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# Applications of quantum dots for single molecule imaging in cells and substrate-supported planar membranes

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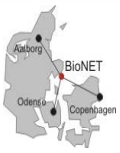


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## ABSTRACT

Quantum dots (Qdots) have several advantages compared to other probes for single molecule imaging. These include enhanced brightness and photostability as well as in some cases smaller size. Perhaps the major advantage of Qdots for single molecule imaging is the possibility of simultaneous imaging of multiple species at fast repetition rates over long periods of time. With this in mind, we have begun assembling a microscopy system eventually capable of imaging multiple colors of single quantum dots at high repetition rates over long periods of time in cells and substrate-supported planar membranes. With our current system, which consists of an Olympus IX81 microscope equipped with a 100 W Hg arc lamp for excitation and an electron-multiplied CCD (Andor DV887-ECS) for detection, we can image single Qdots with 100 µs signal integration or at rates up to about 250 Hz. These results are however very dependent on the particular emission color characteristics of the Qdots, as we find that certain quantum dot colors are dimmer and/or primarily in a non-fluorescent state. Here we present data on the intensity and on/off characteristics of a variety of Qdots. We also give examples of single molecule imaging with Qdots for tracking membrane proteins in cells.

## INTRODUCTION

The hypothesized existence of spatially enriched signaling platforms, also known as lipid rafts, in the cellular plasma membrane has generated much interest [1]. Yet, despite a decade long search, that started with the original definition of lipid rafts as the *in vivo* equivalent of the low buoyancy detergent resistant membrane (DRM) fractions, the existence of lipid rafts in intact plasma membranes has not been proven. As a result, their identification require either lipid or protein markers, all of which to date have been defined by the DRM composition. It is now thought that lipid rafts in cells are very small (≤ 50 nanometer diameter), very short lived (≤ 30 ms) or both.

One of only a few methods that is in principle capable to simultaneously operate at these size and time scales is single particle tracking (SPT). SPT is typically done with 40 nm diameter gold particles. In contrast, we are interested in using Qdots for SPT. Qdots have several advantages over conventional fluorescent probes and gold particles. These include photostability, brightness, and efficient multicolor detection. In comparison with standard gold particles, Qdots also have a roughly two-fold size advantage (Qdots are 20 nm diameter). In this work, we were interested in identifying optimum conditions for using Qdots for SPT and to identify the best color combinations for high speed multicolor SPT. A major limitation of particles, including gold and Qdots, is the lack of monovalent particles. Because of this, we are also performing FRAP experiments in parallel with SPT experiments.

## RESULTS AND FUTURE EXPERIMENTS

In this work, the data presented is limited to streptavidin conjugated quantum dots from Invitrogen. These materials were previously sold by Quantum Dot Corporation. In order to identify the best Qdots for SPT, we imaged single Qdots that had been non-specifically immobilised on a glass coverslip. For these measurements stock quantum dots (~1 µM) were diluted to 1 nM in 50 mM sodium borate, pH 8.2 with 1% BSA. Imaging specimens were prepared by applying 65 ml of a diluted solution of streptavidin Qdots to a sample chamber consisting of a 22 x 22-mm No. 1 1/2 glass coverslip, two strips of doubly adhesive tape and a 3 x 1-in glass slide. Qdots were allowed to adhere for 10 min, after which samples were extensively washed with 50 mM sodium borate, pH 8.2. Specimens were imaged on an Olympus IX81 microscope equipped with a 100 W Hg arc lamp for excitation and an electron-multiplied CCD (Andor DV887-ECS) for detection.

