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Dynamics of Kv2.1 channel cluster formation in mammalian neurons.

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Kv2.1 are potassium channels that play an important role in multiple organs and tissues. In particular, in mammalian neurons Kv2.1 channels have an enormous neuroprotective function attained by their ability to form large clusters on the surface of the neuronal cell body. The regulation of Kv2.1 channel clusters is a key factor in protecting the brain, particularly under sudden ischemic conditions such as those encountered in stroke. It is speculated that this kind of stimulus induces channel declustering in order to suppress neuronal hyperexcitability (i.e. seizures). However, the physical mechanism that forms and maintains Kv2.1 clusters has remained largely unknown. We are investigating the dynamics of channel clusters at the single molecule level using particle tracking with nanometer accuracy in live cells. Here, the cluster structure and individual channels are imaged simultaneously in a total internal reflection microscope. While most Kv2.1 channels in the cell are labeled with green fluorescent proteins (GFP), only a few individual channels are tagged with red quantum dots. This approach allows us to track single molecules and probe their interaction with the cluster perimeter. Different models for the molecular mechanism that localizes Kv2.1 clusters on the cell surface and the implications of our data will be discussed.