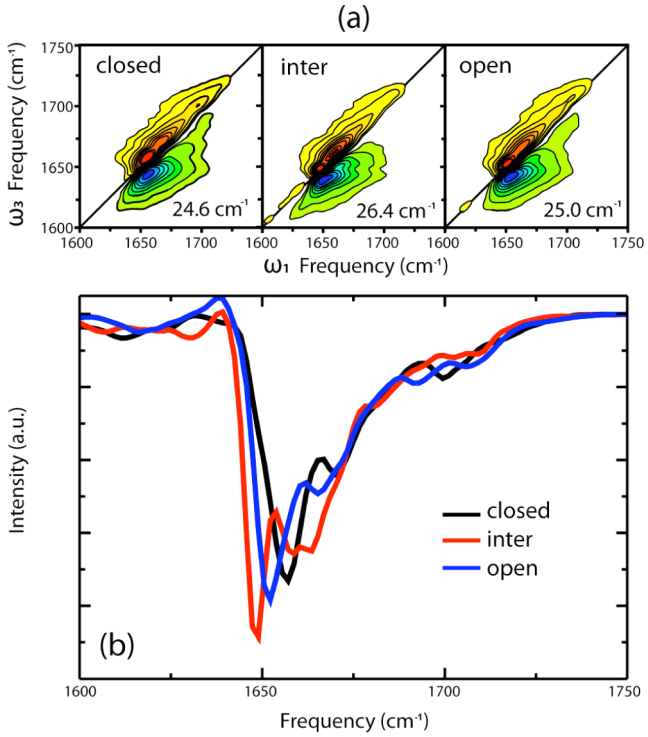

Figure 4. (a) The parallel polarization 2DIR spectra normalized by the maximal intensity amplitude. Each color contour represents 10% of the maximal amplitude. The horizontal axis gives the pump frequency ω_1, and the vertical axis gives the probe frequency ω_3. The overall fwhm diagonal line width of each state is shown in the right bottom corner of the spectrum. (b) The diagonal slices of the 2DIR spectra with identical normalization to allow comparing the peak intensities.

clear comparison, we also extract the diagonal slices of the 2DIR spectra as also shown in the these figures. The spectra are very extended along the diagonal, which indicates that different amide I sites experience very different surroundings.[20] By measuring the overall diagonal line width (the full width at half-maximum (fwhm) is reported in Figures 4 and 5), one can determine the system's disorder quantitatively, which reflects the membrane protein's structural and environmental heterogeneity. By examining the contributions from different structural elements, one finds that the TM1 and TM2 helices mainly give rise to rather sharp peaks at low frequencies typical for α-helices,[13] while the other elements give rise to the diagonally broad feature (see Figures S3, S4, and S5 of the Supporting Information for details). This leads to the

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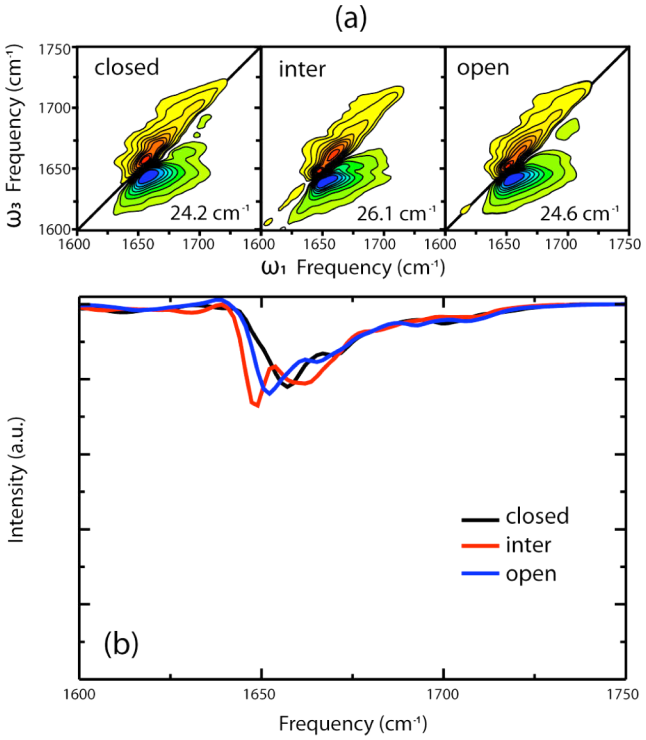

