

# Fluorescence Resonance Energy Transfer in Bioconjugated Quantum Dot-Dye Systems

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## ABSTRACT

We employ time resolved fluorescence, steady state fluorescence resonance energy transfer (FRET) and single-particle fluorescence resonance energy transfer (spFRET) measurements to study multivalent quantum dot (QD)/dye systems and determine how inhomogeneity affects the FRET dynamics of the acceptor dye molecules.

**Keywords:** quantum dots, fluorescence resonance energy transfer, steady state fluorescence, time resolved fluorescence, single-pair FRET

## 1 INTRODUCTION

In this work we address the description of Fluorescence Resonance Energy Transfer (FRET) in a complex multivalent quantum dot (QD)/dye system by understanding how inhomogeneity affects the FRET dynamics of the acceptor dye molecules. Most previous FRET studies of QD-dye systems have focused on describing the QD donor because its fluorescence response tends to be simpler than for the acceptor [1]. However, a full understanding of the acceptor FRET dynamics is also important, and will impact the future design of nanomaterials for diverse applications, including biosensing and solar energy harvesting. Here, we experimentally characterize the FRET interactions of a semiconductor QD/dye system using a combination of ensemble steady state and time resolved fluorescence measurements in conjunction with single-particle FRET (spFRET) measurements. Taken together they resolve the inhomogeneity in the material system, which will improve the modeling and understanding of the experimental FRET dynamics.

## 2 RESULTS

FRET system that we investigate consists of a central QD donor with peptide-modified Alexa 647 (A647) dye bioconjugated to the QD surface by a peptide intermediary. We explore two types of QD donor materials: QD625,

which is a standard CdSe core, ZnSe shell system, and QD616, which is a ternary CdSe core, ZnSe shell, ZnS shell system; the latter material is relatively unexplored for FRET interactions.

Figure 1 shows the fluorescence spectra and time-resolved fluorescence of each QD in buffered solution. Clearly the QD616 shows a significantly broader emission, suggesting a distribution of QD sizes in the ensemble. Figure 2 (bottom) re-enforces this interpretation where the QD616 fluorescence lifetime (measured using time-correlated single photon counting) shows a strong dependence on the emission wavelength. In contrast, Figure 2 (top) shows that the QD625 fluorescence lifetime has much smaller dependence on the emission wavelength. These measurements highlight the inhomogeneities that can be present in the QD part of the QD-dye system.

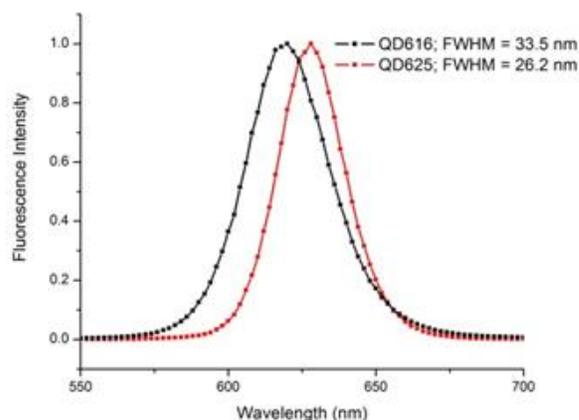


Figure 1. Emission spectra of QD616 and QD625, both in aqueous buffer solution.

Single particle FRET measurements were performed on an Axiovert 200 (Carl Zeiss) confocal microscope setup. 200  $\mu$ L of 160 pM buffered solutions were excited by 20-40  $\mu$ W argon ion laser at 458 nm. Fluorescence signals of the QD donors and dye acceptors were detected by two single-photon counting avalanche photodiodes. The FRET efficiency  $\eta$  of a single donor (D)-acceptor (A) pair is given by:

$$\eta = \frac{I_A}{I_A + I_D}, \quad (1)$$

where  $I_D$  ( $I_A$ ) is the donor (acceptor) fluorescence level. Population distributions were then characterized by their histograms evaluating the fraction of QD/dye conjugates signal bursts exhibiting specific emission ratios.

The spFRET measurement in Figure 3 for QD616 conjugated to N A647 dyes shows the population distribution of FRET efficiency,  $\eta$ . Clearly, at intermediate valence number the spFRET indicates broad distribution of valence states, which may be modeled in terms of Poisson statistics [2]. A Similar trend was observed in the spFRET of the QD625-(N)A647 system.

