Charge Transfer Between Quantum Dots and Peptide-Coupled Redox Complexes

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Introduction: Nanotechnology has great potential for creating a new generation of multifunctional hybrid bio-inorganic assemblies that are capable of enhancing Navy capabilities and DoD battle systems in general. The unique properties of luminescent quantum dots (QDs) have made them an integral building block in this burgeoning field.[1] In addition to their well known size-dependent emission spectra, QDs are extremely sensitive to the presence of additional charges either on their surfaces or in the surrounding environment, which can alter both their photoluminescence (PL) and absorption properties.[2] Since the advent of successful techniques to interface QDs with biological molecules, there has been a strong desire to understand the interactions of QDs with redox-active complexes to create new sensors capable of monitoring specific biological and abiotic processes.[2] However, as there is only a minimal understanding of these systems, rational design of QD-redox assemblies with control over both architecture and redox levels is needed to provide insight into the underlying mechanisms. Here we label peptides with a variety of metal complexes expressing different oxidation potentials and ratiometrically self-assemble them on the QD surfaces. This unique configuration allows us to gain insights into the underlying quenching processes involved and exploit them for biosensing.

Quantum Dot Conjugate Architecture: We have previously shown that polyhistidine-terminated peptides can rapidly self-assemble onto QDs with high affinity and control over conjugate valence (i.e., number of peptides per QD).[2,3] In this study, we exploit this self-assembly process and use CdSe-ZnS QDs capped with either negatively charged dihydrolipoic acid (DHLA) or neutral poly(ethylene glycol)-terminated DHLA ligands (DHLA-PEG).[3] Peptides expressing terminal hexahistidine (His_6) tags and unique cysteine or aminolated residues at the opposite end were labeled with reactive metal complexes including a ruthenium chelate (Ru), a bis-bipyridine ruthenium chelate (ruthenium-bpy), and a ferrocene metal complex. These were ratiometrically self-assembled to the QDs for subsequent spectroscopic analysis; see Fig. 1.

Cyclic Voltammograms and Energy Levels: We first determined the redox levels of the QDs and metal complexes used. Figure 2(a) shows the cyclic voltammograms collected from layers of unconjugated QDs and the metal complex–labeled peptides immobilized on indium tin oxide (ITO) electrodes. The ruthenium and ferrocene peptide complexes show reversible forward (oxidation) and reverse (reduction) scans while the ruthenium-bpy appears to be predominantly oxidized. In comparison, the QDs exhibit a single irreversible broad peak centered between 0.2 and 0.24 V regardless of surface ligand present, which is attributed

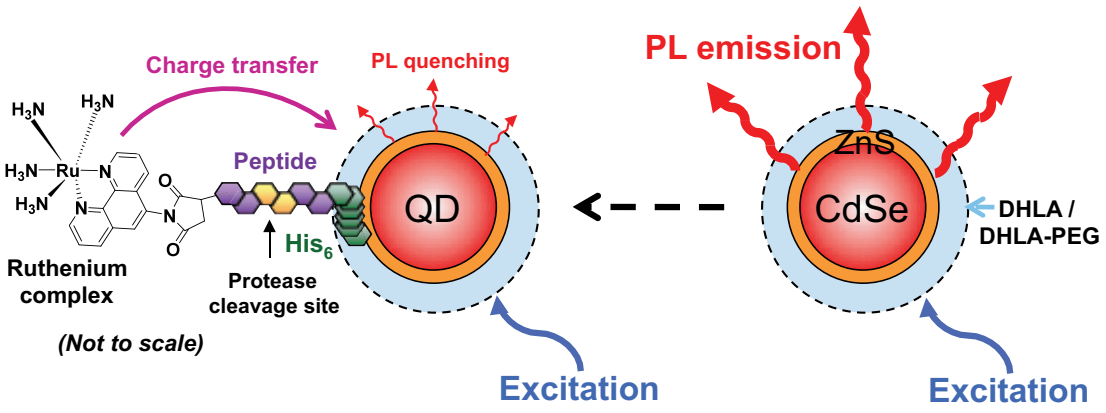

FIGURE 1
Schematic of the charge-transfer-induced quenching of QD PL. Hexahistidine (His_6)-appended peptides are labeled with redox-active metal complexes and self-assembled on CdSe-ZnS QDs capped with either DHLA or DHLA-PEG ligands. The structure of the ruthenium complex is shown. As a result of proximity and redox interactions, charge transfer to the QD results in a quenching of its PL, with a magnitude that depends directly on the number of peptides assembled per QD. Using an amino acid sequence in the peptide that is recognized by a protease allows the assembly of a specific protease sensor. By exploiting the changes in PL quenching following enzyme digestion we are able to realize quantitative monitoring of the enzymatic proteolysis involved.

