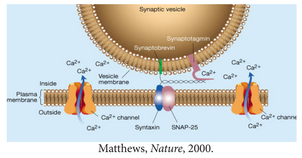
Quantum dot-enabled tracking of single synaptic vesicle protein Synaptotagmin-1 in live neurons

Jenna A. Dombroski[1], Kristina E. Kitko[2,3], Qi Zhang[3]

[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

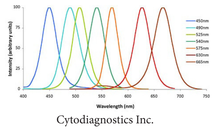
Synaptic vesicles
- Required for neurotransmission
- Ca^{2+} surge triggers vesicle release
- Synaptotagmin-1 is one of the Ca^{2+} sensors[1]



Matthews, *Nature*, 2000.

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

Sigma-Aldrich Co, LLC.



Cytodiagnostics Inc.

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

Preparation of Qdot-antibody complexes

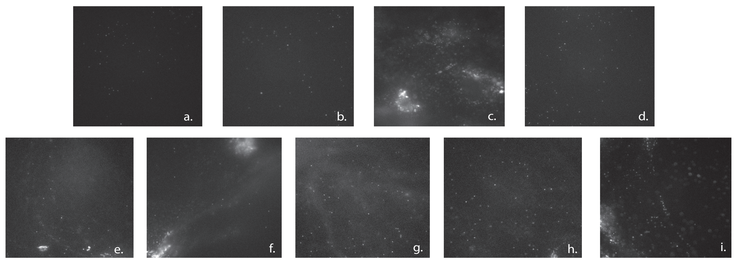
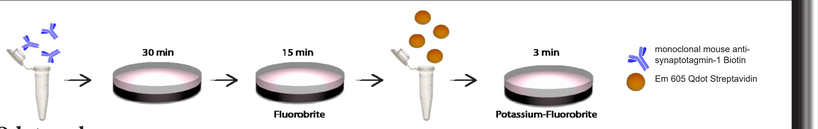


Figure 1. Determination of volume of Qdot 605 streptavidin conjugate and α Syt-1 Biotin. Perfusion was performed for cells with a constant volume of 2μL Qdots and a variable antibody volume: 0.5μL (a), 1.0μL (b), 1.25μL (c) and 1.5μL (d). Once a concentration of 1.25μL antibody was selected, a similar 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.

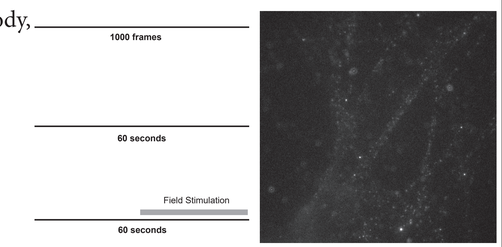
Perfusion with Epi-Fluorescent Microscopy



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 plug-in
- 8-20 labeled Qdots/FOV
- Tracks generated in TrackMate and exported to MATLAB for further analysis

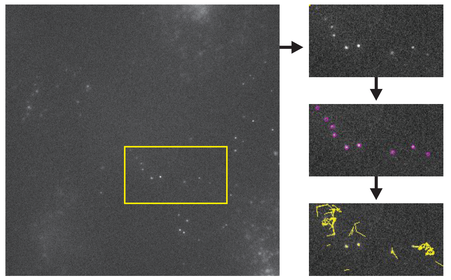


Figure 2. Tracking using FIJI and TrackMate. First, the ROI is selected from the FOV. A threshold is applied to select the Qdots by size and intensity. The particle tracks are then calculated using TrackMate.

Equations for calculating diffusion coefficient and alpha value using MATLAB[2]

$$MSD(ndt) = (N-1-n)\sum_{j=1}^{N-1-n}\{[x(j\Delta t + n\Delta t) + x(j\Delta t)]^2 - [y(j\Delta t + n\Delta t) - y(j\Delta t)]^2\}$$

