Quantum dot-enabled tracking of single synaptic vesicle protein Synaptotagmin-1 in live neurons

Jenna A. Dombroski¹, Kristina E. Kitko²,³, Qi Zhang³

¹ Department of Biomedical Engineering, The State University of New York at Buffalo, Buffalo, NY 14228
² Program in Interdisciplinary Materials Science, Vanderbilt University, Nashville, TN 37235
³ Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232

Introduction

Synaptic vesicles
- Required for neurotransmission
- Ca²⁺ surge triggers vesicle release
- Synaptotagmin-1 is one of the Ca²⁺ sensors

Quantum dots
- Photo-luminescent semiconductor nanocrystals, ranging from 2-10nm in diameter
- High quantum yields, narrow emission wavelengths, excellent photostability

Quantum dot tracking of Syn-1 allows for a better understanding of how synaptic vesicle dynamics may play a role in function.

Preparation of Qdot-antibody complexes

Figure 1. Determination of volume of Qdot-605 streptavidin conjugate and Qdot-605 Biotin. Perfusion was performed for cells with a constant volume of 2μl. Qdots and a variable antibody volume: 0.5μl (a), 1.0μl (b), 1.25μl (c) and 1.5μl (d). Once a concentration of 1.25μl antibody was selected, a similar titration of Qdots was performed: 0.5μl (e), 1.0μl (f), 1.5μl (g), 2.0μl (h), and 2.5μl (i). A concentration of 1.5μl of Qdots was selected. During perfusion, concentrations were increased to 1.5μl antibody and 2.0μl Qdots.

Perfusion with Epi-Fluorescent Microscopy

1. Qdots only
   - Incubate neurons without antibody
   - Add 2.0μl Qdots
2. Qdots + antibody
   - Incubate neurons with 1.5μl biotinylated antibody for 30min
3. Qdots + antibody + stimulation
   - 10Hz field stimulation

Results

Particle tracking via TrackMate
- Images analyzed using TrackMate, a Fiji plugin
- 8-20 labeled Qdots/FOV
- Tracks generated in TrackMate and exported to MATLAB for further analysis

Equations for calculating diffusion coefficient and alpha value using MATLAB

Figure 2. Tracking using Fiji and TrackMate. First, the ROI is selected from the FOV. A threshold is applied to select the Qdot by size and intensity. The particle tracks are then calculated using TrackMate.

Figure 3. Histograms of TrackMate data for Qdot movement. The diffusion coefficient (Log[D(μm²/s)]) for non-specifically uptaken Qdots (a), Qdot-antibody conjugates (b), and Qdot-antibody conjugates + stimulation (c) describes the type of diffusion the particles are taking. The alpha values, depicting the type of motion of the particles, are shown in the figure (d-f) for the non-specifically uptaken Qdots, Qdot-antibody conjugates, and Qdot-antibody conjugates + stimulation, respectively.

Figure 4. Mean and median diffusion coefficients for Qdots, antibody, and field stimulation.

Future Research
- Non-specific binding had not previously been studied as a parallel control in Qdot tracking
- Vesicle release upon field stimulation may cause Qdot loss as conjugates become separated inside the acidic synaptic vesicle environment
- Potential to impact results of previous findings using field stimulation

References and Acknowledgements

3. We thank Danielle Bailey for helpful advice and assistance with analysis. This work was supported by NIH OD008761 (QZ) and NSF 1560414.