Single-particle Cryo-EM study of human connexin 26 channel in the form of native cell membrane nanoparticles

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Maintenance of tissue and organ homeostasis through the cellular communication is vital for multicellular organism. One type of rapid communication between cells is mediated by gap junctions. Gap junctions channels form by two single hemichannels, each formed by six connexin proteins. More than 20 different connexins have been identified in human. The importance of gap junction channels is highlighted by plethora of pathologies known to directly result from gene mutation of the connexin protein. M34T mutation in connexin 26 (Cx26) is well known to be associated with hereditary hearing loss[1]. A 3.5 Å crystal structure of Cx26 has been reported[2]. The crystal structure is a significant step toward understanding the properties of gap junction channels. However, the crystal structure didn't provide any structural information of the important protein-lipid interaction of this membrane protein channel.

For membrane proteins interactions with membrane lipids often have dramatic functional and structural consequences[3-11]. For connexin channels this is no doubt true; A number of lipids which associated with Cx26 hemichannel and gap junctions have been identified which includes anionic lipids as well as cholesterol[9].We have shown that detergent-based method for membrane protein structural biology has significant drawbacks in that detergents can damage the important protein-lipid interaction which are often crucial for understanding the active mechanism of the function of membrane protein[11].

The structural conformation and function of Cx26 is regulated by both calcium and pH conditions[12, 13]. More high-resolution structures of Cx26 is warranted to understand its active mechanism. For the purpose of our detergent – free method development, Cx26 is also an ideal model protein because we can use it to test our developed membrane active polymers that are compatible to divalent ions and various pH conditions. Structures of multiple conformations of Cx26 in different conditions will also provide us more detailed structural information to understand this important channel. Currently, we have successfully prepared Cx26 samples in the form of native cell membrane nanoparticles with our development detergent-free system. We have also screened our sample using both negative stain EM (**Figure 1**) and single particle cryo-EM (**Figure 2**). Our preliminary data tell us that it is very promising to obtain high-resolution cryo-EM structure of Cx26 in truly detergent-free native cell membrane nanoparticles given access to Krios time. The study is important not only for technology development but also in understanding the biological function of this important membrane protein channel.

Titan Krios time request

It will be ideal to have a 24 hour or more data collection time.

