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Differential Uptake and Trafficking of Nanoparticles by Living Cells

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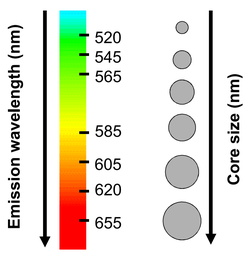
Motivation & Background

- "At the nanoscale material properties vary as a function of size, which not only enables new benefits, but also may lead to unintended health and environmental risk." – National Nanotechnology Initiative, 9/2006
- Goal: Characterize the cellular membrane interactions, uptake, and biomolecular responses of cells to Quantum Dot (QD) nanoparticles (NPs)
- Fluorescence emission spectra of QD depends on size, making it easy to identify and follow in living cells
- Previous work has shown interaction and uptake of NPs, but no systematic study of early dynamics [1-3]
- We investigated cellular uptake and trafficking of three different sizes of carboxylic acid terminated quantum dots in a mast cell line (RBL-2H3)

QD Characterization

Qdot spectral emission vs. size



QD Size and Shape

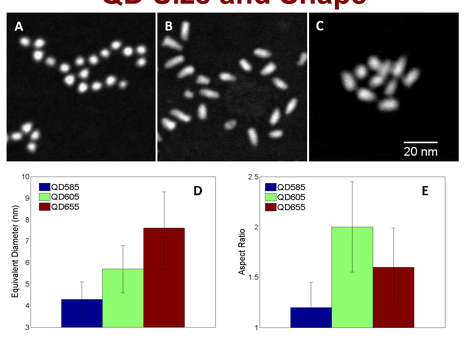


Figure 1: Left panel: Relationship between QD emission maxima and QD core size. Right panel: STEM images of QDs with fluorescent emission peak maxima of (A) 585nm, (B) 605nm, and (C) 655nm acquired using a Tecnai F-30ST TEM/STEM (FEI Company) operating at 300kV. (D) Image analysis reveals average particle diameters of 4.3, 5.7, and 7.6nm for the 585, 605, and 655nm-emitting QDs. (E) The average particle aspect ratio, however, was found to be highest for the 605nm-emitting QDs at 2.0, vs. 1.2 and 1.6 for the 585 and 655nm-emitting QDs.

Uptake, Trafficking, and Localization is Influenced by Size & Shape

Diffusion within the Cell Membrane

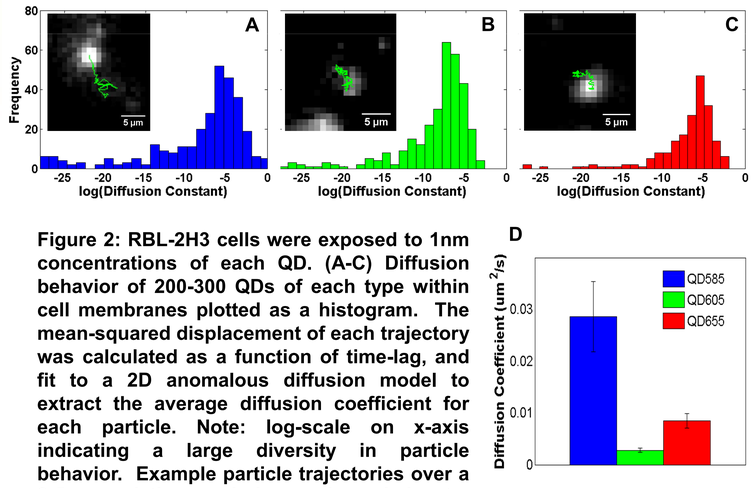


Figure 2: RBL-2H3 cells were exposed to 1nm concentrations of each QD. (A-C) Diffusion behavior of 200-300 QDs of each type within cell membranes plotted as a histogram. The mean-squared displacement of each trajectory was calculated as a function of time-lag, and fit to a 2D anomalous diffusion model to extract the average diffusion coefficient for each particle. Note: log-scale on x-axis indicating a large diversity in particle behavior. Example particle trajectories over a 3-5 second time period are shown in insets.
(D) The average diffusion coefficients for each particle type (with uncertainties expressed as standard error of the mean) reveal that 605nm-QDs diffuse an order of magnitude slower that the 585nm QDs, and ~ 5-fold slower than the 655nm QDs. This behavior mirrors the average aspect ratio measured for each particle type (see Figure 1E).