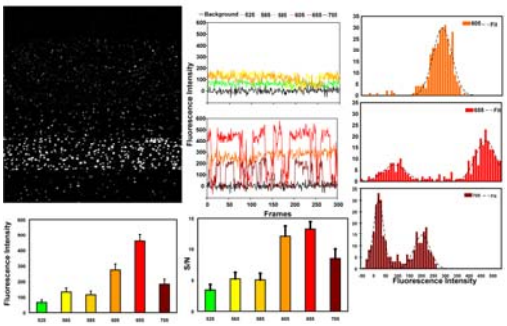

FIGURE 1: Intensity comparison of streptavidin Qdots adsorbed on glass. Images were acquired with 10 ms integration using a Hg arc lamp, a GFP long pass fluorescence filter set, an Andor EMCCD at 100nm projected pixel size. (top left) Background subtracted image of from top to bottom, 525 nm, 565 nm, 585 nm, 605 nm, 655 nm and 705 nm Qdots. (top center) Integrated intensity of representative single Qdots a function of frame number (acquisition frame rate was 94 Hz) showing on/off characteristics. (top right) Histograms of integrated intensity of representative single 605 nm, 655 nm and 705 nm Qdots. (bottom left) Average on intensity and (bottom center) average signal-to-noise of representative single Qdots. Under these conditions, the order of Qdot brightness is 655 nm > 605 nm > 705 nm > 585 nm, 565 nm > 525 nm while the Qdot "on" frequency is maximal for the 525, 565, 585 and 605 nm Qdots with the 655 nm Qdots and especially the 705 nm Qdots are much more frequently in an "off" intensity state.

Based on these experiments, we have decided to primarily use 605 nm or 655 nm Qdots for SPT experiments with biotinylated lipids and membrane proteins targeted either conventionally by antibodies or by fusion proteins incorporating the peptide substrate for bacterial biotin ligase [3]. In order to control for artificial probe induced cross-linking, we are also performing parallel experiments by FRAP by use of dye labeled streptavidin.

EXAMPLE 1: SPT of CD73, a GPI-anchored protein, with antibody targeting

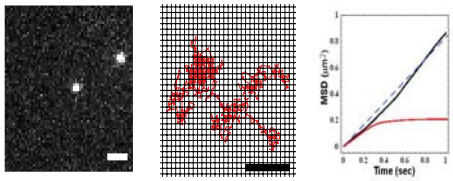


**FIGURE 2: In SPT, a time series of the point spread function (PSF) of a single molecule is imaged (left) and the centroid of the single molecule is determined by curve fitting to the theoretical PSF resulting in sub-pixel spatial resolution. Resulting centroids are linked into trajectories (center) from which the mean squared displacement (MSD) is calculated (right). In the case of free random (Brownian diffusion (blue dash), MSD=4Dt where D is the diffusion coefficient. The presence of nanostructures will result in confined diffusion (red solid) in which the MSD reaches steady state at the area of the confining nano-domain. In this example, 5'-nucleotidase (CD73), a GPI-AP, on a live IMR-90 human fibroblast were specifically labeled with biotinylated monovalent Fab antibody fragments and sAv-Qdots and imaged at 189 Hz (left). In this case, the diffusion is Brownian with D = 0.2 µm2/sec (Scale bars = 1 mm).**

**EXAMPLE 2: SPT of angiotensin type 1 receptor (AT1R), A G-protein coupled receptor, with bacterial biotin ligase targeting.**

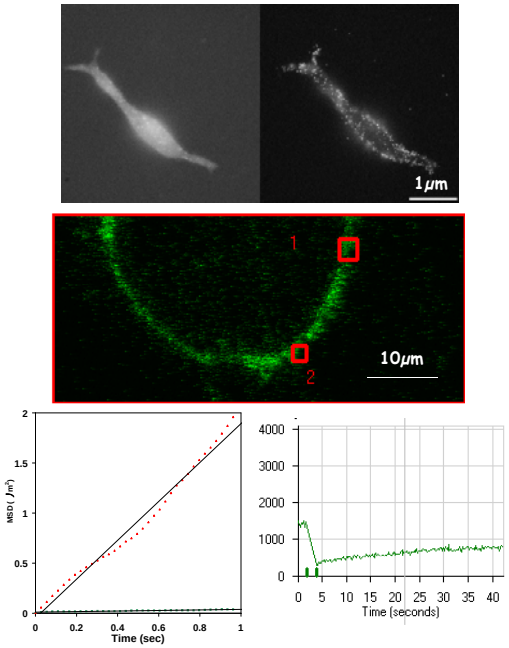


FIGURE 3: Qdots conjugated to AT1R in live HEK-293 cells showed two populations of movement patterns: One moved freely and one was stationary. These experiments were backed up by FRAP data where we saw limited recovery and therefore limited movement of AT1R.

## CONCLUSION

- Qdots are good for SPT
- It is possible to do two colour experiments with life time Qdot imaging
- The major problem is cross-linking

## REFERENCES

1. Lagerholm et al. (2005) Ann. Rev. Phys. Chem. 56, 309; 2. Simons and Ikonen (2000) Science 290, 1721; 3. Howarth et al. (2005) PNAS 102, 7583.

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