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# Single molecule FRET detection in CdSe-QD donor and Cy5-labeled molecular chaperone acceptor complex by imaging microscopy

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ABSTRACT

We report single molecule spectroscopic evidence of FRET in CdSe quantum dot (QD) conjugated with Cy5-labeled molecular chaperone systems in buffer solutions. Donor QDs are core-shell type nanocrystals covered with organic surfactants on their outermost surfaces, i.e. CdSe/ZnS/TOPO's. As prototype molecular chaperones, we adopt prefoldins (PFDs), on which Cy5's are labeled as acceptors. Donor QDs possess two-fold degenerate emission dipoles perpendicular to the *c*-axis, due to their Wurtzite crystal structures, while acceptor Cy5's possess linear absorption and emission dipoles. Thus, their combination provides novel features to those in conventional FRET systems. PFDs are jellyfish-shaped hexameric co-chaperones of group II chaperonins, which recognize hydrophobic portions of denatured proteins and encapsulate them within their central cavities. Hence, PFDs will also capture the CdSe/ZnS/TOPO QDs due to its surface similarity to the denatured proteins. By introducing simple microscope setup for single QD-PFD-Cy5 spectroscopy, we have successfully captured the emission spectra in FRET regime. We also have observed peculiar features in time evolution profiles of single QD emissions conjugated with Cy5-labeled PFDs under polarization modulation measurements. Notable point of our hybrid conjugates is that they are biochemically in living action. We describe our present results in relation to possible protein reactions.

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## 1. Introduction

Among various semiconductor quantum dots (QDs), CdSe/ZnS/Trioctylphosphineoxide (TOPO) nanocrystals are attracting due to its core-shell structures as well as organic ligand surfactant on the outermost surfaces [1]. They acquire not only high quantum yield of photoluminescence with high optical stability but also possess unique advantage of possible high biochemical affinities. In order to quest for novel functions in future molecular devices [2], we have been providing the QDs conjugated with proteins keeping in their bioactivities.

For this purpose, we are concerned with molecular chaperones as prototype proteins. They recognize hydrophobic region of denatured proteins and encapsulate them internally for transporting, refolding and other related functions. It thus seems possible that they can also capture the QDs due to its surface similarity. Such complex of the QDs and Cy5-labeled molecular chaperones can actually be prepared [3] and their photophysical investigations are ongoing. Among them, detection of resonant energy transfer (FRET) from the donor QDs to the acceptor Cy5-labeled proteins [4] is significant and also a most direct evidence for complex formation. In this work, we report our recent preliminary results on single QD-PFD spectral observation in FRET regime with single molecule imaging technique, which would eventually deduce the dynamics in the bioactive functions of the complex.

Experimentally, we have used PFD, CPN and sHsp for our molecular chaperones. Butanol is adopted as a water-soluble catalytic solvent to dissolve the QDs into aqueous solution in order to avoid severe aggregations as possible [3]. While the complex formation of QDs with molecular chaperones in aqueous buffer solution is a hard task, we can provide observable amount of hybrid complex successfully for the single molecule imaging technique [5].

The emission transition dipole of CdSe colloidal QDs is two-fold degenerate at room temperature [6,7]. As the absorption and

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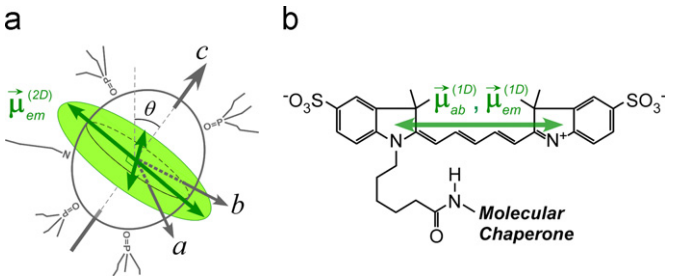


**Fig. 1.** Schematics of (a) QD donor and (b) Cy5 acceptor labeled on molecular chaperone and their emission dipoles, respectively, which concern with our present FRET experiments. Present QDs are core-shell structure CdSe/ZnS/TOPO-HDA nanocrystals with uniaxial Wurtzite structure, which therefore possess degenerate two dimensional emission dipoles in their *a*–*b* planes perpendicular to the *c*-axis. Cy5 labeling dyes, on the other hand, possess one dimensional linear emission dipoles, which are almost parallel to the long molecular axes.

emission dipoles of Cy5 are linear and almost parallel to the long molecular axes, formation of novel FRET system is also expected (Fig. 1). Furthermore, this implies that the 3D orientation of the unique crystal *c*-axis of QDs can be determined at room temperature with polarization microscopy [8]. It would be useful to disclose behaviors ranging from conformational changes in QD-PFD systems to slow as well as fast relaxation dynamics at the molecular level in its living bioactive states. Such indication in the experiments will be described.

## 2. Experiments

Monodispersive CdSe/ZnS/TOPO-HDA nanocrystals (QDs) are synthesized in molten coordination solvents (TOPO mixed with 30% of hexadecylamine, HDA) by pyrolysis of organometallic compounds at 240 °C [9]. The CdSe core-diameters used for the FRET experiments are ca. 4.0 nm, mainly due to the tuning of the spectral overlap. We have also observed the isolated QDs of ca. 3.8 nm core-size for comparison. As for the shell thickness, we used ca. 1.7-monolayers for all in common. High-resolution TEM observations confirm the size, shape and crystal phase homogeneity (aspect ratio ≈1.3, Wurzite structure). Photoluminescent quantum yields are roughly 30% in average. TOPO as well as HDA surfactants ensure high solubility of the QDs in nonpolar solvents such as toluene, while some solubility in *n*-butanol is also reserved. Mother solutions for conjugations are eventually prepared by *n*-butanol and its concentration is ca. 5 μM.

