BL-1A, BL-5A, BL-17A, NW12A and NE3A/ 2014G179

# Crystal structures of a large-conductance mechanosensitive channel captured in two different conformational states

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## 1 Introduction

Mechanosensitive Channels of Large Conductance (MscL) and Small Conductance (MscS) are the emergency pressure-release valves protecting bacteria against acute hypo-osmotic challenge from the environments [1]. They exist mainly in bacteria and archaea, and some MscS homologs are also found in plants [2]. MscL has a large conductance at about 3 nS, around three times of MscS conductance. MscL and MscS are well-recognized model systems for studying the molecular mechanism of mechanosensation. MscL forms a homopentameric channel, and each monomer consists of a N-terminal amphipathic helix (N-helix) parallel to the membrane, two transmembrane helixes (TM1 and TM2) and a C-terminal helix at cytosolic region (C-helix). MscL responds to membrane tension at a level close to the lytic limit of the membrane. It will open a large non-selective pore with a diameter at about 30 Å [3]. The transition from closed to full open state requires large conformational changes and rearrangement of the protein. So far, the closed-state structure of MscL was solved with the homolog from *Mycobacterium tuberculosis* at pentameric state [4], while its expanded-state structure was obtained with a *Staphylococcus aureus* homolog at tetrameric state [5]. Toward a full understanding of the molecular mechanism of MscL gating, it is indispensible to obtain structures of MscL trapped at different conformational states, ideally from the same species and of the same oligomeric state.

## 2 Experiment

*Protein expression and purification*

The *mscl* gene from *Methanosarcina acetivorans* was fused with an exogenous gene encoding a soluble protein named riboflavin synthase of *Methanocaldococcus jannaschii* (MjRS) through overlapping PCR. The fusion gene was ligated into pET-15b vector. Expression of the fusion protein was carried out in C41(DE3) cells with 1 mM IPTG for 3 h at 37℃. The cells were harvested and the membrane protein was extracted by 1.5% Triton X-100. Purification of the target protein was achieved through nickel-affinity chromatography in solutions with nonyl-β-D-maltoside (NM). The eluted protein from nickel-column was concentrated and further purified by passing through the gel filtration column Superdex 200 10/300 GL. The protein was concentrated to 10-20 mg/ml for crystallization trials.

*Crystallization*

The crystal of closed-state MaMscL-MjRS was obtained through sitting-drop vapor diffusion method by mixing 17 mg/ml protein with a reservoir solution containing 7% PEG4000, 0.4 M $NH_4SCN$ and 0.1 M citric acid (pH4.3) at 1:1 (v:v) ratio. The expanded-state MaMscL-MjRS fusion protein was crystallized through hanging-drop vapor diffusion by mixing 1μl 15 mg/ml protein with 1 μl of a reservoir solution containing 1.6 M $(NH_4)_2SO_4$, 0.1 M NaCl and 0.1 M HEPES (pH 7.5).

*Data collection and structure determination*

Screening of crystals were performed on BL1A, BL5A, BL17A, NE3A and NW12A beamlines at PF. A complete dataset of the expanded-state MaMscL-MjRS were collected at BL-17A of PF, while the data for the closed-state structure was collected at BL17U of Shanghai Synchrotron Radiation Facility (SSRF). The structures were solved through molecular replacement and refined by using CNS and Refmac programs.

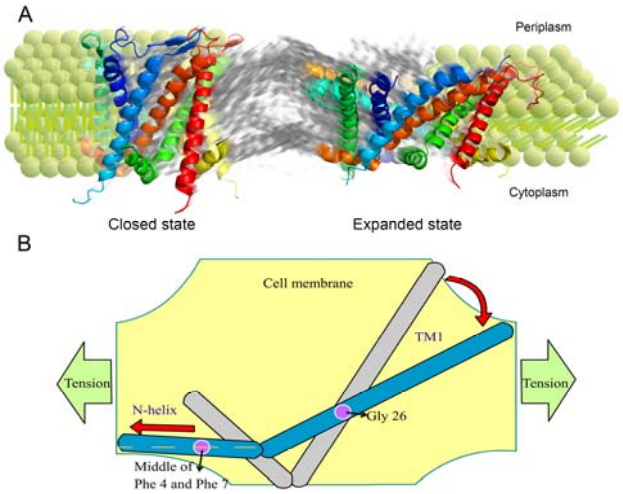


**Fig. 1 Structures of MaMscL at two different conformational states**. (A) Structures of MaMscL in closed (left) and expanded (right) states. (B) A model describing the coupling between N-helix and TM1. The gray rods shows TM1 and N-helix in the closed state structure and the blue one represents the expanded state.

## 3 Results and Discussion

MscL is well-known for the difficulty to yield high-quality crystal sample due to the fact of being an integral membrane protein with high flexibility. MaMscL naturally lacks the C-helix (unlike the other homologs such as the one from *E. coli*), suggesting it is probably more likely to be trapped in an expanded state [5]. To promote crystallization of MaMscL, we have screened a number of different soluble proteins being fused with

MscL. MjRS was identified as the one forming a stable fusion protein with MaMscL. It provides restraints limiting the flexibility of MaMscL channel without abolishing its function. As a consequence, MjRS contributes a large scaffold mediating crystal packing interactions, and may also helps to stabilize the channel at two discrete states. After screening hundreds of crystals, we have obtained two distinct crystal forms of the fusion protein diffracting X-ray to 3.5 Å and 4.1 Å resolution. One form is with MaMscL stabilized at the closed state, and the other contains MaMscL trapped at the expanded state (Fig. 1A).

By analyzing the two structures, we have found that the expansion of MscL was achieved through dramatic tilting of the two transmembrane helices (TM1 and TM2). Tilting of TM1 and TM2 is coupled to a pivoting motion of N-helix toward membrane plane and stretching of the periplasmic loop between TM1 and TM2. The N-helix is an amphipathic α-helix positioned at membrane surface and is known for its role to serve as an anchor to the membrane. Through the structural studies, we have raised a model describing the coupling mechanism between N-helix and transmembrane helices (Fig. 1B). In this model, N-helix rotates and slides along the membrane plane, at the same time it pulls on TM1 so that it will tilt around the pivot point on Gly26. TM1 forms a rigid-body anti-parallel pair with the adjacent TM2 (TM2') and they will tilt together toward the membrane plane. The overall motions of TM1, TM2 and N-helix resemble the opening process of an umbrella, with the N-helices serving as ribs and TM1-TM2' pairs acting like the stretchers [6].

The PDB codes for the two structures of MaMscL-MjRS fusion protein are 4Y7K (closed state) and 4Y7J (expanded state).

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References

[1] N. Levina, *et al*., *EMBO J*. **18** ,7(1999).
[2] E. S. Haswell, *et al. Structure.* **19**, 10 (2011)
[3] C. C. Cruickshank, *Biophy. J.* **73**, 1925 (1997).
[4] G. Chang, *et al*., *Science* **282**, 2220 (1998).
[5] Z. Liu, *et al*., *Nature* **461**, 120 (2009).
[6] J. Li, *et al*., *PNAS* **112**, 10726-10731 (2015)

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