Structural basis of interacting complexes in the intracellular relocalisation of Aquaporin-4

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Water homeostasis is essential in the central nervous system (CNS) for normal function. This is maintained by a homotetrameric membrane channel called Aquaporin-4 (AQP4) in which it facilitates water flow across the membrane of astrocytic endfeet as part of the glymphatic system that eliminates waste products from the CNS (**1**). Reduced activity of this process is prevalent in ageing brains which can result in Alzheimer's disease which makes AQP4 an interesting drug target (**2**). Furthermore, AQP4 also has implications in the development of CNS oedema after brain injury (**3**). To date, drugs that target the pore of AQP4 has been unsuccessful since there are 13 different AQP isoforms in humans with similar characteristics (**4**). To affect the activity of AQP4, one can instead control the abundance of AQP4 molecules in the membrane and this is regulated by interaction of AQP4 with Calmodulin (CaM) which is specific for astrocytic cells (**5**). This interaction shuffles AQP4 from intracellular storage vesicles to the astrocytic membrane after which it is anchored through interaction with α-syntrophin (α-syn) as part of the dystrophin complex (**6**). Structural information is critical for efficient drug development and we have collected multiple cryo-electron microscopy (cryo-EM) data sets of the AQP4-CaM complex both with and without crosslinking. In our reconstructions, it is evident that CaM interacts with the C-terminus of AQP4 which also has been confirmed earlier from binding studies (**5**). CaM consists of two lobes, the N- and C-lobes, and based on previous studies using AQP4 termini it is believed that the C-lobe preferentially interacts with the C-terminus of AQP4 while the N-lobe interacts with the N-terminus (**7**), although the N-lobe interaction is not as evident in our reconstructions since that interaction is weaker than the C-lobe interaction. Furthermore, the AQP4 tetramer is well resolved in the reconstructions while the CaM is worse since the interaction seems to be very dynamic.

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