ABSTRACT

Quantum dots (Qdots) are fluorescent nanoparticles that have far superior signal intensity and signal stability compared to more conventional fluorescent molecules. We find that imaging with Qdots can easily be extended to the simultaneous visualization of up to four different molecular species at single molecule sensitivity and milliseconds time integration. We find that this technique can easily be adapted towards studying the spatial and temporal organization of various combinations of lipids and proteins in the cellular plasma membrane.

SUMMARY

Our main interest is in using Qdots in single molecule imaging microscopy for investigating the spatial and temporal nanoscale organization of the cellular plasma membrane. As part of this work, we are interested in the single molecule behavior of Qdots (i.e. brightness, photostability, and spectral separation), and in investigating various methods for targeting Qdots with high specificity and avidity to a variety of lipids and membrane proteins.

MICROSCOPY SET UP

Our microscope is an inverted Olympus IX-81 fluorescence microscope equipped with a very sensitive Andor EMCCD. On this microscope, we can illuminate specimen with either a conventional Hg arc lamp, a CoolLED (465 and 525 nm), or laser illumination (473 nm) in epi or TIRF mode. On the detection side, we also have a QuadView image splitter for simultaneous image acquisition in up to 4 separate color channels.

EXAMPLES OF CURRENT PROJECTS

QDOT INTENSITY AND BLINKING STATISTICS

Qdot intensity comparison of from top to bottom, 565, 605, 655 and 705 emitting Qdots adsorbed on glass. Time lapse images in each instance were acquired with 10 ms integration using a Hg arc lamp. In these image sequences, we observe frequent Qdot intensity fluctuations between an on and an off state for all Qdots studied to date. We find that the observed Qdot brightness and intermittency is color dependent, where 605 nm Qdots exhibits the best combination of Qdot brightness (~9000 counts above background with 10 ms resolution) and on frequency (~90/10 on/off frequency). In contrast, 705 nm Qdots are moderately bright (~4000 counts above background) and are primarily in an off state (40/60 on/off frequency). Consistent with previous results, we do also find that small reducing agents (β-mercaptoethanol, DTT) in the 1-10 mM range improves the on/off frequency with no effect on the Qdot brightness.

TARGETING OF QDOTS TO SPECIFIC MEMBRANE PROTEINS AND LIPIDS

We are applying Qdots for tracking the spatial and temporal dynamics of lipids and membrane proteins (MPs). In the case of membrane proteins, we are currently exploring three targeted schemes: 1) Targeting of recombinant and native membrane proteins by genetic insertion of a 15 amino acid biotin ligase acceptor peptide (BLAP) substrate to bacterial biotin ligase in combination with streptavidin (sAv) conjugated Qdots, 2) Targeting of recombinant membrane proteins by genetic insertion of a 77 amino acid acyl carrier protein (ACP) substrate to ACP Synthase in combination with custom CoA conjugated Qdots, and 3) Custom antibody Qdot conjugates. In the case of lipids, we are exploring the use of cholesteryl toxin conjugates for tracking the sphingolipid (Gp, bottom right), sAv conjugated Qdots (605 nm) for tracking epidermal growth factor receptor (EGFR) BLAP (bottom left), and yellow fluorescent protein (YFP) plasma membrane marker for visualizing the plasma membrane.