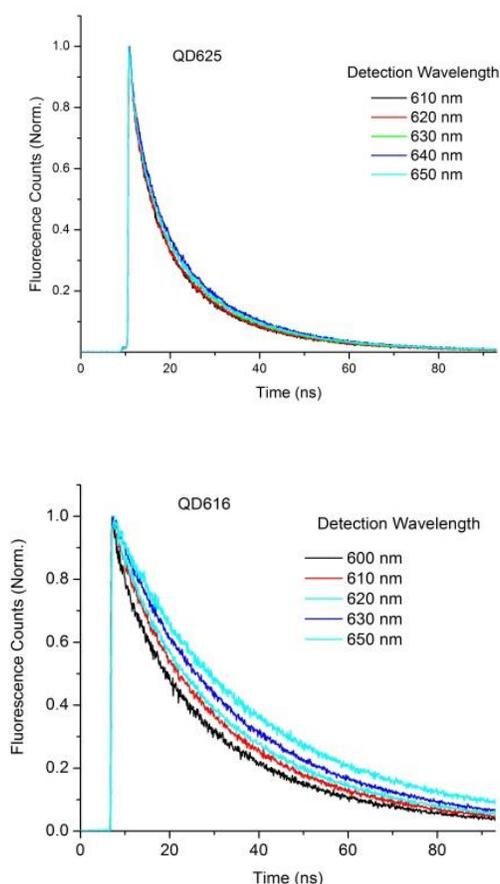


Figure 2. Time-resolved fluorescence of QD616 (bottom) and QD625 (top) in buffered solution. For each case the fluorescence decays are measured at a specific wavelength within the steady state emission spectrum.

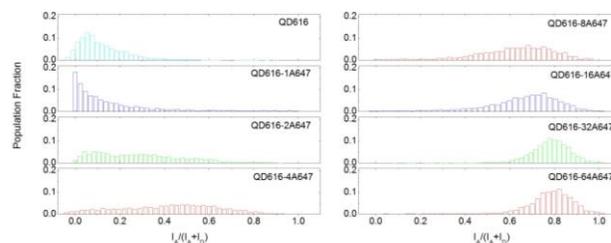


Figure 3. spFRET characterization of the series QD616-A647 for N conjugated A647 dyes.

Figure 4 shows the FRET dynamics of the A647 dye acceptor measured via the time-resolved fluorescence at 675 nm. Each curve shows a rise time in the acceptor fluorescence which accelerates as the number of A647s increase. In addition, the decay time also accelerates as the number of A647s increase. In general, the complex acceptor FRET dynamics depend on the QD excited state lifetime, the FRET rate between the QD and N acceptors, and the excited state lifetime of the acceptor. We are currently exploring modeling the complex FRET dynamics based on standard rate equations using these quantities, and including the inhomogeneities shown in Figs. 1-3. It will also be important to explore the potential of homo-FRET interactions, which might become important as number of A647 dyes conjugated to the central QD increases.

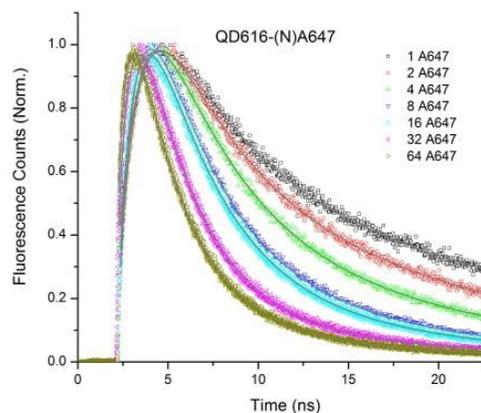


Figure 4. A647 acceptor FRET dynamics for QD616-(N)A647. The solid lines are fits to the data using a sum of three exponentials.

### 3 CONCLUSION

Both steady state FRET and spFRET methods confirm energy transfer between QDs and fluorescent dye and that process is mostly driven by dipole-dipole coupling that is within the Förster formalism. Single particle spFRET measurements provide insight on heterogeneity of individual QD-dye conjugate structures and revealed FRET distribution within macroscopically homogenous structure.

## REFERENCES

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