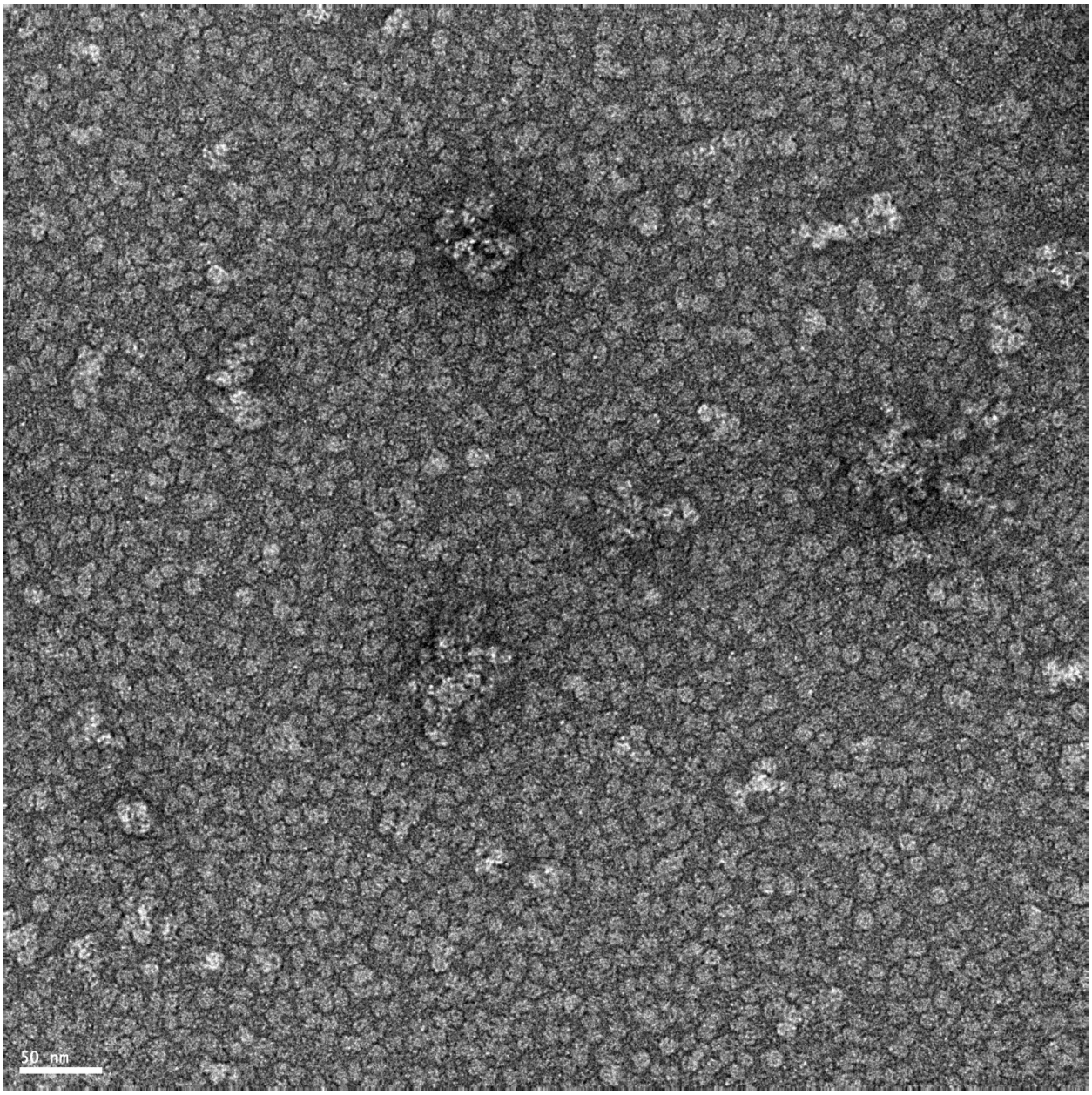

Figure 1. Negative stain of Cx26 native cell membrane nanoparticles

The negative stain EM image was taken at UVA on FEI Tecnai F20, equipped with a CCD camera.

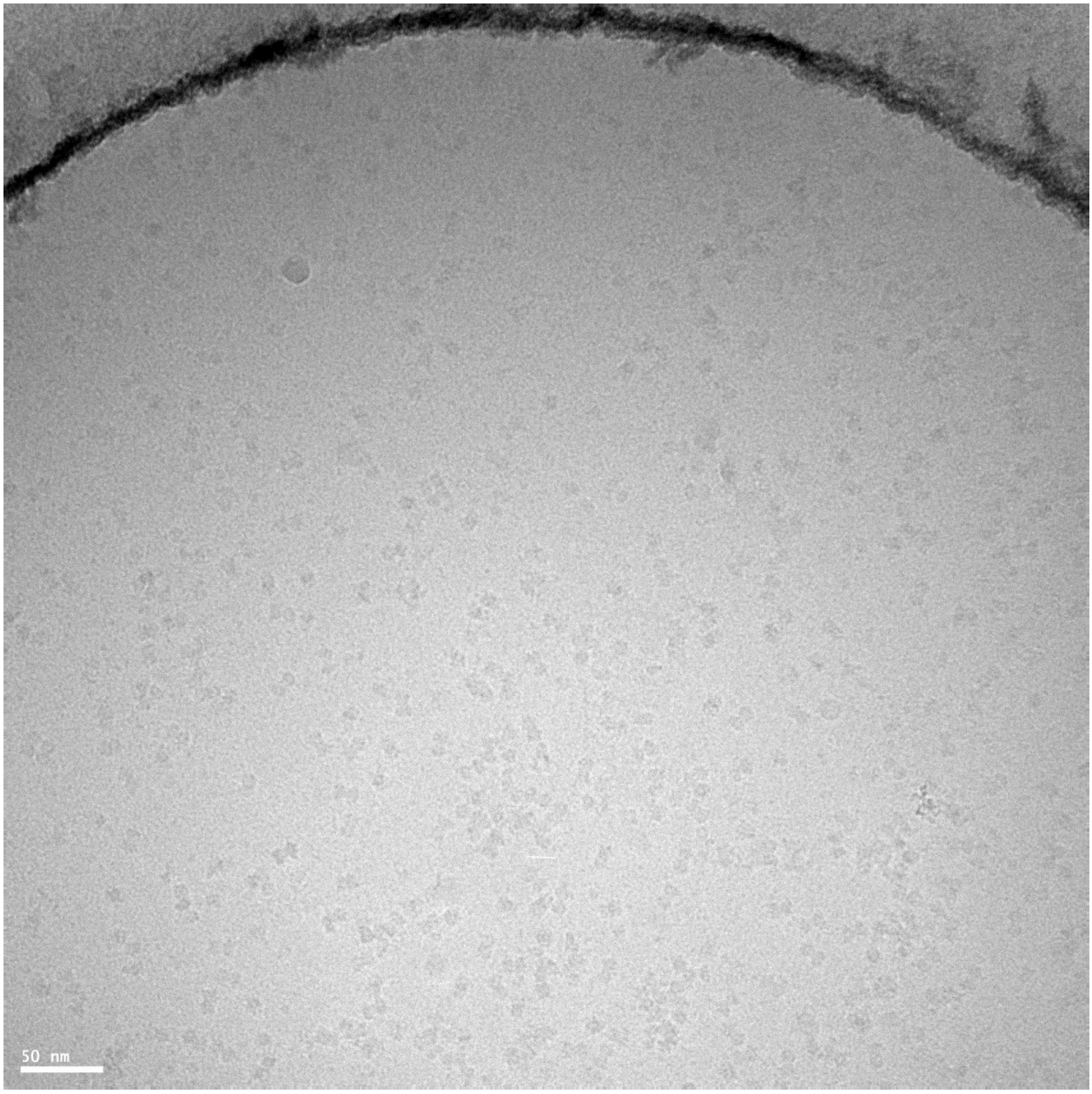

Figure 2. Single particles of Cx26 in the form of native cell membrane nanoparticles under Cryo-EM

The single-particles cryo-EM micrograph was taken at UVA on the FEI Tecnai F20 equipped with a CCD camera.

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