$$MSD(t) = 4D\Delta t^{\alpha}$$

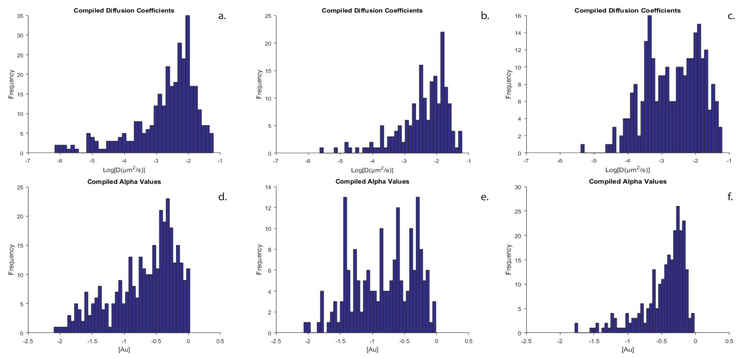


Figure 3. Histograms of TrackMate data for Qdot movement. The diffusion coefficient (Log[D(um²/s)]) for non-specifically uptaken Qdots (a), Qdot-antibody conjugates (b), and Qdot-antibody conjugates + stimulation (c) describes the type of diffusion the particles are taking. The alpha values, depicting 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 alpha values for Qdots, antibody, and field stimulation.

	Qdots	Antibody	Stimulation
Mean (Au)	0.3311 ± 0.0148	0.2507 ± 0.0169	0.4189 ± 0.0136
Median (Au)	0.2793	0.1718	0.4285

Figure 5. Mean and median diffusion coefficients for Qdots, antibody, and field stimulation.

	Qdots	Antibody	Stimulation
Mean (Log[D(μm²/s)])	0.0072 ± 5.4088x10^{-4}	0.0093 ± 7.9925x10^{-4}	0.0076 ± 6.9011x10^{-4}
Median (Log[D(μm²/s)])	0.0038	0.0059	0.0027

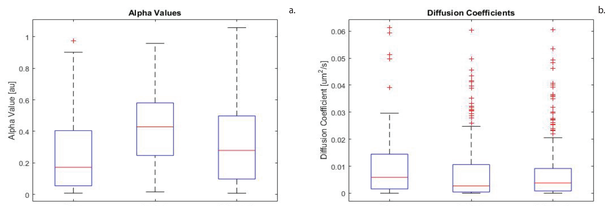
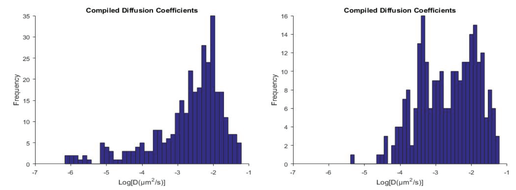


Figure 6. Boxplots of TrackMate data for Qdot movement. Average alpha values (Au) are displayed for non-specifically uptaken Qdots, Qdot-antibody conjugates, and Qdot-antibody conjugates + stimulation, respectively (a). Average diffusion coefficients are displayed for non-specifically uptaken Qdots, Qdot-antibody conjugates, and Qdot-antibody conjugates + stimulation, respectively (b). The center red lines indicates the median of the data, the edges of the boxes correspond to the first and third quartile, the whiskers indicate the most extreme points, and the red crosses represent any outliers in the data.

Similarity between Qdots and Field Stimulation Data

Diffusion coefficient data for non-specifically uptaken Qdots and field stimulation



	Qdots	Stimulation
Mean (Log[D(μm²/s)])	0.0072 ± 5.4088x10^{-4}	0.0076 ± 6.9011x10^{-4}
Median (Log[D(μm²/s)])	0.0038	0.0027

Loss of Qdots over time during field stimulation

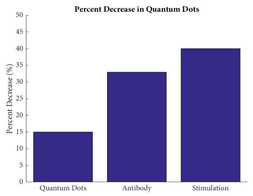
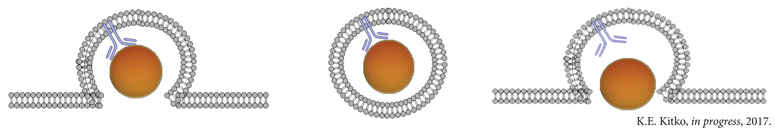


Figure 7. Total percent decrease in quantum dots from Frame 1 to Frame 1000. Stimulation data had the greatest loss in Qdots during perfusion.

Conclusions and Future Research

Proposed Model



K.E. Kitko, *in progress*, 2017.

- 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

Future Research
- Increase the number of biological replicates
- Run a trial with Qdots and field stimulation (no antibody)

References and Acknowledgements

1. Li, YC and ET Kavalali."Synaptic vesicle-recycling machinery components as potential therapeutic targets." *Pharmacological Reviews*. 69.2 (2017): 141-160.
2. Chang, J. C. and S. J. Rosenthal. "Real-time quantum dot tracking of single proteins." Biomedical Nanotechnology. *Methods in Molecular Biology*. 726 (2011): 51-62.
3. We thank Danielle Bailey for helpful advice and assistance with analysis.

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