Figure 5. (a) The perpendicular polarization 2DIR spectra normalized by the maximal intensity amplitude. Each color contour represents 10% of the maximal amplitude. The horizontal axis gives the pump frequency ω_1, and the vertical axis gives the probe frequency ω_3. The overall fwhm diagonal line width of each state is shown in the right bottom corner of the spectrum. (b) The diagonal slices of the 2DIR spectra with identical normalization to that in Figure 4 to allow comparing the peak intensities.

interpretation that the diagonal elongation is due to the presence of many different fixed local environments mostly present in the more disordered parts of the protein. In the intermediate state, the first peak at low frequency (1650 cm^{-1}) is narrower than in the closed and open states. This arises from changes in the TM2 helix configuration (see Figures S3, S4, and S5 in the Supporting Information). The second peak (between 1655 and 1675 cm^{-1}) is more pronounced in the intermediate state than in the closed and open states. This is because the spectrum of the individual TM1 helices is broader in the intermediate state than in the closed and open states (see Figures S3, S4, and S5 in the Supporting Information). The observed spectral changes thus primarily originate from changes in the TM1 and TM2 helix configurations.

While the differences in the 2DIR spectra may seem subtle, they are much larger than the changes that have previously been used to characterize protein aggregation[29] and temperature dependent unfolding,[30] and to determine structure by studying individually isotope labeled sites in proteins.[18] Here we have only discussed the parallel polarization 2DIR spectra. The perpendicular 2DIR spectra are often considered to be more sensitive to vibrational couplings.[13,14] We did not find significant differences except that the perpendicular 2DIR spectra are 3 times weaker. We thus find that 2DIR spectroscopy is capable of distinguishing between all three different states of the Tb-MscL channel examined.

In summary, we have modeled the IR spectra of an MS channel and illustrated that LDIR spectroscopy is sensitive to the orientation of the transmembrane helices, which is tilting during the opening process, so that the open state spectrum has much lower intensity. SFG spectroscopy can be used to distinguish the intermediate state, because it is sensitive not only to the magnitude of the tilting, but also to the direction of the amide I transition dipoles changing their orientation along the channel axis. We also showed that 2DIR spectroscopy is capable of distinguishing all three studied gating states, because the structural changes of the transmembrane helices result in different lineshapes. The advantage of the 2DIR experiment is that the samples can be prepared in an isotropic solution containing micelles/liposomes with MS channels, which is much easier than preparing samples where the MS channels are orientationally ordered, as is necessary for the LDIR and SFG measurements. Such micelle/liposome solutions are also closer to the situation in vivo. In the present study, we limited ourselves to studying relatively short trajectories of three representative structures for the opening process of the MS channel pore. In reality, many more structures may be present and of importance. However, we have shown that infrared spectroscopies can be applied to distinguish between three essential states, and it may be able to distinguish others as well. Future experimental studies will probably require additional spectral simulations to aid the interpretation. Furthermore, future studies may make use of advanced isotope labeling techniques allowing one to highlight protein segments by shifting the spectrum of a complete segment to lower frequencies.[31] In this way, structural changes in the individual structural segments may be highlighted. Such studies may also make use of the spectral variations with temperature[32] and advanced line shape analysis.[33] MS channels have been engineered to contain chemical groups that allow opening them using phototriggering.[4] We propose studying those with LDIR, SFG, and 2DIR for following the structural changes induced by phototriggering. Our results suggest that such experiments will provide more detailed information on the MS channel gating mechanism than phototriggering combined with FTIR.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details on the computational methods of all-atom MD simulations and the spectrum modeling is provided together with spectra of the different substructures. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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