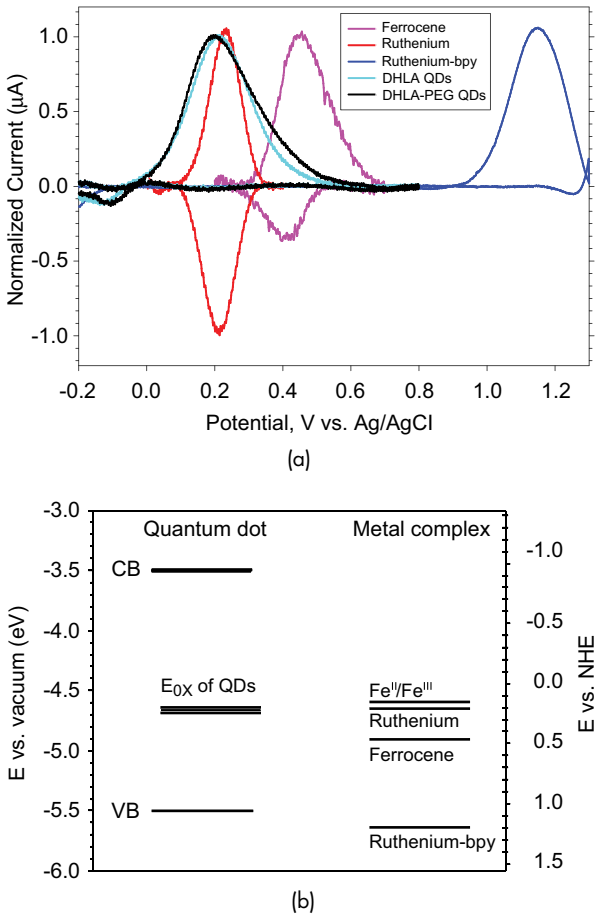

FIGURE 2
(a) Superimposed and background-corrected cyclic voltammograms collected from 590-nm emitting QDs capped with DHLA or DHLA-PEG and peptides labeled with a ruthenium chelate, a bis-bipyridine ruthenium chelate (ruthenium-bpy), and a ferrocene metal complex. (b) Positions of the energy levels corresponding to the QD oxidation peak (E_{ox}), and the oxidation potentials of the metal complexes and the control Fe^{II}/Fe^{III} compound.

to their oxidation. Figure 2(b) shows the extrapolated positions of the QD conduction bands (CB) and valence bands (VB), along with the corresponding QD oxidation peak (E_{ox}) and the oxidation potentials of the metal complex–peptides and of an atomic iron control (Fe^{II}/Fe^{III}). These observations indicate that both the ruthenium complex and atomic iron with their lower oxidation potentials should promote charge transfer from the metal center to the QD when self-assembled into hybrid conjugates. In contrast, ferrocene and ruthenium-bpy with their higher oxidation potentials present less favorable configurations for charge transfer to the QDs.[2]

Spectroscopic Analysis: We next examined the effects that assembling the metal complexes had on the QD photoemission. Figure 3(a) shows representative PL spectra collected from solutions of conjugates having an increasing average number of Ru-peptides per DHLA-functionalized QD. Data clearly show that the proximity of Ru-labeled peptides to the nanocrystals produces a significant and progressive loss of PL; this loss also directly tracks the Ru-peptide valence in the conjugate. Further insight was provided by examining the effects that self-assembling Ru-peptides had on QD absorption properties. The differential absorption plots shown in Fig. 3(b) show that there is bleaching of the QD absorption along with a long red tail contribution in the presence of Ru-peptide, the magnitude of which increases progressively with QD-conjugate valence. This broad red tail change in absorption which accompanies the observed quenching is attributed to electrons populating the QD surface states. Interestingly, QDs functionalized with neutral DHLA-PEG ligands displayed similar quenching from Ru-peptides while the changes in absorption suggested charge-transfer to both the QD surface and core-states. These results were confirmed by using atomic iron as a control, as its low oxidation potential suggested it should also promote charge transfer–based QD quenching.[2] In comparison, the other metal complexes exhibiting higher oxidation potentials did not elicit any significant changes to the QD absorption or PL properties. We also found that the magnitude of Ru-peptide quenching was dependent upon QD size, with larger, red-emitting QDs less quenched on average than smaller, blue-green QDs.[2]

Proteolytic Assays: We exploited this charge transfer process to demonstrate the use of Ru-peptide-based quenching as a signal-transduction modality for monitoring enzymatic proteolysis. Peptides incorporating sequences recognized by the protease chymotrypsin were self-assembled to QDs and exposed to increasing amounts of this enzyme in the absence and presence of a specific inhibitor. Concentration-dependent protease cleavage of the Ru-peptides resulted in concomitant QD PL increases which were converted into enzymatic velocity by correlating quenching efficiencies into the number of intact Ru-peptides per QD-conjugate following enzymatic digestion. This also allowed the derivation of standard Michaelis-Menten kinetic values which describe the enzymatic processes; see Fig. 4. The activity of several other proteases was also monitored with this sensing configuration and in each case the kinetic data agreed reasonably with the expected values.[2]

Conclusions: We combined the electrochemical properties of redox-active metal complexes, peptide-driven self-assembly, and the surface-sensitive quantum dot photoluminescence to elucidate and understand a few aspects of charge transfer between metal complexes and luminescent CdSe-ZnS QDs.