We use prefoldin (PFD, *Pyrococcus horikoshii* OT3, 86 kDa) [10] in this experiment. Our protocol for the conjugation in Hepes buffer solution of pH 7.5 with or without potassium chloride (HK) is in Ref. [3], however just briefly, notable points are as follows. The QDs dislike any trace amount of waters primarily in every step of synthesis to its preservation. While *n*-butanol is a suitable as water-soluble carrier solvent for the QDs, its solubility to water is still 7.7% at 20 °C. In addition, from time to time, a trace amount of alcohol works toxically against proteins even slowly. Thus, the protocols for conjugation are quite delicate and we need to optimize every step sharply. In such biochemical procedures, addition of *t*-butanol seems to improve the stability of the QDs against aggregation and improve the yield of final products as well.

For the moment, a typical yield of conjugation is roughly 5%. This is not high enough for ordinary macroscopic biochemical evaluations, thus single molecule detection is significant. To capture the proteins optically in visible region, Cy5's are labeled through amide bonds to the lysine residues of the proteins. Labeling modulus is ca. 1.2 or less. Fig. 1 illustrates schematic

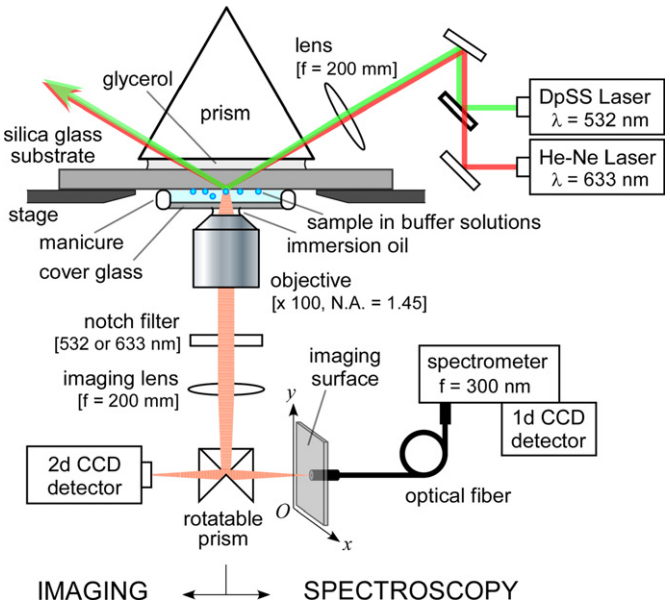


**Fig. 2.** Schematic micro-photoluminescence setup for single molecule imaging and spectroscopy with two-color evanescent light coaxial excitations ($\lambda_{ex}$: 532 and 633 nm). For the detection of single QD-Cy5 FRET spectroscopy, the system utilizes large-scale optical fibers (core diameter: ∅800 μm) placed at the imaging surface to cut the specific spot signal out to the spectrometer. In case of polarization-rotating modulation microscopy, rotating analyzer is inserted between the notch filter(s) and objective lens.

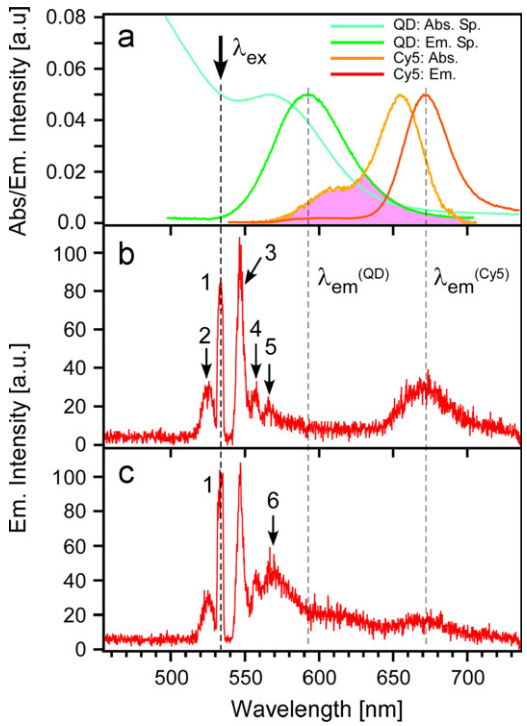


**Fig. 3.** (a) Macroscopic absorption and emission spectra of the QDs in toluene and Cy4-PFDs in buffer solutions at room temperatures. Core diameters of the QDs are ca. 4.0 nm. Arrows marked with $\lambda_{ex}$=532 nm followed with broken line indicate the excitation wavelength used for single molecule spectroscopy. (b and c) Typical two examples of the emission spectra of single QD and Cy5-labeled PFD complex with evanescent 532 nm laser light excitation of ca. 47 W/cm$^2$ for 100 s exposure time. Sharp peaks appeared at 1 are the leakage components of the excitation source. Other peaks at 3–5 appearing around 1 are Raman scattering components from silica glass substrate including anti-Stokes line 2. Two broken lines marked with $\lambda_{em}^{(QD)}$ and $\lambda_{em}^{(Cy5)}$ are the peaks of the emission bands of QDs and Cy5s, respectively.

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