Kinetics of Internalization

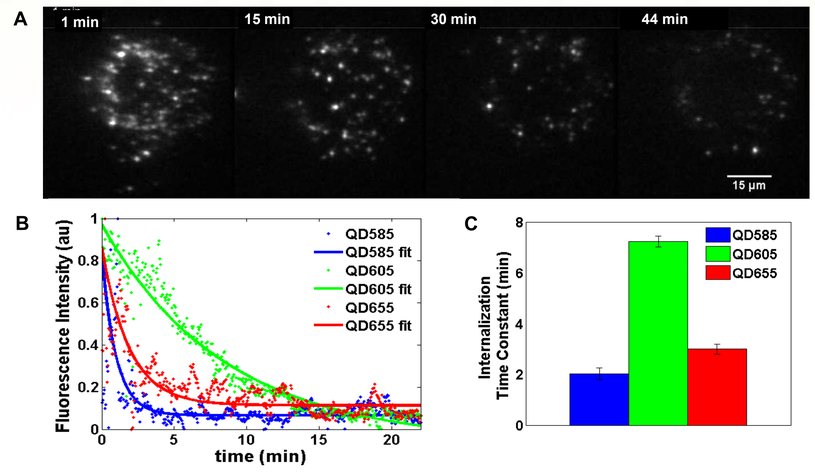


Figure 3: Time-lapse TIRF microscopy of individual RBL-2H3 cells after exposure to 1nM solution of QDs. (A) Example images showing uptake of 605nm-emitted QDs Images were acquired at 3 second intervals for 45 minutes. The overall signal intensity decreases over time as particles are internalized moving beyond the TIRF evanescent field of view. (B) Total signal intensity (background removed) was plotted for 585nm-, 605nm-, and 655nm-emitting QDs and fit to a single exponential decay model (solid line). (C) Average decay constants for each QD type, averaged over 3-5 cells, indicate that the 605nm-emitting QDs reside in the cell membrane, on average, for approximately 7 minutes, vs. 2-3 minutes for the 585nm- and 655nm-emitting QDs.

Multiplexed Visualization of QD Localization

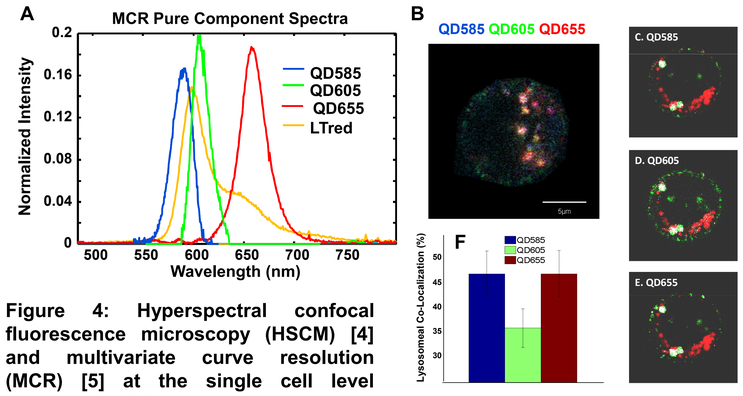


Figure 4: Hyperspectral confocal fluorescence microscopy (HSCM) [4] and multivariate curve resolution (MCR) [5] at the single cell level resolves the QD spectra and a lysosome label simultaneously. RBL cells were labeled with LysoTracker Red (Invitrogen) after 15 minute exposure to 100nM solution containing QD585, QD605, and QD605 NPs and returned to incubator for an additional 15 min. Total exposure time = 30 min. (A) Spectra recovered from HSCM & MCR analysis. (B) Three-color image overlay depicting QD585, QD605, and QD655. (C-E) QDs and lysosome-specific dye are shown in green and red, respectively. Areas of co-localization as defined by minimum signal threshold and ratio are white. Note the increased presence of QD605 on the cell membrane, relative to the other QD types. (F) Overall fraction of each QD type that is co-localized with the lysosome.

Summary Points

- TIRF micrscopy and hyperspectral imaging allow examination of QD interaction with and diffusion within cell membrane as well as ultimate fate within the cell
- QD NPs interact with RBL cell membranes, becoming internalized
  - Expected mechanism is not endocytosis
  - Differential interaction
  - Shape plays a measurable role in uptake and trafficking of QD NPs in RBL cells
- Our results show the higher aspect ratio particles are:
  - More immobile in RBL cell membrane
  - Slower to be internalized by RBL cells
  - Less likely to be colocalized with the lysosomal compartments

References:
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