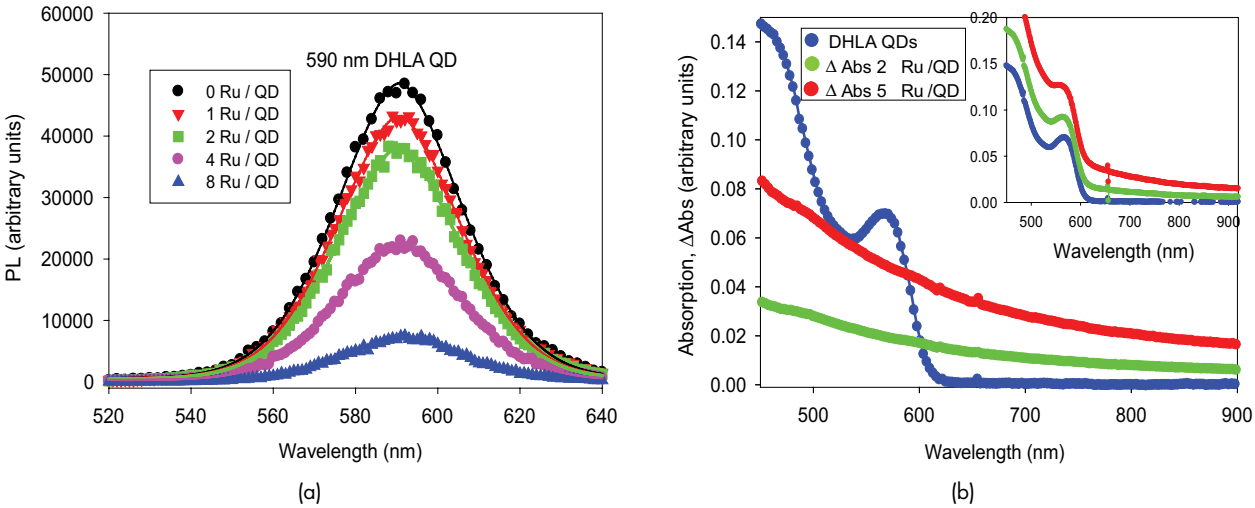

FIGURE 3
(a) Photoluminescence spectra collected from solutions of DHLA-capped QDs at increasing Ru complex-to-QD ratio. (b) Effects of redox coupling on the QD absorption profile. Plots of QD absorption together with differential absorption (ΔAbs) spectra for DHLA QDs self-assembled with increasing number of Ru-peptides. Inset shows the raw absorption data.

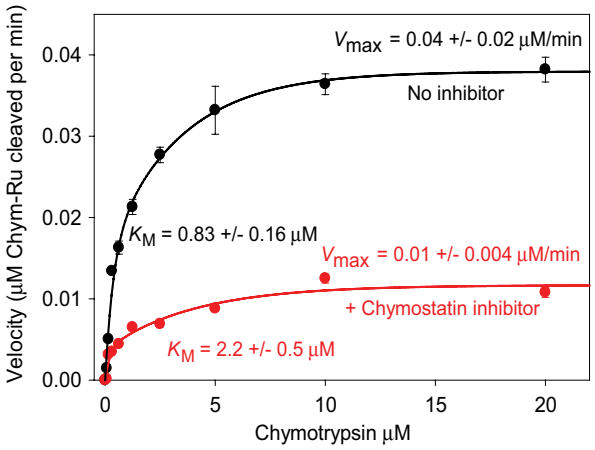

FIGURE 4
Enzymatic assay of chymotrypsin activity utilizing QD–Ru peptide conjugates, showing enzymatic velocity versus increasing chymotrypsin concentration in the presence and absence of a specific chymostatin inhibitor. The changes in kinetic parameters (higher Michaelis constant K_M and lower maximal velocity V_{max}) are characteristic of a mixed inhibition process.

We find that charge transfer and quenching are highly dependent on the relative position of the oxidation levels of QDs and metal complex used; they are also directly responsive to the controlled number of metal complexes brought in close proximity to the nanocrystal surface. The ability to control the number of metal complexes is not something easily achievable with other experimental approaches such as spin-coating or layer-by-layer assembly. Exploiting the underlying PL quenching processes allowed us to demonstrate specific enzymatic sensors. This approach highlights NRL's unique capability to combine an understanding of nanoscale materials and biological functionalities to create new hybrid bio-inorganic sensors. These results strongly suggest that other QD charge transfer configurations may be viable and will allow similar architectures to be designed for a variety of nanoscale roles of direct relevance to the Navy, including sensing, catalysis, and energy harvesting.

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