Structure determination of Cx26/Calmodulin/Ca^{2+} complex using NCMN system

Intercellular communication is crucial for cellular processes in the human body. To that end, gap junction channels (GJCs) directly regulate the cell-to-cell exchange of ions, metabolites, and second messengers. Mutations of one such channel, connexin 26 (Cx26), can cause severe hearing loss. Although several structures in open states of connexin family members are reported, the close state is still elusive, which could be essential for understanding channel gating and dysfunction of Cx26 variants. Calcium and calmodulin as a chemical modulator play a crucial role in regulating Cx26 gating. The calcium-calmodulin Cx26 complex structure may imply a plausible gating mechanism. However, there is no complex structure available.

Additionally, the known structures of Cx26 within the endogenous lipid environments are also missing because detergents often lead to over-delipidation. Moreover, we are specifically interested in the Cx26-R75W mutant, which is typically expressed and trafficked onto the plasma membrane but cannot function properly as a channel to transfer the dyer based on the biochemical data. This evidence suggests that some conformation changes of Cx26-R75W may cause the dysfunction of gating. High-resolution atomic structure data for Cx26 that includes the endogenously associated lipids at a close status is crucial for understanding this syndrome and raising a novel strategy for its possible treatment. We have determined the single-particle cryo-EM structures of Cx26 in the presence and absence of calcium ions at 2.6 and 2.8 Å, respectively, using the data sets collected at NCCAT on Mar 12 and Oct 13, 2020. We recently successfully purified the potential Cx26 complex with human calmodulin. Compared with the single particles of Cx26 in the absence of calmodulin and calcium ions (Fig. 1A), the Cx26/calmodulin/Ca^{2+} complex particles look like in a close state (Fig. 1B). The representative particles in green squares (Fig. 1B) showed that the Cx26 channels have no clear holes, suggesting they are in a close state. This contrasts with the representative particles of Cx26 in the absence of calmodulin and calcium ions (Fig. 1A) that showed clear holes in the center of Cx26 as marked by red squares. Then we hypothesized that human calmodulin interacts with Cx26 and in the presence of calcium ions and drives the channel into a close state.
We have prepared the Cx26/calmodulin/ Ca2+ complex in good quality, as shown in Fig. 1B and Fig. 2. We plan to collect a cryo-EM data set for structure determination. Based on this new structural information, we might provide a plausible gating mechanism to understand the structural basis of Cx26 gating, which could be a universal phenomenon within the connexin family members.

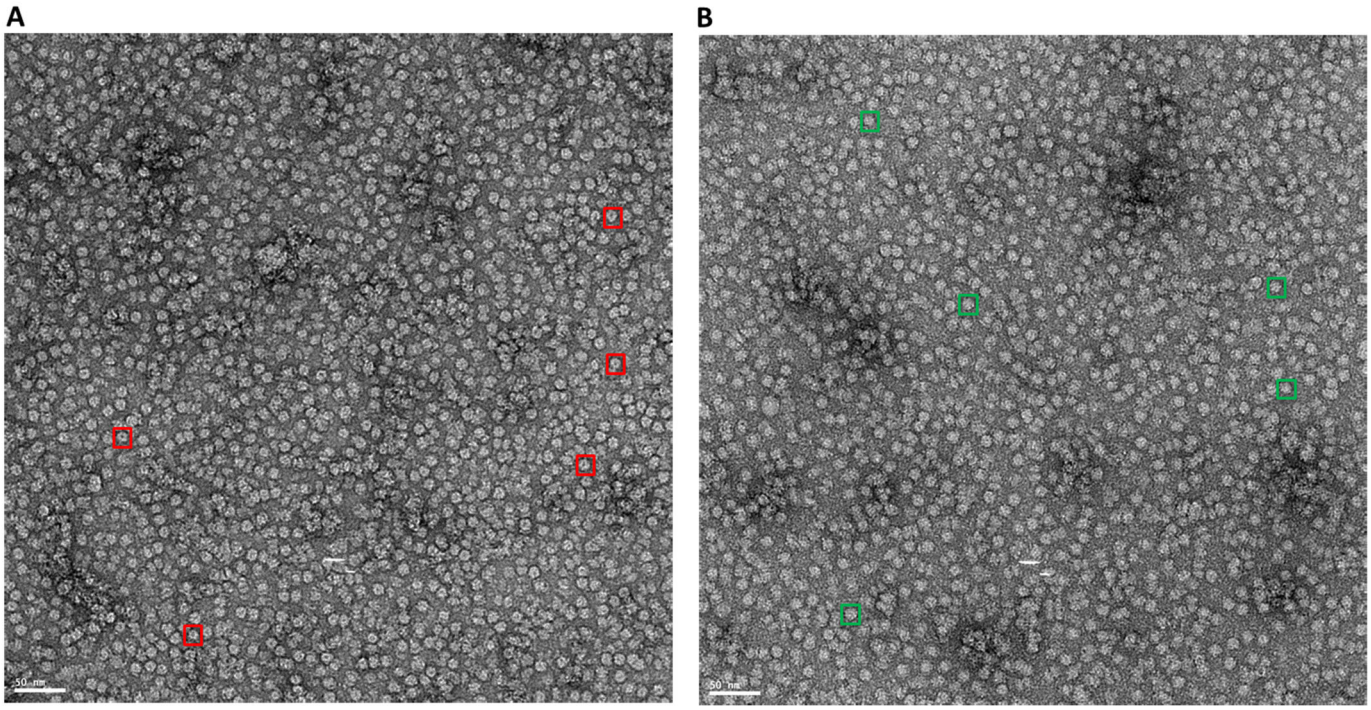

Fig. 1. Representative micrographs of negative stain analysis of Cx26 in the presence of 3 mM calcium ions without calmodulin (A) and with calmodulin (B). The micrograph in Fig. A showed Cx26 with obvious central holes in red squares. In comparison, the micrograph in Fig. B showed the homogenous particles without the holes in the center in green squares.

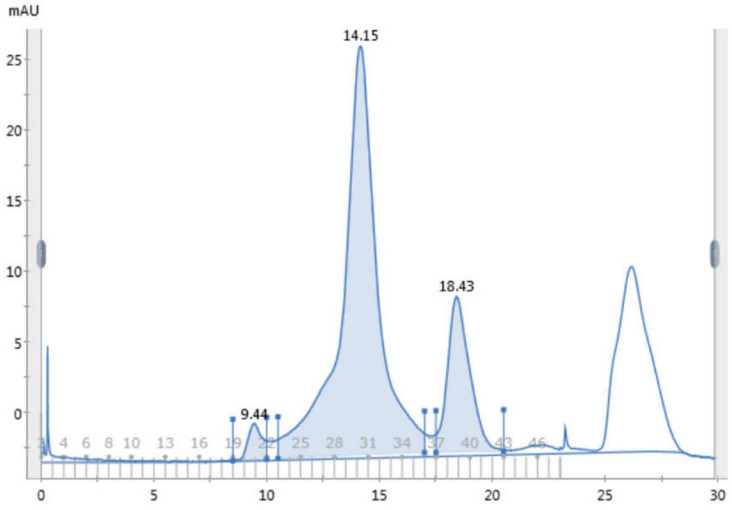

Fig. 2. Size exclusion column purification profile of Cx26/Calmodulin/Ca^{2+} complex.
The elution peak at 14.15 mL represents the Cx26/calmodulin/Ca2+ complex, the elution peak at 18.43 mL represents the free calmodulin/Ca^{2+}.