Translational Laboratory in Genetic Medicine

NUS National University of Singapore

# Seminar Announcement
# -All Are Welcome-

**Speaker :** **Professor Hyokeun Park**
**Division of Life Science and Department of Physics**
**Hong Kong University of Science and Technology**

**Title :** **Relationship of Vesicle Positioning to Pool Identity and Exocytotic Fusion Mode: Insight from Real-time Three-dimensional, Nanometer-Accuracy Tracking of Single Synaptic Vesicles in Live Hippocampal Neurons**

**Date :** **10 May 2016(Tuesday)**

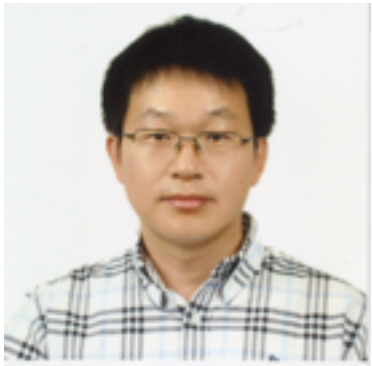
**Time :** **2.00pm – 3.00pm**

**Venue :** **Creation @ Matrix Level 4**

**Host :** **Dr Mahmoud Pouladi**
**(Tel: 64070700; e-mail: pouladi@tlgm.a-star.edu.sg)**

***Abstract of the Seminar:***
The position and movement of synaptic vesicles in presynaptic terminals are important for synaptic transmission. However, accurate three-dimensional tracking of single synaptic vesicles in presynaptic terminals has remained a challenge. Using dual-focus imaging optics, we have been able to track quantum dot (Qdot)-labeled single vesicles in three-dimensions, with an accuracy of 20 nm in x-y and 30 nm in z in 10 Hz imaging. We observed three typical patterns of movement of single vesicles up to the moment of exocytosis – minimal (almost stationary), intraboutonic, and intersynaptic movement. Interboutonic movement was the most prevalent whereas intersynaptic movement was the least prevalent and sometimes appeared as largely unidirectional motion, in some cases for distances larger than the average interbouton spacing.
Using different loading protocols, we tracked the dynamics of synaptic vesicles derived from either the readily releasable pool (RRP) or total recycling pool (TRP). The vesicles from the RRP were located much closer to fusion sites than those from the TRP. This spatial disparity determines the identity of the synaptic vesicles pool but is not mutually exclusive of other possible sources of functional differences. Also two different modes of exocytosis - full-collapse-fusion and kiss-and-run - were distinguished using the degree of quenching of quantum dot photoluminescence by micromolar trypan blue. Vesicles that underwent kiss-and-run traveled shorter distances before fusion than those which underwent full-collapse fusion; vesicles originating from the RRP before Qdot loading showed more kiss-and-run than ones drawn from the whole recycling pool. Both findings support the idea that the choice between fusion modes is not solely determined at the last moment but depends on prior vesicle state, including starting position before stimulation and pool of origin.

In order to determine the location of releasing position relative the center of the active zone, we localized the centroids of spectrally separable markers, FM 4-64 (presynaptic sites) and PSD 95-GFP (postsynaptic sites) in three dimensions using the same methods as Qdot loaded vesicles. The vector connecting these centroids approximated the central axis of the synapse, and allowed us to estimate the degree to which fusion occurred at positions away from the center of the active zone. We found that synaptic vesicles undergoing kiss-and-run tend to fuse close to the center of the active zone whereas vesicles undergoing full-collapse fusion tend to fuse all around the synapse. This difference of fusion mode may be related to the difference in the spatial distribution of AMPA and NMDA receptors, implying the relation between fusion mode and synaptic transmission.



***About the Speaker*:**

Professor Park's main research interests focus on the development of new single-molecule fluorescence imaging techniques and their application to address biological questions. In particular, he is interested in understanding how the transport of vesicles and organelles is disrupted in neurodegenerative diseases such as Huntington's diseases. Using his state-of-the-art three-dimensional nanometer-accuracy microscope, he investigates the transport of vesicles in Huntington's disease mouse models.