

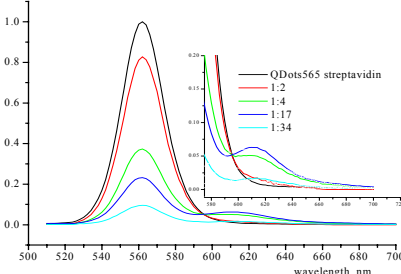
Individual colloidal semiconductor quantum dots nanocrystal (NC) are attractive FRET donors because their photostability and ~20 times larger brightness than single organic fluorophores. They possess broad blue-shifted excitation and narrow, tuneable emission spectra that greatly reduce the bleed-through of donor fluorescence into the acceptor channel. These properties guarantee an improved selectivity for FRET detection in the acceptor channel. Also, the possibility of fine-tuning NC emission permits choosing optimal donor/acceptor pairs and multiplexing at the single-NC scale by attaching different acceptor fluorophores. Sensing will involve either donor/acceptor separation or the modulation of the acceptor fluorescence through changes in the local microchemical environment of the functionalised NC. Our goal is to create a family of stably assembled NanoFRET sensors with optimal donor/acceptor match (spectral overlap, orientation and stoichiometry) while minimizing their separation distance to increase the FRET efficiency.

Sensor design

Benefits of biotin-streptavidin binding assay

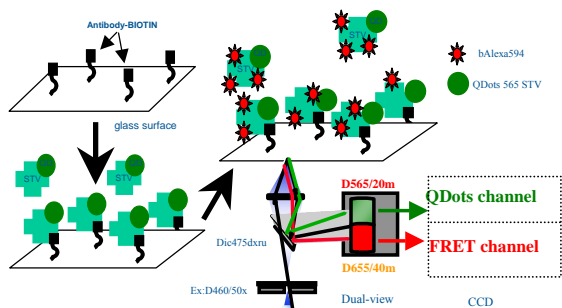
- Streptavidin is a tetrameric protein (4 x 13kDa) with 4 binding sites for biotin
- High affinity ($K_d = 4 \times 10^{-14} M$) and low non-specific binding
- Strongest noncovalent interaction between protein and ligand
- Tolerates a range of temperature, pH conditions and organic solvents
- NCs have 15-20 streptavidin per nanoparticle
- Open platform for multicolor assays and multiplexed analysis

Spectroscopy measurements



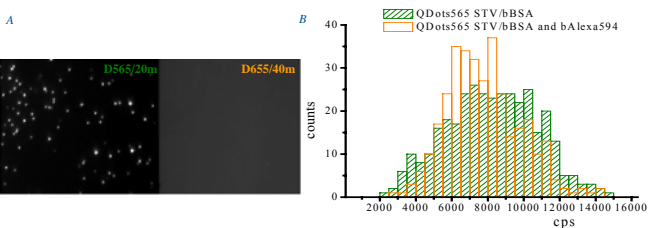
Evolution of emission spectra of 10 nM QDots565 STV with increasing concentration of bAlexa594. Excitation at 440nm for all samples. *Inset* zoom on acceptor fluorescence.

Detection of FRET on single molecules level

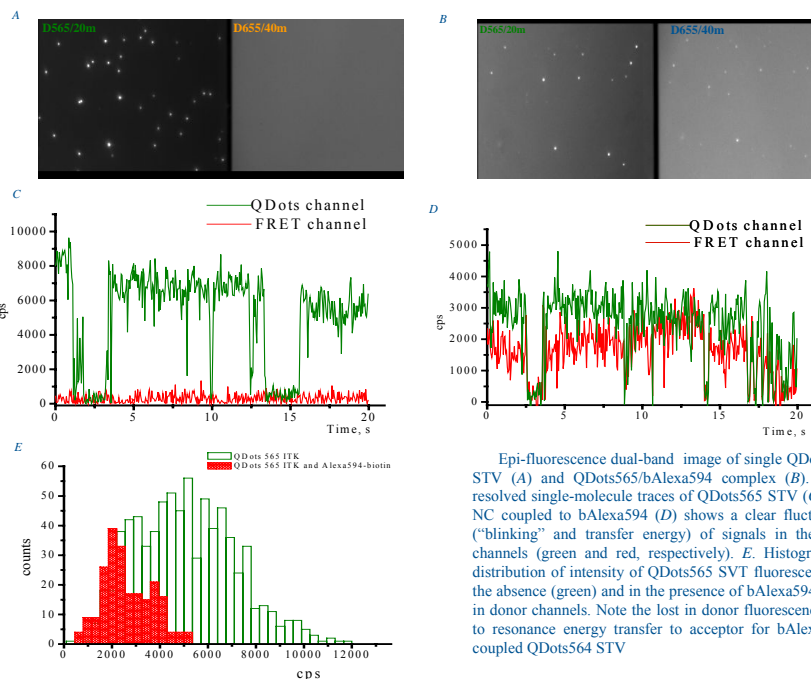


Experimental setup for dual-band single-molecule fluorescence imaging. QDots565 SVT/bAlexa594 complexes are immobilized on a glass surface using a biotinylated antibody. The dual-view optics splits the fluorescence into two spatially identical but spectrally distinct images (QDots and FRET channel) on the CCD camera, permitting the simultaneous donor and acceptor detection

Negative FRET control



The dual-view fluorescence image of negative control for single bare QDots565 STV/bBSA and bAlexa594 in solution (A). Histogram of fluorescence intensity of single QDots565 SVT conjugated to bBSA (green) and in presence of bAlexa594 in solution (orange) in donor channels (B).

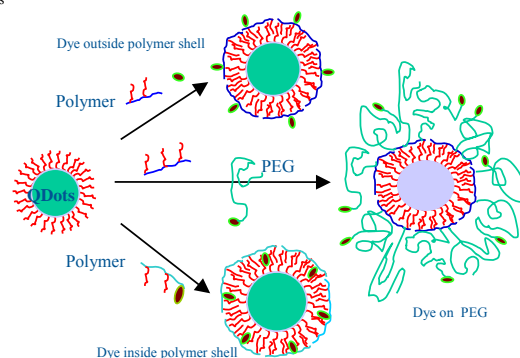


Epi-fluorescence dual-band image of single QDots565 STV (A) and QDots565/bAlexa594 complex (B). Time resolved single-molecule traces of QDots565 STV (C) and NC coupled to bAlexa594 (D) shows a clear fluctuation ("blinking" and transfer energy) of signals in the both channels (green and red, respectively). E. Histogram of distribution of intensity of QDots565 SVT fluorescence in the absence (green) and in the presence of bAlexa594 (red) in donor channels. Note the lost in donor fluorescence due to resonance energy transfer to acceptor for bAlexa594-coupled QDots565 STV

Perspectives

We are currently exploring strategies for the direct attachment of the acceptor to nanocrystal surface. The advances of this complex are:

- Optimization of QD-acceptor FRET separation distances
- Development of QD-surface ligands for water compatibility and covalent conjugation
- Controlled donor-to-acceptor ratio



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